MOLECULAR BIOLOGY OF THE HUMAN BRAIN

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Neuronal Diversity and its Expression in Disease

S 001 MONOCLONAL ANTIBODIES IN STUDIES OF NEURONAL DIVERSITY.

Colin J. Barnstable, The Rockefeller University, New York, NY 10021.

Monoclonal antibodies provide sensitive tools with which to understand the range and patterns of expression of molecules unique to the nervous system. In one relatively simple portion of the CNS, the retina, it has been possible to identify molecular markers of each of the major classes of cells. The groups of cells identified by particular antibodies are often those predicted from known properties, but sometimes new and potentially important groupings are found. One important feature of the nervous system, the polarisation of neuronal structure, is shown by antibodies that identify molecules restricted to particular subcellular compartments.

Using multiple markers of a single cell type, the rod photoreceptor, we have been able to follow its development and to identify some of the cell interactions necessary for the sequential appearance of particular antigens. By combining anatomical studies of antigen appearance with biochemical studies of the molecules recognised we have begun to translate these developmental studies into a description of gene regulation during neural development.

We have extended our analysis of the visual system to the identification of cell markers in area 17 of several mammals. One cell surface antigen, VC1.1, has been found to identify a subpopulation of GABAergic interneurons in middle and deep cortical layers. Other markers preferentially recognise determinants expressed by pyramidal cells in layers 3 and 5. These, and other markers, are allowing us to define cortical structure in molecular terms that can act as a valuable adjunct to other methods available. They also provide the opportunity of extrapolating results in experimental animal models to the more important but less accessible human brain

S 002 MOLECULAR BIOLOGY OF THE HUMAN BRAIN, Floyd E. Bloom, M.D., Div of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Our laboratories have used multidisciplinary methods to study the localization, circuitry and transmission mechanisms of neuropeptide and other transmitters. This communication will describe the functional diversity in signalling that can arise from interactions between the transmitters of convergent synaptic inputs on common target cells. Two systems will be highlighted: 1) the combination of norepinephrine, the transmitter of the globally directed coeruleo-cortical projection, and Vasoactive Intestinal Polypeptide (VIP), one of the peptides attributed to the intrinsic local interneurons of the rodent cerebral cortex, the bipolar neurons; and 2) interactions between pro-somatostatin derived peptides, found within several neuronal elements of the limbic system, and acetylcholine, long a presumptive transmitter for the septohippocampal pathway. In addition, I will describe our strategies employing molecular genetic approaches to the isolation of novel neuropeptides and other neural markers. We interpret our early findings with this strategy as potentially important in unraveling the chemical and functional organization of the primate brain. Although the nature and number of neurotransmitters including peptides is still incompletely defined, a limited repertoire of response mechanisms is postulated. Interactions among these response mechanisms can provide an enriched diverse array of signalling possibilities.

S 003 THE NEUROBIOLOGICAL EXPRESSION OF CELLULAR DIVERSITY, Edward G. Jones, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Neurons are all the same but different. The fundamental similarity of neurons, despite a wide-range of morphological diversity has been evident to morphologists for many years. No matter how diverse their form, most (but not all) neurons have dendrites, a soma and an axon and thus are morphologically and functionally polarized. Within this morphological framework, the fundamental chemistry of one type of neuron is not greatly different from that of another. What factors, then, determine the vast morphological differences among neurons? Can these differences by explained, as has been done up to the present, solely in terms of inter-neuronal connectionism? Even if so, is this an expression of differential gene regulation and can molecular markers be identified in development or in adulthood that bespeak the individuality of neuronal types? In another vein, how does the differential expression of transmitters, peptides and phosphoproteins reflect neuronal specificity and how fixed is this expression for a given cell type?

This presentation will explore these questions from a background of morphological, immunocytochemical and in situ hybridization studies.

S 004 INDIVIDUAL CELL TYPES AS TARGETS OF NEURAL DISEASE PROCESSES, Joseph B. Martin, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, 02114.

Neuronal cell death is the principal neuropathologic feature in the neurodegenerative diseases. Recent techniques for assessing specific cell types (radioimmunoassay of neuroactive substances, and immunocytochemistry) have clarified the selectivity and specificity of cells affected. Two disorders, Huntington's disease (HD) and Alzheimer's disease (AD) will be discussed.

HD is an autosomal dominant disorder of high penetrance and low mutation rate that causes clinical signs of choreiform movement disorder, psychiatric changes, and dementia. Neuronal cell loss in the striatum (putamen and caudate nucleus) is prominent. Further characterization of cells affected show loss of spiny neurons (containing GABA, substance P and opioid peptides) and sparing of aspiny neurons (containing somatostatin and neuropeptide Y). This selective neuronal loss is mimicked by administration of quinolinic acid, a glutamate receptor active neurotoxin, which is normally found in brain as a metabolite of tryptrophan. The implication of these findings will be discussed.

AD is usually sporadic in occurrence, although families with autosomal dominant inheritance have been reported. Neuropathologic changes include formation of senile plaques and neurofibrillary tangles in the cerebral cortex. Again selectivity of cell involvement occurs. Loss of acetylcholine (and choline acetyl transferase), norepinephrine and serotonin are a reflection of cell loss in subcorticocortical projection systems. Loss of somatostatin and neuropeptide Y occurs in cerebral cortex with preservation of neuronal elements containing cholecystokinin and vasoactive intestinal polypeptide.

The investigation of these disorders with these approaches allows a new delineation of the neuropathologic process and may provide clues to the pathogenesis of the symptoms, or perhaps even of the disease itself.

Regulation of Gene Expression for Transmitters and Neuropeptides

S 005 REGULATION OF NEUROTRANSMITTER MESSENGER RNA, Ira B. Black, Cornell University Medical College, New York NY 10021.

Recent work from our laboratory has indicated that extracellular signals differentially regulate co-localized transmitters in a variety of neuronal populations. For example, in sympathetic neurons, impulse activity with attendant depolarization increases the steady-state levels of messenger RNA (mRNA) encoding tyrosine hydroxylase (TH), while decreasing mRNA coding for substance P peptide precursors. Consequently, similar environmental stimuli evoke entirely different responses in the steady-state levels of mRNA's coding for transmitter molecules. We have used the adrenal medulla to determine whether regulation occurs at the level of gene readout. In fact, nuclear run-off assays indicate that impulse activity decreases transcription of the preproenkephalin gene. In summary, it appears that impulse activity regulates transmitter metabolism by altering steady-state levels of messenger RNA through the alteration of gene transcription.

Parallel studies performed in the locus coeruleus and in the substantia nigra in the brain suggest that similar regulatory mechanisms are operative. Implications of these findings will be discussed.

S 006 REGULATION OF GENE EXPRESSION FOR CATECHOLAMINE ENZYMES
Tong H. Joh, Joanne M. Carroll, Marian Evinger and Onyou Hwang, Cornell University Medical College, New York, N.Y. 10021

Catecholamine neurotransmitters, dopamine, norepinephrine and epinephrine, are synthesized in the central and pheriphral nervous systems as well as in adrenal medulla. Biochemical, neurobiological and pharmacological studies on the regulation of catecholamine biosynthetic enzymes, namely tyrosine hydroxylase, aromatic L-amino acid decarboxylase, dopamine B-hydroxylase and phenylethanolamine N-methyltransferase have been well established in the past. Recently, we have identified and characterized DNAs complementary to mRNAs specific for these enzymes, and the studies on their structure/function relationship are in progress in our laboratory.

It has been found that the primary structure of phenylalanine hydroxylase and tyrosine hydroxylase are more than 70% homologous, suggesting that they are members of a multigene family. Tryptophan hydroxylase which catalyzes the first step of serotonin biosynthesis, may belong to the same gene family. Structural analyses of catecholamine enzyme cDNAs reveal that partial amino acid sequence homologies also exist among them.

In this study, we present our current data on these enzymes' structure/function relationship, and gene regulation by hormones and drugs.

Receptors and Their Interactions with Intracellular Processes

S 007 EXPRESSION AND ACTIVATION OF pp60$^{c\text{-}src}$ IN NEURONAL AND NON-NEURONAL CELLS.

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Cultured neurons from day 15 rat embryo striatum contain two structurally distinct forms of pp60$^{c\text{-}src}$. The 60 kDa form is similar to pp60$^{c\text{-}src}$ from cultured rat fibroblasts or astrocytes. The 61 kDa form is specific to neurons and differs in the N-terminal 18 kDa of the molecule. In undifferentiated neurons the predominant phosphorylated species of pp60$^{c\text{-}src}$ is the fibroblast form. Upon differentiation the neuron-specific form is also found to be phosphorylated. This form has two or more additional sites of serine phosphorylation within the N-terminal 18 kDa region of the molecule, one of which is Ser 12. However, treatment of the neuron-specific form with phosphatase does not change its gel mobility, implying that the alteration in the N-terminal region is not due to phosphorylation. We are investigating whether the neuron-specific form differs in primary structure in the N-terminal region by isolating and sequencing cDNA clones for pp60$^{c\text{-}src}$ from brain cDNA libraries. The specific protein-tyrosine kinase activity of the total pp60$^{c\text{-}src}$ population is increased 7-fold, as measured by phosphorylation of an exogenous substrate, in striatal neurons differentiated in culture compared to undifferentiated neurons. Normally the activity of fibroblast pp60$^{c\text{-}src}$ is negatively regulated by phosphorylation at Tyr 527. In addition oncogenically-activated forms of pp60$^{c\text{-}src}$ with high protein kinase activity are phosphorylated at Tyr 416 and not Tyr 527. The elevation in protein kinase activity of pp60$^{c\text{-}src}$ from neurons, however, occurs without a detectable decrease in Tyr 527 phosphorylation or increase in Tyr 416 phosphorylation. Thus the the mechanism of activation is unclear. In support of the results obtained with cultured cells, we have been able to detect the neuron-specific form in striatal tissue, and show that there is a 5-fold increase in the total pp60$^{c\text{-}src}$ protein kinase activity from day 15 to day 20 of embryonic development in the striatum. The expression of the neuron-specific form of pp60$^{c\text{-}src}$ and the increase in specific protein kinase activity may be important for the differentiation and/or the function of neurons.

Treatment of quiescent mouse fibroblasts with PDGF leads to rapid phosphorylation of a small fraction of the pp60$^{c\text{-}src}$ population on an unknown tyrosine in the N-terminal 18 kDa of the molecule, causing a slight gel mobility retardation. We are testing whether this N-terminal tyrosine is phosphorylated directly by the PDGF receptor. There is also an increase in phosphorylation of Ser 12 of all pp60$^{c\text{-}src}$ molecules, due to the activation of protein kinase C by PDGF-induced PI turnover, and of the altered form of pp60$^{c\text{-}src}$ at two novel, but unassigned, serine phosphorylation sites, as well as at Tyr 416. These changes in phosphorylation are correlated with a 2-3 fold increase in pp60$^{c\text{-}src}$ protein kinase activity. Thus some of the PDGF-induced tyrosine phosphorylation events may be due to activated pp60$^{c\text{-}src}$.

S 008 TRANSDUCTION OF SIGNALS BY BRAIN PROTEIN KINASES, Mary B. Kennedy, Division of Biology, California Institute of Technology, Pasadena CA 91125.

The brain contains at least four major protein kinase classes that are regulated by distinct, but interacting second messenger systems; cyclic AMP, cyclic GMP, diacylglycerol, and calcium ion. The organization and specific functions of these kinases and second messenger systems varies in different types of neurons. The concentrations of both the kinases and the molecules responsible for generating their respective second messenger activators can vary widely. In addition, protein substrates that are regulated by these transduction pathways, such as ion channels and cytoskeletal elements, are located in distinct cell types and subcellular locations. Much current research on signal transduction pathways in the brain is directed toward understanding the specific location and organization of protein kinases, their activators and their substrates.

Calcium signals are widely used in the CNS and are often highly localized. Neuronal activity can raise the concentration of cytosolic calcium ion by opening voltage dependent calcium channels. Receptors for some classical neurotransmitters, such as the acetylcholine receptor and the NMDA-type glutamate receptor, are linked to channels through which calcium passes into the cell. In addition, activation of receptors linked to the hydrolysis of phosphatidylinositol phosphate produces inositol triphosphate (ITP) which can release calcium ion from internal stores and transiently raise the concentration of calcium. Among the several regulatory targets for calcium ion is a broad specificity calcium/calmodulin-dependent protein kinase that is particularly highly concentrated in the telencephalon (forebrain). This molecule is present throughout the cytosol of many forebrain neurons, in dendrites, in axons, and in some nerve terminals, where it can potentially phosphorylate several different proteins, many of them associated with the cytoskeleton. Cell biological studies suggest that it is particularly highly concentrated in postsynaptic densities where it may respond specifically to receptor-generated calcium signals. When it is activated by calcium and calmodulin *in vitro*, this kinase undergoes extensive autophosphorylation. When an average of 3-5 phosphates have been incorporated into each multisubunit holoenzyme, the kinase acquires a new calcium-independent activity. It will then remain active until its activation is terminated by a protein phosphatase. It is possible that *in vivo* it may continue to generate changes in the functions of CNS molecules after the decay of an initial activating calcium signal. We are testing the hypothesis that this mechanism may participate with others in a cascade of regulatory events initiated by transient increases in calcium concentration to produce various forms of synaptic plasticity and perhaps alterations in the shape and structure of the neuronal cytoskeleton.

S 009 MOLECULAR AND REGULATORY PROPERTIES OF ADENYLATE CYCLASE COUPLED ADRENERGIC RECEPTORS, Robert J. Lefkowitz and Marc G. Caron, Departments of Medicine, Biochemistry and Physiology, Duke University Medical Center, Durham, North Carolina 27710.

Several pharmacologically distinguishable subtypes of adrenergic receptors are coupled to second messenger generating systems in diverse cell types. β_1 and β_2-adrenergic receptors stimulate the membrane bound enzyme adenylate cyclase thus regulating intracellular levels of cyclic AMP. α_2-adrenergic receptors inhibit the activity of the enzyme lowering levels of cyclic AMP. α_1-Adrenergic receptors appear to regulate the activity of phospholipase C which in turn controls the rate of hydrolysis of polyphosphoinositides leading to generation of two putative second messenger candidates, diacylglycerol and inositol trisphosphate. We have purified the adrenergic receptor subtypes to homogeneity and studied their molecular properties. Most information is currently available for the β_2-adrenergic receptor, the gene and cDNA for which we have cloned and sequenced from both hamster and human material. There is a remarkable homology observed between the β-adrenergic receptor and the family of visual pigments such as rhodopsin. In addition to sequence homology, the two proteins have identical membrane topology including seven putative transmembrane spanning domains.

Another common feature between the β-adrenergic receptor and rhodopsin is that the function of both receptor proteins is controlled by phosphorylation by specific protein kinases. The two enzymes are the β-adrenergic receptor kinase and rhodopsin kinase. Each enzyme phosphorylates its receptor substrate only when it is in its active form, i.e., occupied by agonist in the case of the β receptor or bleached by light in the case of rhodopsin. Each enzyme, moreover, will phosphorylate the other's preferred substrate. In each case, such phosphorylation diminishes receptor activity. This phosphorylation likely occurs at serine/threonine clusters which are present in the carboxy termini of both proteins. The function of the phosphatidylinositol cycle coupled α_1-adrenergic receptors is also regulated by phosphorylation reactions. However, in this case, receptor phosphorylation appears to be mediated by protein kinase C. The similarities in structure and function observed between the β-adrenergic receptor and the visual light receptor rhodopsin provide new clues for understanding the functional activity of such receptor proteins and the structural basis for important regulatory reactions.

Regulation of Gene Expression for Receptors

S 010 THE ACETYLCHOLINE NICOTINIC RECEPTOR : FUNCTIONAL ORGANISATION AN REGULATION, Jean-Pierre Changeux, Molecular Neurobiology, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France.

The nicotinic receptor (AChR) is a heterologous pentamer $\alpha_2\beta\gamma\delta$ which contains the ion channel and all the structural elements engaged in the regulation of its opening by ACh and by a variety of allosteric effectors. The ACh binding site has been localized on the amino acid sequence of the α-subunit from T. marmorata by a photolabile antagonist (DDF) primarily, though not exclusively, in the 179-207 AA domain. The unique high affinity site for the noncompetitive channel blocker chlorpromazine comprises Ser 262 in the putative transmembrane helix II of the δ subunit and the homologous serine in the β-subunit. The relationship of this site with the ion channel is discussed.

The evolution of topological distribution and properties of the AChR during the formation of the neuromuscular junction in the chick involves a complex sequence of processes which include several distinct regulations of gene expression. After an initial onset of AChR gene expression consecutive to the fusion of myoblasts into myotubes, an activity-dependant repression of AChR biosynthesis takes place in extra-junctional areas. Both can be reproduced in cultured muscle cells and are analysed with probes derived from genomic clones encoding the α-subunit gene. The 5' promoter region of this gene was characterized and shown by transfection experiments to contain sequences important for its tissue specific and developmentally controlled expression.

Calcitonin-gene-related peptide, a coexisting peptide present in spinal cord motoneurons, increases the number of AChR molecules (1.6x) in chick cultured muscles via intracellular signals distinct from those elicited by electrical activity, pointing to possible differential regulation of receptor genes in junctional vs nonjunctional nuclei.

The role of the 43000 v_1 protein in the subneural "epigenetic" localisation and stabilisation of the AChR is discussed.

S 011 IDENTIFICATION OF A GENE FAMILY CODING FOR ACETYLCHOLINE RECEPTORS EXPRESSED IN MUSCLE AND BRAIN, Stephen Heinemann, Jim Coulter, Danny Goldman, Evan Deneris, John Connolly, Paul Gardner, Sylvia Evans, Keiji Wada, Walter Luyten and Jim Patrick, Molecular Neurobiology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Most plausible theories of higher brain function such as memory and learning depend upon changes in the efficiency of chemical synapses. It seems unlikely that these theories will be testable until we have learned more about the structure and function of the molecules essential for chemical transmission. We have used a molecular genetic approach to clone the genes and their RNA products (i.e. cDNA) that code for the nicotinic acetylcholine receptor expressed in mouse skeletal muscle. The cDNA clones were sequenced and the complete primary structure of the receptor deduced. Models relating structure to function have been proposed and are being tested by site specific mutagenesis using an oocyte expression system to meausure function. The cDNA clones have been used to study the regulation of receptor synthesis in muscle. These experiments indicate that the receptor genes are regulated by the level of electrical activity. The promoter for one subunit has been isolated and is being analyzed in order to understand how electrical activity can regulate gene activity. These results may have implications for mechanisms by which input to the brain may change brain function. Nicotinic acetylcholine receptors are known to be expressed in nerve cells in the peripher yand the brain. The muscle cDNA clones have been used as probes to screen cDNA libraries made from various regions of the rat brain. We have identified a gene family with at least five members which code for proteins with sequence and structural homology to the muscle nicotinic acetylcholine receptor. In situ hybridization experiments show that his gene family is expressed in areas of the brain known to contain cholinergic pathways. Recently we have shown that some of the genes expressed in the brain code for functional acetylcholine receptors.

S 012 STRUCTURE AND FUNCTION OF ACETYLCHOLINE RECEPTORS.
Masayoshi Mishina, Tai Kubo and Shosaku Numa, Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Kyoto 606, Japan.
The nicotinic acetylcholine receptor from the electric organ of Torpedo californica consists of four kinds of subunits assembled in a molar stoichiometry of $\alpha_2\beta\gamma\delta$. The primary structures of the acetylcholine receptor subunits from fish electric organ as well as from mammalian skeletal muscle have been elucidated in our laboratory by cloning and sequencing the cDNAs or genomic DNAs. The acetylcholine receptor subunits exhibit marked amino acid sequence homology and are similar in hydropathy profile and predicted secondary structure, thus being oriented presumably in a pseudosymmetric fashion across the membrane to form the ionic channel.

In an attempt to understand the molecular basis for acetylcholine receptor function, we have developed a cDNA expression system which produces functional receptor in Xenopus laevis oocytes. Functional analysis of acetylcholine receptor mutants generated by site-directed mutagenesis allows functional regions of the receptor to be localized. Furthermore, single-channel current measurements on hybrid receptors composed of Torpedo and bovine subunits, produced by expression of the corresponding cDNAs, suggest that at least the δ-subunit is involved in the gating of the channel.

By cDNA cloning, we have discovered a novel muscle acetylcholine receptor subunit (named the ε-subunit). The single-channel properties of bovine acetylcholine receptors of different subunit compositions produced by expression of the corresponding cDNAs, in conjunction with the developmental changes observed in the muscular contents of the subunit mRNAs, suggest that replacement of the γ-subunit by the ε-subunit is responsible for the functional alteration of the receptor during muscle development.

The primary structure of porcine brain muscarinic acetylcholine receptor has been deduced by cloning and sequencing the cDNA. The muscarinic receptor is homologous with the β-adrenergic receptor and rhodopsin in both amino acid sequence and suggested transmembrane topography. Expression of the cDNA produces functional muscarinic receptor in Xenopus oocytes. The tissue location of the RNA hybridizing with the cDNA, as well as the high apparent binding affinity for pirenzepine of the muscarinic receptor expressed from the cDNA, is consistent with the notion that the cDNA encodes the muscarinic receptor of the M subtype.

S 013 MOLECULAR BIOLOGY OF GABA RECEPTORS, Richard W. Olsen and Allan J. Tobin, Departments of Pharmacology and Biology, UCLA, Los Angeles, CA 90024.
The GABA receptor protein complex has been purified to near homogeneity from mammalian brain in milligram quantities by benzodiazepine affinity chromatography. Two major stained peptides on SDS-PAGE were identified as receptor subunits on the basis of photoaffinity labeling: the α subunit (52 kD) was photolabeled with [^{3}H]flunitrazepam (identifying the benzodiazepine binding site), and the β subunit (57 kD) was photolabeled with [^{3}H]muscimol (identifying the GABA binding site). Two minor peptides (49 kD and 31 kD) were suspected break-down products of the two major peptides. The purified protein contained GABA binding (1750 pmol/mg), benzodiazepine binding (700 pmol/mg), convulsant [^{35}S]TBPS binding, and chloride-dependent allosteric modulation of the three receptor sites by barbiturates, indicating the presence of all native activities associated with the receptor in the neuronal membrane, probably including the chloride channel. Rabbit antiserum was produced to this protein and in microliter amounts precipitated soluble GABA-benzodiazepine receptor activity and reacted with receptor subunits on Western blots. This antiserum was employed to obtain cDNA clones from a λGT11 expression library from human and mouse brain. Antibody purified with the antigenic fusion protein reacted with purified receptor subunits on Western blots. These cDNA clones are being further characterized. In addition, quantities of the two subunits have been prepared by preparative SDS-PAGE for structural studies and antibody production. Work by other laboratories has produced monoclonal antibodies to the GABA-benzodiazepine receptor that react with our purified receptor subunits on Western blot (gift of H. Mohler, Basel, Switzerland). Other groups have demonstrated that crude mRNA from vertebrate brain can express drug-modulated GABA receptor-chloride channels in frog oocytes, laying the foundation for future expression of engineered message for this important brain neurotransmitter receptor/ion channel.

Growth, Maturation and Maintenance Factors

S 014 BRAIN-DERIVED NEUROTROPHIC FACTOR, Yves-A. Barde and H. Thoenen, Max-Planck Institute, Dept. of Neurochemistry, 8033 Martinsried, FRG.

Our work on brain-derived neurotrophic factor (BDNF) is based on the concept that during neurogenesis, some vertebrate neurons require specific neurotrophic proteins if they are to survive. This requirement is particularly critical at the time of target innervation. In addition, we think that these proteins have a restricted distribution and are present in very small, limiting amounts in the targets of innervating neurons.
Classical transplantation and ablation studies in neuroembryology show that there is a need for proteins with such biological properties, and work on nerve growth factor (NGF) strongly supports this concept.
The data obtained with BDNF suggest that functionally, it belongs with NGF to the same category of neurotrophic proteins. BDNF is a small protein that has been isolated from mammalian brain after a purification factor of over one million fold. It selectively supports the survival in vitro of some but not all neuronal populations, at a time when these neurons send processes in vivo to their targets. These neurons include not only placode-derived sensory neurons, not responding to NGF, but also neural crest-derived sensory neurons responding to NGF. This and other findings have led to the suggestion that first order sensory neurons, with their double projections, require the combination of 2 different neurotrophic proteins in order to survive. In some cases, one is BDNF that reaches the neurons by their central processes and the other NGF, present in the periphery. Amongst the neurons not responding to NGF, but to BDNF, are the embryonic rat retinal ganglion cells.
In addition to the functional relatedness between NGF and BDNF, it is interesting to note that BDNF, both in terms of molecular weight and isoelectric point is very similar to the monomer of NGF. However, partial amino acid sequence of BDNF has not revealed any similarities with the primary structure of NGF, nor with that of any other known proteins.

S 015 ROLE OF NERVE GROWTH FACTOR IN THE BRAIN. F. Hefti, J. Hartikka and C. Montero. Dept. of Neurology, University of Miami School of Medicine, Miami FL, 33101.
Nerve growth factor (NGF) has been established as a neurotrophic factor for peripheral sympathetic and sensory neurons. Despite its ability to promote survival, fiber growth and function of peripheral catecholaminergic neurons, NGF fails to affect catecholaminergic neurons of the CNS. However, findings obtained in recent years have provided strong evidence that NGF acts as a neurotrophic factor for cholinergic neurons of the mammalian forebrain. NGF and the mRNA coding for NGF are located in the brain and their anatomical distribution corresponds to the anatomy of cholinergic neurons. Furthermore, NGF stimulates choline acetyltransferase (CAT) activity by cholinergic neurons in vivo and in vitro. Recent findings from our laboratory suggest that NGF, besides increasing CAT activity in cholinergic neurons, is able to promote survival and fiber elongation of these neurons in vivo and in vitro. Dissociated neurons from the septal area of fetal rats were grown in low density cultures. Cultures grown in presence of NGF contained more cholinergic cells than control cultures. Cholinergic neurons grown in presence of NGF were found to have a higher total length of fibers, total number of branching points, and average length between two branching points than cholinergic neurons surviving in control cultures. In adult rats, intraventricular injections of NGF were found to attenuate the lesion-induced degeneration of forebrain cholinergic neurons. A lesion of the septohippocampal pathway produced by transection of the fimbria resulted in loss of 50% of the cholinergic neurons in the medial septal nucleus and the diagonal band of Broca. Intraventricular injections of NGF to such animals during four weeks reduced the loss of these neurons to 10%. Using monoclonal antibodies against rat and human NGF receptors in an immunohistochemical procedure, we found that NGF receptors were located on cholinergic neurons. None of the other populations of neurons in the mammalian forebrain expressed these receptors. The findings suggest that NGF plays a role in survival, fiber elongation, and maintenance of function of forebrain cholinergic neurons. In the forebrain, these actions of NGF seem to be selective for cholinergic neurons.

Molecular Biology of the Human Brain

S 016 NEUROPEPTIDES: MULTIPLE REGULATORY MECHANISMS AND THEIR ROLES IN MEDIATING SIMPLE BEHAVIORS, Richard H. Scheller, Department of Biological Sciences, Stanford University, Stanford, CA 94305. The central nervous system of the marine mollusk Aplysia is a useful model system for studies of the cellular basis of simple behaviors. We have isolated several genes encoding the precursors for biologically active peptides used as intercellular messengers by identified neurons in the abdominal ganglion. Cell specific expression of the neuropeptide genes is being studied by microinjection of cloned genes into the neurons. The large size of Aplysia neurons makes it possible to analyze the expression of microninjected genes in single cells. Antibodies to synthetic peptides define the anatomical and subcellular distribution of the molecules. The peptide products are expressed in subsets of central neurons and are packaged in multiple populations of dense core vesicles. The distribution of immunoreactive processes suggests three anatomical locations of neurosecretion, the neuropile, the connective tissue sheath and specific peripheral targets. The structure of the neuropeptide precursors defined by analysis cDNA clones suggest sites of proteolytic processing which liberate mature, biologically active to define peptides. HPLC in conjunction with protein microsequencing are used to define the precise proteolytic processing pathway. Once cleavage products of the precursors are defined, physiological analysis of their actions is possible. The peptides act directly as excitatory and/or inhibitory transmitters and so modulate the actions of other substances on both neuronal and muscle targets. Voltage and patch clamp analysis are being used to define the cellular mechanisms of action of the peptide products. The multiple roles of biologically active peptides in the nervous system are being defined by this multidisciplinary approach aimed at understanding the simple behaviors of Aplysia.

Interactions Between the Neuronal Genome and Processes of Learning and Memory

S 017 BIOCHEMICAL AND ELECTROPHYSIOLOGICAL MECHANISMS OF HIPPOCAMPAL LONG-TERM POTENTIATION, Michel Baudry, Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 97717.
Long-term potentiation of synaptic transmission has been an increasingly popular model for studying learning and memory mechanisms in mammalian CNS and in particular in the hippocampus. Several reasons explain this popularity as well as justify the validity of this model: i) causes and substrates of LTP can be easily studied in *in vitro* preparations, ii) the LTP effect is produced by very brief events and persists for very long periods of time, iii) LTP is selective for the population of synapses experiencing the high frequency stimulation and is optimally induced by physiological patterns of stimulation similar to those occurring in behaving animals, iv) it is prominent in hippocampus and cortex, structures that are central to memory storage, and v) preventing LTP produces marked alterations in some forms of memory. The mechanisms involved in the production of LTP are now fairly well understood. The initial phase of electrical stimulation sets up a transient disinhibition that allows a temporal summation of synaptic depolarization elicited by subsequent stimulation. The large synaptic depolarization thus obtained allows the activation of a transmitter-dependent voltage-dependent receptor (the NMDA receptor) that produces a large influx of calcium in postsynaptic elements. Increased calcium concentrations stimulate a variety of calcium-dependent processes in dendritic spines, including a calcium-dependent protease (calpain), whose activation results in proteolysis of cytoskeletal elements and in a disassembly/reassembly process of the synaptic structure. This is postulated to produce lasting changes in the functional properties of synaptic contacts. The various elements of this sequence can now be analyzed separately. The in vitro slice preparation is useful to study the role of inhibitory processes and to test for the effects of pharmacological compounds interfering with specific mechanisms. Functional receptors for glutamate have been reconstituted in artificial lipid bilayers providing detailed understanding of their gating and regulatory mechanisms. The role of calpain in the regulation of cell shape is best studied in homogeneous cell populations. It remains to be clearly established whether the stabilization of new structures resulting from LTP induction requires proteins newly synthesized and rapidly transported or locally synthesized on dendritic polyribosomes.

Dementia and the Aging Brain

S 018 MOLECULAR PATHOLOGY OF INTRANEURONAL AND EXTRANEURONAL FILAMENTS IN ALZHEIMER'S DISEASE, Dennis J. Selkoe, Center For Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston MA 02115. The formation of highly stable intra- and extraneuronal protein filaments accompanies progressive neuronal degeneration in Alzheimer's disease (AD). Qualitatively indistinguishable fibrous lesions occur in much smaller numbers and in restricted distribution in the brains of most normal aged humans. Inside neurons, paired helical filaments (PHF) and antigenically related straight filaments (10-20 nm) accumulate as perikaryal tangles (NFT) and in the dystrophic axons and dendrites that comprise neuritic (senile) plaques. The insolubility of PHF and the inability to purify them fully have hampered direct protein chemical analyses and led to reliance on immunocytochemical approaches. Whereas PHF and straight filaments share certain epitopes with the neurofilament (NF) 145 and 200 Kd and MAP2 proteins, recent studies reveal that the microtubule-associated phosphoprotein, tau, appears to be their principal antigenic determinant. Moreover, we have found that several monoclonal antibodies against phosphorylated NF previously shown to label PHF cross-react with tau; independent evidence for the presence of NF proteins in PHF is now required. In addition to tau reactivity, PHF antisera contain antibodies to as-yet-unidentified proteins. Also, certain PHF monoclonal antibodies do not recognize tau or any other known protein. Thus, neuronal proteins other than tau are also found in PHF.

Since NFT that are structurally and immunochemically indistinguishable from those in AD occur in numerous unrelated brain diseases, the neuritic plaque with its central deposit of extracellular amyloid filaments is a more specific lesion for AD. Neuritic plaques occur principally in 3 conditions: AD; normal brain aging; and the AD-type degeneration occuring late in Down's syndrome. The amyloid that forms the core of the plaque is composed in part of a ~4.5 Kd hydrophobic protein that readily aggregates into filamentous polymers having β conformation. Antibodies to the core amyloid crossreact with amyloid deposits in cortical and meningeal microvessels in AD but show no reaction with NFT. Although the amino acid compositions of purified amyloid cores and meningovascular amyloid are highly similar, the latter protein can be sequenced whereas the former appears to be blocked at its n-terminus. The sequence is not found among known proteins. Antibodies raised to either amyloid filaments, their 4.5 Kd protein or a synthetic amyloid peptide recognize microvascular and plaque amyloid in old rhesus monkeys and 4 other aged mammmals in a highly similar fashion to AD amyloid. These animals develop neuritic plaques late in life but form no PHF. The amyloid antibodies also recognize certain normal plasma proteins, raising the possibility that AD amyloid originates in part from proteins synthesized outside the CNS. AD and Down's syndrome may represent an acceleration of an age-related involutional pathology that is conserved in other mammals and involves progressive deposition of altered proteins in cerebral microvessels and the cortical neuropil.

DNA Polymorphisms and Genetic Markers

S 019 DNA MARKERS IN NEUROLOGICAL DISORDERS. JAMES F. GUSELLA, PH.D., NEUROGENETICS LABORATORY, MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MA 02114.

The use of restriction fragment length polymorphisms as high quality genetic markers has made genetic linkage analysis a powerful tool for the investigation of neurogenetic disorders. The exact strategy for applying this method varies depending on the disorder, with approaches that include the use of randomly chosen markers, candidate genes, chromosomal linkage maps and investigation of tumor tissue. Our application of DNA markers to determining the chromosomal locations of the primary defects in Huntington's disease, Familial Alzheimer's disease and bilateral acoustic neurofibromatosis illustrates each of these approaches. The discovery of DNA markers linked to a disease gene permits a definition of the extent of heterogeneity in the disorder and offers the possibility of presymptomatic or prenatal diagnosis in many cases. The greatest impact of a successful linkage analysis, however, is the avenue it opens to cloning and characterizing the genetic defect based only upon its chromosomal position, without a knowledge of the affected protein product. We are currently attempting to identify the primary defects in Huntington's disease, familial Alzheimer's disease, and bilateral acoustic neurofibromatosis using chromosome-specific cloning methods.

S 020 MAPPING HUMAN CHROMOSOMES BY GENETIC LINKAGE, Ray White, Mark Leppert, Peter O'Connell, G. Mark Lathrop, and Jean-Marc Lalouel, Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City UT 84109.

The rationale for the effort to develop a complete linkage map of the human genome is to cover each chromosome with markers at close enough intervals that any unknown gene can be located by its co-inheritance with a mapped marker within a family. Genetic diseases in which the biochemical defect is unknown can be assigned to a specific chromosomal region by linkage mapping if a specific allele of an already mapped marker segregates with the disease; a recent example is cystic fibrosis. When the gene is located, strategies for its isolation and cloning can be put into motion with the ultimate aims of elucidating the disease mechanism and designing appropriate therapy. We have developed primary linkage maps for several chromosomes (1,2), and are developing a series of new and highly polymorphic DNA markers that are based on loci containing a variable number of tandem repeats of a defined oligonucleotide sequence. A large panel of cell lines from three-generation families, involving more than 800 individuals, provides DNA for genotyping new markers as they become available for mapping. The normal chromosome maps are serving as a reference base for linkage studies in the relatively rare families that segregate genetic diseases, such as familial polyposis, ataxia telangiectasia, and X-linked spastic paraplegia. Our mapping studies have shown that the frequency of recombination events between loci -- the basis for genetic linkage studies -- usually varies according to sex, and apparently not in a constant ratio from one chromosome to the next, or even from one interval to another. A hypothesis concerning the mechanism of the sex effect on recombination will be discussed. As a practical matter, we conclude that two genetic linkage maps must be prepared for each chromosome, one for each sex, showing a constant gene order but different recombination distances between loci.

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2. Leppert, M. Cavenee, W., Callahan, P., Holm, T., O'Connell, P, Thompson, K., Lathrop, G.M., Lalouel, J.M., and White, R. (1986). A primary genetic map of chromosome 13q. Am. J. Hum. Genet. 39:425-437.

Discussion: Human Brain Imaging

S 021 HUMAN BRAIN IMAGING, Michael J. Kuhar, NIDA Addiction Research Center, Baltimore, Maryland 21224

Imaging studies of brain have often been powerful extensions of *in vitro* biochemical molecular studies. The imaging approach provides biochemical measurements in an anatomical context. Also, measurements can be made in clinical populations and can be directly applied to human disease.

Some imaging techniques are microscopic, provide resolution in the micron range, but are invasive and utilize tissue obtained at autopsy. These include receptor autoradiography and *in situ* hybridization. Other techniques are relatively noninvasive, use external detectors with living humans, but provide resolution in the millimeter range. These include positron emission tomography (PET) and single photon emission computed tomography (SPECT); these are sensitive tools capable of measurement in the nanomolar range. Yet another imaging tool, magnetic resonance imaging (MRI), is primarily useful for measuring brain structure and tissue density, although single cells can be examined by spectroscopic imaging.

An example of where the imaging approach has been applied fruitfully is receptor mapping. It has been used with autopsy tissues (autoradiography) and in living human (PET scanning). More recent approaches include immunohistochemistry with monoclonal antibodies and *in situ* hybridization with nucleic acid probes. These recent developments have solved some difficult problems in receptor mapping.

Many other problems have been explored with brain imaging and yet other problems will be explored in the near future. A summary of these efforts will be presented.

Role of Viruses in Brain Disorders

S 022 SV40 IN TRANSGENIC MICE, Albee Messing, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706. Transgenic mice have proven to be valuable models for studing the pathological consequences of atypical gene expression in the nervous system. We have produced several types of transgenic mice carrying the early region from simian virus 40 (SV40) which codes for the small and large T antigens. Depending upon modifications in adjacent regulatory sequences, these mice consistently develop neoplasms in either choroid plexus, liver, endocrine pancreas of exocrine pancreas. In addition, one of these groups develops widespread hypomyelination and demyelination in the peripheral nervous system. Results of developmental, immunohistochemical, and morphometric analyses of thes lesions will be presented. We have found a close correlation between the onset of SV40 large T antigen gene expression and morphologic abnormalities in the tissues. In some lines of mice tissues appear to progress through a hyperplastic phase before the development of neoplasia, while in other lines no distinct hyperplastic phase is seen. The peripheral neuropathy may result from expression of large T antigen in Schwann cells without transformation. The consequences of expression of SV40 large T antigen elsewhere in the body, and of expression of other papovavirus genomes in the brain, will be reviewed.

S 023 PRIONS CAUSING BRAIN DEGENERATION. Stanley B. Prusiner. Department of Neurology, University of California, San Francisco, CA 94143, USA.
Scrapie and Creutzfeldt-Jakob disease (CJD) are caused by prions. These infectious pathogens are composed largely, if not entirely, of protein molecules. No prion-specific polynucleotide has been identified. Purified preparations of scrapie prions contain high titers ($\geq 10^{9.5}$ ID_{50}/ml), one protein (PrP 27-30) and amyloid rods (10-20 nm in diameter x 100-200 nm in length). Considerable evidence indicates that PrP 27-30 is required for and inseparable from scrapie infectivity. PrP 27-30 is encoded by a single copy cellular gene and is derived from a larger protein, designated PrP^{Sc} or PrP 33-35^{Sc}, by protease digestion. A cellular isoform, designated PrP^{C} or PrP 33-35^{C}, is encoded by the same gene as PrP^{Sc} and both proteins appear to be translated from the same 2.1 kb mRNA. The PrP gene is comprised of two exons separated by a 10 kb intron; the second exon contains the entire open reading frame. Monoclonal antibodies to PrP 27-30 as well as antisera to PrP synthetic peptides specifically react with both PrP^{C} and PrP^{Sc}, establishing their relatedness. PrP^{C} is digested by proteinase K while PrP^{Sc} is converted to PrP 27-30, under the same conditions. Prion proteins are synthesized with signal peptides and are integrated into membranes. Detergent extraction of microsomal membranes isolated from scrapie-infected hamster brains solubilizes PrP^{C} but induces PrP^{Sc} to polymerize into amyloid rods. This procedure allows separation of the two prion protein isoforms and the demonstration that PrP^{Sc} accumulates during scrapie infection while the level of PrP^{C} remains constant. The prion amyloid rods generated by detergent extraction are identical morphologically except for length to extracellular collections of prion amyloid filaments which form plaques in scrapie- and CJD-infected brains. The prion amyloid plaques stain with antibodies to PrP 27-30 and PrP peptides. PrP^{C} does not accumulate in the extracellular space. Prion rods composed of PrP 27-30 were dissociated into phospholipid vesicles with full retention of scrapie infectivity. Digestion of these phospholipid vesicles with nucleases did not alter prion titers. All attempts to identify a scrapie-specific nucleic acid in purified preparations of prions have been unsuccessful, to date. The murine PrP gene (*Prn-p*) was mapped to chromosome 2 and is linked to the *Prn-i* gene which controls scrapie incubation times. The human PrP gene (PRNP) was mapped to chromosome 20 which is homologous to murine chromosome 2. Human and hamster PrP cDNA sequences are 90% homologous. While the central role of PrP^{Sc} in scrapie pathogenesis has been established, the chemical and/or conformational differences between PrP^{C} and PrP^{Sc} are unknown but probably arise from post-translational modifications.

S 024 ROLE OF VIRUSES IN BRAIN DISORDERS: AN OVERVIEW, Leslie P. Weiner, Depts. Neurology and Microbiology, University of Southern California, Los Angeles, CA 90033

In the past 20 years, there has been a significant change in the fundamental thinking about viral infections of the nervous system. The clinical syndromes resulting from acute, persistent, latent chronic and slow viral infections are better understood and modern techniques have stimulated research into immune-mediated disorders such as multiple sclerosis and degenerative diseases such as Alzheimer's disease and Parkinsonism. The effect that viruses have on the nervous system is multifactorial. Whether the infection is clinically apparent, inapparent or subtle relates to both the nature of the organism and the host response. Of importance is the viral structure and replication. Host factors include the immune response, the blood-brain-barrier and CNS cell tropism as they relate to viral disease. The multiplication cycle of viruses can have a number of outcomes depending on the genetics of the virus, the virus-host cell interaction and the overall state of the host immune system. Perhaps of greatest interest are the processes of selective vulnerability of CNS cells and the establishment of latent or persistent viral states. Viral-receptor interactions and viral replication including transcription and translation of structural and nonstructural viral proteins appear to be under the control of the viral genome, with host factors influencing the expression of viral genes. A number of model systems of both RNA and DNA viruses have been studied with recent attention to the lentivirus, human immunodeficiency virus, the putative cause of AIDS and the AIDS dementia complex. The molecular genetics of reoviruses, the murine coronaviruses, the herpes viruses, and measles have given some understanding of viral-CNS malfunction and cell death.

Neuronal Diversity and Neural Disease

S 100 MOLECULAR CLONING AND PRIMARY STRUCTURE OF MYELIN-ASSOCIATED GLYCOPROTEIN
Monique Arquint, Jim Down, Loo-Sar Chia, John Roder and Rob Dunn

Myelin associated glycoprotein (MAG) may be involved in the interaction of oligodendrocytes and Schwann cells with neurons. In order to gain more insight into the structure of MAG two rat cDNA clones were isolated from a brain lamda gt11 library using two monoclonal and two polyclonal antibodies specific for MAG. The cDNAs hybridized to two mRNA species, approx. 2.5 and 3.0 kb in size, which are present in rat brain but not liver. These transcripts are more abundant in actively myelinating rat brains compared to mature brains and are present at very low levels in hypomyelinating mutant mice, "jimpy", compared to their normal littermates. Independent evidence for the identity of the isolated MAG cDNA clones was obtained by sequencing two cyanogen bromide fragments prepared from gel-purified MAG. The two peptide sequences, 13 and 9 amino acids long, exactly matched sequences within the single open reading frame deduced from the DNA sequence of the two cDNA clones. The open reading frame of the MAG cDNA clones consists of a 17 amino acid hydrophobic leader peptide, followed by an external domain (499 aa's), a membrane-spanning domain (20 aa's) and a cytoplasmic domain (90 aa's). The external domain contains triple internal repeats (90 aa's) and each repeat is flanked by cysteines suggesting a domain-like structure analogous to that seen in members of the immunoglobulin supergene family. The MAG repeat is 30% homologous to an internal repeat of the neural cell adhesion molecule N-CAM (Arquint et al., PNAS, in press).

S 101 VISUALISATION OF PREPROCHOLECYSTOKININ mRNA IN RAT BRAIN BY *IN SITU* HYBRIDISATION USING COMPLEMENTARY DNA AND RNA PROBES, J. de Belleroche, K. O'Brien, B.K. Premi and A.D.B. Malcolm, Charing Cross & Westminster Medical School, London, W6 8RF, U.K.

Neuronal perikarya containing CCK-immunoreactivity are located in the cerebral cortex, hippocampus, ventral tegmental area and periaqueductal grey matter. The distribution of the mRNA coding for the CCK precursor, preprocholecystokinin was characterised in rat brain in this study using both cDNA and cRNA probes.

Plasmid pCCK (kindly donated by Dr Jack Dixon), derived from the CCK cDNA plasmids pCK 2AB5 and pCK 16AB5 (Deschenes et al, 1984) contains a 535 bp cDNA fragment complementary to the mRNA of rat preproCCK. A double digest with HindIII and EcoRI restriction enzymes excised the cDNA fragment which was separated from the rest of the plasmid by gel electrophoresis and labelled with ^{32}P dCTP or ^{35}S. Hybridisation with the labelled cDNA probe was carried out using cryostat sections (15μM) of frozen rat brain mounted on gelatinised slides following fixation and prehybridisation. Sense and anti-sense cRNA probes were also obtained by insertion of CCK cDNA into an SP6 promoter polymerase vector.

High concentrations of CCK mRNA were seen throughout the cerebral cortex and hippocampus, the highest concentrations being in cingulate cortex, claustrum, piriform cortex and entorhinal cortex. Several thalamic nuclei labelled strongly. Within the cerebral cortex a laminar distribution was evident, high levels of CCK mRNA being present in two bands with little labelling between the bands. The CA1-3 cell layers of hippocampus also showed up as intense bands of labelling. Low levels of CCK mRNA were found in caudate/putamen, kidney and heart.

S 102 MOLECULAR ANALYSIS OF SYNAPSIN I: THE PROTEIN AND THE GENE.
Louis J. DeGennaro, Dept. Neurochemistry, Max Planck Institute for Psychiatry, D-8033 Martinsried, West Germany.

Synapsin I is a neuron-specific phosphoprotein associated with neurotransmitter vesicles in the presynaptic terminals of virtually all neurons. Both its abundance (synapsin I constitutes 0.4% of total brain protein) and its subcellular localization in the presynaptic terminal, attest to the importance of this protein in neuronal function.

We have employed molecular genetic techniques to analyze the structure and function of synapsin I, and the structure and expression of the gene encoding it. This poster will describe the use of synapsin I-specific cDNA clones previously isolated in our laboratory for:

1). the determination and analysis of the primary structure of synapsin I.
2). the isolation and characterization of synapsin I genomic clones.
3). the localization of the synapsin I gene to the X chromosome in mouse and man, and the assessment of possible links between synapsin I gene structure or expression and X-linked neuronal disorders.
4). the characterization of synapsin I gene transcripts and the regulation of their expression during neuronal development.

S 103 ISOLATION,CHARACTERIZATION AND CHROMOSOMAL LOCALIZATION OF HUMAN BRAIN cDNA CLONES CODING FOR THE PRECURSOR OF THE AMYLOID OF ALZHEIMER DISEASE AND AGING BRAIN.
D.Goldgaber,M.I.Lerman,W.O.McBride,U.Saffiotti,D.C.Gajdusek,NIH,Bethesda,MD 10892.We have isolated cDNA clones coding for the polypeptide which forms paired helical filaments of neurofibrillary tangles within neurons,extraneuronal amyloid plaque cores and vascular wall amyloid in Alzheimer disease,adult Down syndrome,Guamanian amyotrophic lateral sclerosis/parkinsonism-dementia and aging brain.Computer analysis of the Glenner 28 amino acid (aa) sequence of amyloid revealed that the first 20 aa included unique regions not found in known sequences deposited in protein banks.Four clones were isolated from adult human brain library made in λgt11,screened with a 59-mer oligonucleotide probe synthesized using deoxyinosine in every third position.Restriction maps of the four positive cDNA clones revealed their identity,except for one c.100 bp longer.On Southern analysis the 59-mer probe hybridized to the 5'fragment.The whole 1kb EcoRI insert was subcloned in pGEM-3 or pGEM-blue and sequenced by the Sanger method.The translated 99 aa sequence for the first 300 nucleotides included Beyreuther's 42 aa sequence of amyloid followed by 57 aa and a TAG termination codon.A 126 kDa fusion protein was produced in E.coli Y1089;the 12 kDa recombinant part is consistent with 99 aa.Northern analysis of poly-A RNA under high-stringency conditions showed a single band of c.3kb in mouse,rat,bovine brains and human thymus,but not in human placenta,epithelium,liver.Southern analysis revealed two EcoRI fragments of 2.9 and 8.7-8.9 kb in human genomic DNA and in DNA from apes,monkeys,rats and mice.The data are consistent with existence of a single copy gene, highly conserved in evolution.Chromosomal mapping localized the gene on chromosome 21.

S 104 DETECTION OF TYROSINE HYDROXYLASE mRNA IN YOUNG AND AGED MICE BRAINS USING IN SITU HYBRIDIZATION, M. Gupta, M.H. Stoler, L.H. Fossom, D.L. Felten and A.W. Tank, University of Rochester School of Medicine, Rochester, NY 14642

Aging is associated with decreased catecholamine levels in the brain. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of catecholamines. It has been reported previously that the activity of TH in the striatum decreases in aged rats compared with their younger counterparts. The present study was undertaken to investigate if this decrease is due to alterations in coding for TH mRNA in the substantia nigra and if such a decrease also may be present in the ventral tegmental area of aged mice compared with the young adults. Male C57BL/6 mice at 3 and 24 months of age were perfused intracardially with 4% paraformaldehyde. The brains were removed, 10 um thick sections were cut through the substantia nigra and the ventral tegmental area and processed for in situ hybridization using (^{3}H) RNA probe complementary to TH-mRNA. (^{3}H)-labeled sense probe was used as negative control. Alternate sections were stained immunocytochemically for tyrosine hydroxylase. In both substantia nigra and the ventral tegmental area, silver grains were observed in dopaminergic cells that were immunocytochemically positive for TH in the alternate sections. Although the number of cells expressing TH mRNA in the substantia nigra and the ventral tegmental area were similar in both the young and aged mice, the expression of TH per cell in the two age groups may vary. Results of these studies will be presented.

S 105 IN SITU HYBRIDIZATION QUANTIFICATION: POMC EXPRESSION IN HUMAN POST-MORTEM TISSUES Guadalupe Mengod[1], Alphonse Probst[2] and José M. Palacios[1], 1) Preclinical Research, Sandoz Ltd., Basle and 2) Institute of Pathology, University of Basle, Switzerland

In situ hybridization techniques combined with quantitative autoradiographic procedures have been used to study POMC expression in human postmortem tissues and animal models. Synthetic oligonucleotides, complementary to the human POMC mRNA were used as hybridization probes. Appropriate standards were used to quantify the hybridization. The following parameters were analyzed: a) Influence of postmortem delay, age and gender in human pituitary POMC expression; b) Effects of chronic drug administration and disease, particularly schizophrenia and Parkinson's disease. The data will be compared with animal models where an experimental dopaminergic denervation was produced or after chronic treatment with different classes of neuroleptic drugs.

S 106 HETEROGENEOUS DISTRIBUTION OF "BRAIN SPECIFIC" mRNAs IN MONKEY CORTEX, C.C.G.Naus, G.H.Travis, J.G.Sutcliffe, R.J.Milner, J.H.Morrison, F.E.Bloom, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Neocortex is characterized by a systematic neuronal diversity that is correlated with its lamination, allowing certain cells to be identified and categorized according to their morphology. To address the molecular basis of this heterogeneity, a cDNA library was constructed in pUC18 using cytoplasmic poly(A)+ RNA prepared from primary visual, motor and dorsal prefrontal cortex of young adult monkeys (Macaca fascicularis). "Brain specific" (absent in liver) clones were analyzed by regional Northern blots and in situ hybridization. A heterogeneous distribution pattern was observed with each clone. One "brain specific" clone which, by Northern blot analysis, was relatively abundant in all cortical regions, produced a consistent laminar pattern in each region, with hybridization being most intense in layers 5 and 6, least in layer 4, and intermediate in layers 2 and 3. In the temporal lobe, visual association cortex (inferior temporal gyrus) contained more labeled cells than auditory association cortex (superior temporal gyrus). Also, in the occipital lobe, the density in the primary visual cortex was significantly greater than in the peristriate visual association cortex. Another clone, which represented a low abundance mRNA species shown by Northern blot analysis to be absent in cerebellum, hybridized intensely to cells in most cortical layers and displayed little regional heterogeneity. The observed differences in the distribution of several "brain specific" mRNAs reveals an underlying molecular heterogeneity in neuronal populations which, we believe, will provide valuable insight into the understanding of the cellular organization of neocortex.

S 107 CHARACTERIZATION OF HUMAN NEUROFILAMENTS: PROMOTER AND GENE STRUCTURES, David L. Nelson, Michael W. Myers and Robert A. Lazzarini, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

We have isolated and sequenced cDNA and genomic clones for two of the three human neurofilaments. Sequence data obtained from the medium subunit, NF-M, reveals a striking protein repeat in the C-terminal half of the protein. The repeat is serine and proline rich, constitutes the major site of phosphorylation, and may be involved in altering the conformation of the protein. The gene structure of NF-M is unique; containing only two introns in positions identical tothose of the first two introns of the mouse small neurofilament. The exon structure of the neurofilaments is quite different from that of other known intermediate filaments, leading to confusion regarding their evolutionary origins. We are currently investigating the promoter regions of these two genes in order to delinate the control elements conferring neuron specific and developmental expression.

S 108 ISOLATION OF CELL SPECIFIC cDNA CLONES FROM MOUSE CEREBELLUM, Daniel T. Nordquist, Harry T. Orr, University of Minnesota, Mpls., MN 55455.

To study molecules involved in neuron function, we are using a cDNA cloning strategy to isolate sequences absent in cerebella of mice with neurological mutations. A cDNA library of 100,000 recombinants has been constructed in the vector pUC118 using total poly(A)$^+$ RNA from normal mouse cerebellum. The method of construction used allows: (1) production of antisense single strand cDNA plasmids, and (2) removal of non-recombinant plasmids by oligo(dA) cellulose chromatography. A selected cDNA library can be constructed from the single strand cDNA by PERT hybridization to as little as 2 µg of poly(A)$^+$ RNA, followed by transformation of nonhybridizing cDNA. Cell specific cDNA clones will be identified with a similarly subtracted cDNA probe, which we estimate will detect sequences representing as low as .005% of total poly(A)$^+$ RNA. Using this strategy we have constructed a selected cDNA library using cerebellum poly(A)$^+$ RNA from 6 week pcd mice, which have virtually no Purkinje cells. This library is being probed with ^{32}P-cDNA to detect Purkinje cell specific clones.

S 109 MYELIN BASIC PROTEIN GENE EXPRESSION IN DYSMYELINATING AND TRANSGENIC MICE. Brian Popko, Carol Readhead, Carmie Puckett, Eric Lai, Naoki Takahashi*, David Shine+, Richard Sidman+, and Leroy Hood. California Institute of Technology, Pasadena, CA 91125, *University of Tokyo Faculty of Medicine, Tokyo, Japan, +Children's Hospital, Boston, MA 02115

Myelin basic protein (MBP) is an important structural component of central nervous system myelin. The dysmyelinating mouse mutants shiverer and myelin defecient (mld) appear to contain defects of their MBP genes. In shiverer mice a major portion of the MBP gene is deleted. We have examined the expression and organization of the MBP gene in mld mice. The mld MBP gene is expressed at very low levels relative to normals and this expression is not regulated in the proper developmental manner. Genomic blots indicate that there are multiple copies of the MBP gene in the mld genome. We have constructed a cosmid library from mld mice and have identified 3 classes of mld MBP genes. Two of the classes contain large internal deletions and the third class appears unaltered relative to the normal MBP gene. We are examining these clones in more detail in an effort to determine the exact molecular defect of mld mice. Utilizing transgenic methods we have introduced the wild-type MBP gene into mld and shiverer mice. The phenotype and CNS myelination of those animals in which the introduced MBP gene is expressed has been drastically improved.

S 110 THE CLONING AND EXPRESSION OF HUMAN PROTEOLIPID PROTEIN. C. Puckett[1], L. Hudson[2], K. Ono[2], J. E. Benecke[3], and R. Lazzarini[2], [1]California Institute of Technology, Pasadena, CA 91125, [2]Laboratory of Molecular Genetics, NINCDS, NIH, Bethesda, MD 20205, [3]Otologic Medical Group, Inc., Los Angeles, CA 90057.

Two different cell types are involved in the elaboration and maintenance of the myelin sheath, the Schwann cell in the peripheral nervous system (P.N.S.) and the oligodendrocyte in the central nervous system (C.N.S). Myelin is composed of 70% lipids and 30% protein, with the protein component consisting of a number of proteins found only in myelin. Some of these proteins such as proteolipid protein (PLP) and PO glycoprotein have been identified as proteins found only in C.N.S. and P.N.S. myelin, respectively. Our group has isolated a 2.8 kb human cDNA representing the human proteolipid protein, the major protein component of central nervous system myelin. By using this cDNA we have investigated the expression of PLP message in the C.N.S. and P.N.S.. Initial experiments revealed that PLP mRNA is expressed at levels comparable to those of the C.N.S. in human acoustic neuromas. These peripheral nerve tumors are of Schwann cell origin, but were not found to express PO. Subsequent work demonstrated that PLP message is expressed in rabbit sciatic nerve. By the use of immunocytochemistry we find this message is translated into proteolipid protein in both rat and human sciatic nerve, but is not incorporated into the myelin sheath. Future work will be directed at understanding the transcriptional regulation of the PLP gene and what post-translational events are involved in the incorporation of proteolipid protein into the myelin membrane.

S 111 MUTATIONS AT THE PLP LOCUS IN X-LINKED DISEASES OF MYELINATION. J.R. Riordan, S. Fahim, A.B. Naismith, R. Simons and C. K. Csiza*, The Hospital for Sick Children, Toronto, Canada M5G 1X8 and New York State Dept. of Health*, Albany, N.Y.

There are X-linked diseases in several animal species in which CNS myelination is completely or partially defective. Having mapped the gene for myelin proteolipid protein (PLP) to the X-chromosome (Willard and Riordan, Science 230: 940,1985), we have investigated the possibility that there are mutations at this locus in these diseases. The following evidence indicates that this is the case in the jimpy (jp) mouse. The PLP gene is localized to the same region of the long arm of the mouse X-chromosome to which the disease locus was previously mapped. The mRNA is reduced in size (by about 100 bp) and is less stable than normal. This small deletion is not detectible in Southern blots of genomic DNA. Examination of RNA from the brains of rats with a similar phenotype, i.e. the myelin deficient (md) rat have now revealed virtually the same changes as in the jp mouse. The mRNA is apparently reduced in size by about the same amount and is even less stable than in the jp mouse. No major rearrangement in the gene could be detected. S1 nuclease analysis is being performed to more precisely define the small deletion in the transcribed sequence. Human PLP cDNA and genomic sequences have been isolated and used to examine DNA from patients with Pelizaeus-Merzbacher disease, the apparent human counterpart of the jp mouse and md rat. Of patients from six different families one exhibits a rearrangement detectible in Southern blots. Sequences from different regions of the 16Kb gene are being used to detect more minor changes in the other patients. These studies have demonstrated the involvement of mutations at the PLP locus in X-linked hypomyelination diseases and confirmed the essential role of this protein in the biogenesis of a functional myelin sheath. (Supported by the Multiple Sclerosis Society of Canada)

S 112 NEURON-SPECIFIC ALTERNATIVE RNA PROCESSING IN TRANSGENIC MICE EXPRESSING A METALLOTHIONEIN-CALCITONIN FUSION GENE.
Andrew F. Russo, Bryan Crenshaw, and Michael G. Rosenfeld. University of California, San Diego. La Jolla, CA 92093.
Alternative RNA processing of the rat and human calcitonin/CGRP gene transcript generates RNAs encoding predominantly calcitonin in thyroid C cells and CGRP in the nervous system. To examine the RNA processing choice of this gene in a wide variety of tissues, we created transgenic mice expressing the rat calcitonin/CGRP transcript from the mouse metallothionein-I promoter. Most cells that do not express the endogenous calcitonin/CGRP gene have the capability to make a clear splicing choice for calcitonin or CGRP transcripts. In the majority of tissues studied, 90-97% of the transgene mRNA encodes calcitonin. In contrast, both calcitonin and CGRP mRNAs were detected in the transgenic mice brains. Immunohistochemical and in situ RNA hybridization analyses show that CGRP transcripts are expressed in a wide variety of neurons, although a few neurons do appear to produce calcitonin transcripts. Based on these results, we suggest that the regulatory machinery required for the processing of CGRP transcripts is restricted primarily to neurons.

S 113 A PRIMATE BRAIN-SPECIFIC POLYMERASE III TRANSCRIPT OF THE ALU REPEAT FAMILY.
Joseph B. Watson and J. Gregor Sutcliffe. Research Institute of Scripps Clinic, La Jolla, CA 92037
Small brain-specific RNAs have been detected previously in cytoplasmic A^+ RNA fractions from rat brain and shown to be transcribed from repetitive identifier (ID) sequences by RNA polymerase III (Sutcliffe et al. 1982, PNAS, 79:4942-4946; Sutcliffe et al. 1984, Nature 308:237-241). To test whether these RNAs are conserved in primates, Northern blots of $PolyA^+$ fractions from Cynomolgus monkey brain were probed at low stringency with a rat ID probe. A small abundant cytoplasmic RNA (200 nt) was detected in monkey brain but not in liver, spleen, kidney, heart, colon, or skeletal muscle. The cDNA sequence of the monkey A^+ RNA was determined by primer extension using a synthetic 3' deoxyoligonucleotide $[(T)_{12}CC]$. A computer search indicated that the cDNA sequence was highly homologous to the Alu repeat family, showing only 43% homology to the consensus rat ID sequence. Both A and B boxes defining an intragenic Pol III promoter and a long 3' poly A tract were evident. This is the first detection in vivo of a stable cytoplasmic transcript of an Alu repeat. Further DNA sequence analysis of clones isolated from a monkey cortex cDNA library with a second synthetic 3' deoxyoligonucleotide confirmed primer extension sequencing. Additional Northern blot experiments using a monkey cDNA probe detected an homologous RNA of the same size in a human brain A^+ fraction. These findings indicate that the sequence of brain-specific Pol III RNAs are not conserved between different classes of mammals (primates, rodents) while the process of brain-specific Pol III transcription of some repetitive families (Alu, ID) is conserved.

S 114 SPINOCEREBELLAR ATAXIA: LOCALIZATION OF AUTOSOMAL DOMINANT, HLA-LINKED FORM BETWEEN TWO MARKERS ON HUMAN CHROMOSOME 6, P. Wilkie, S. Rich, L. Schut, H. Orr, University of Minnesota and V.A. Medical Center, Minneapolis, Minnesota 55455.
A seven generation kindred with HLA-linked autosomal dominant Spinocerebellar Ataxia (SCA) was studied for restriction fragment length polymorphisms (RFLP) to determine the location of the SCA gene. DNA samples from family members were digested with the restriction enzyme Eco RV and probed with an anonymous DNA fragment, 7H4, known to lie near the telomere of chromosome 6. Three-locus linkage analysis of the 7H4 polymorphism, the SCA locus, and the HLA-A locus showed that the most likely gene order was HLA-A:SCA:7H4 (odds of 18:1 of being the correct gene order over SCA:HLA-A:7H4). The LOD score for linkage between HLA-A and SCA in this kindred was increased from 3.71 (0=.18) (Haines et al., 1985) to 8.6 (0=.15) in this study. The calculated genetic distance between the HLA-A locus and the SCA locus of 15 cM is in good agreement with results obtained by others studing HLA-linked SCA, but contrary to an earlier report that placed it centromeric to the glyoxylase locus. The reliability of using both genetic markers (HLA-A & 7H4) in diagnosis is potentially as high as 95%. This is great improvement over using either marker alone in diagnosis since the recombination distance between SCA and either marker is relatively high. Studies of the location of SCA relative to coagulation factor XIIIA (F13A) are currently in progress using isoelectric focusing and immunoblotting. Two-locus linkage analysis of data from 63 family members estimates the distance between SCA & F13A to be 17 cM, although the LOD score was not statistically significant. In addition, two-locus linkage analysis between HLA-A and F13A estimates a genetic distance of 27 cM, which is relatively consistent with the estimate of 20 cM compiled by Lamm and Olaisen (Cytogenet, Cell Genet, 40:142) (combined LOD = 15.65). Further, three-locus linkage analysis between SCA, HLA and F13A suggest that SCA lies centromeric to F13A (33:1 odds).

Neurotoxins, Neuropeptides And Transmitters

S 200 RECEPTOR-MEDIATED REGULATION OF NEUROPEPTIDE TRANSCRIPTION VIA cAMP, Neal C. Birnberg and Michael J. Comb, Yale University, New Haven, CT 06510 and Massachusetts General Hospital, Boston, MA 02144.

We have characterized a cis acting DNA element associated with the human proenkephalin gene that confers inducibility in the presence of elevated cAMP levels. This element can be linked to heterologous genes and retain full function. The element has all the properties that have been described for enhancer elements. We have also been able to demonstrate that specific ligands can induce expression of transfected human enkephalin fusion genes when host cells are treated with the appropriate agonist. We are currently using these properties as a strategy in the isolation of neuropeptide, neurotransmitter and polypeptide hormone receptor genes whose products couple to adenylate cyclase.

S 201 HUMAN TACHYKININ GENES, Tom I. Bonner, Alice C. Young, Marjorie Warden, W. Scott Young III, and Hans-Urs Affolter, National Institute of Mental Health, Bethesda MD 20892

We have cloned and sequenced the two human genes which encode the three known human tachykinins: substance P, neurokinin A (or substance K), and neurokinin B (or neuromedin K). Both genes are expressed in human striatum and *in situ* hybridization histochemistry indicates that both genes are widely expressed in rat brain. Alternative splicing of the substance P-neurokinin A transcript could potentially regulate the relative amounts of the two peptides. However, the relative amounts of the three major mRNAs in striatum show substantial species variation. Comparison of the rat and human substance P promoters shows 85% sequence conservation from bases -67 to -419 with an apparent cAMP responsive element at -381 to -369 and an apparent thyroid hormone responsive element at -127 to -114. There is a single neurokinin B message in striatum but the possibility of a second promoter which may be used in other tissues and which is located 13 kb upstream is under investigation.

S 202 CHARACTERIZATION OF GENOMIC CLONES FOR RAT TYROSINE HYDROXYLASE Joanne M. Carroll, Howard M. Goodman*, Tong H. Joh Cornell University Medical College, New York, N.Y. and *Massachusetts General Hospital, Boston, MA.

Tyrosine hydroxylase catalyzes the rate limiting step in catecholamine biosynthesis. We have isolated TH genomic clones from a rat library constructed in the EMBL3 vector (generous gift or G. Scherer). Three independent isolates, carrying inserts of approximately 20 kb, were identified which hybridize to probes from both the 5' and 3' ends of the cDNA. Restriction analysis of one clone revealed a 1.5 kb BamH1 fragment with strong hybridization to the 5'probe. Sequence analysis demonstrates that this fragment contains the first exon and approximately 550 bases of 5' flanking sequence. The transcription start site was determined by primer extension analysis. Within the upstream region a TATA-like sequence, TTTAAA, is found at -25. Interestingly, a sequence sharing homology with an element proposed to have a role in cyclic AMP regulation (Hanson et al., 1986) resides just upstream of TTTAAA. Whether this represents a functional element is under investigation.

We are currently constructing plasmids containing the 5' flanking region of the TH gene, and by transfection into mammalian cells, examining the promoter and enhancer elements responsible for tissue-specific and regulated expression of TH. (Supported by NIMH grant MH24285; Hoechst AG to MGH)

S 203 POST-TRANSLATIONAL PROCESSING OF TRANSFECTED NEUROPEPTIDE-Y IN CORTICOTROPES, Ian M. Dickerson and Richard E. Mains, The Johns Hopkins School of Medicine, Department of Neuroscience, Baltimore, MD 21205. Many small bioactive peptides are derived from large, biologically inactive precursor molecules. The NPY precursor provides a good model for studying this maturation process, since processing of the precursor probably involves a single cleavage event. To study this process, we have cloned the cDNA for human NPY (kindly provided by Dr. Jack Dixon, Purdue University) into an expression vector under the control of the mouse metallothionein promoter. This vector also confers resistance to the antibiotic G418. This plasmid was transfected into AtT-20 cells, a cell line derived from mouse anterior pituitary corticotropes. AtT-20 cells process and secrete peptides processed from the pro-ACTH/endorphin precursor molecule, but do not produce detectable NPY before transfection. Several stable cell lines have been isolated following G418 selection, and one (Mt.NPY1a) has been more fully characterized for NPY peptide and mRNA production. Cadmium induces NPY production by Mt.NPY1a at least 35-fold to a level about half the molar rate of the endogenous peptides. NPY expression has been examined by biosynthetic labeling with ^{3}H-Tyr, ^{3}H-Leu, or ^{3}H-Pro followed by immunoprecipitation with antibodies raised against NPY or the C-terminal piece. Examination of the cleavages has been performed on labeled NPY, pro-NPY, and the C-terminal piece of pro-NPY. The C-terminal heptapeptide found in pancreatic polypeptide is not released from pro-NPY by AtT-20 cells. Tryptic fragments were analyzed by RP-HPLC, and compared to identified tryptic fragments from synthetic NPY. Microsequencing was performed to determine the sites of signal peptide cleavage and cleavage between NPY and the C-terminal piece.

S 204 Isolation and characterization of genes that play a putatative role in the communication between the neuroendocrine and immune systems. Anders Eriksson, Dan Larhammar and Håkan Persson. Department of Medical Genetics, Uppsala University, Sweden.

Recent studies in many fields have provided evidence of a reciprocal interaction between the neuroendocrine and immune systems through peptides and receptors common to both systems. We have used cloned DNA's as hybridizaton probes to detect mRNA synthesis for neuropeptides in immune cells. Results from such studies showed a high level of neuropeptide-Y (NPY) expression in non-neuronal cells. NPY expression in the spleen correlated with immunoresponse in several different mouse strains. The cell types, in the spleen, responsible for NPY synthesise were identified by in situ hybridization.

Subtractive hybridization and cDNA cloning was used to isolate genes that may be important for the reciprocal interaction between the neuroendocrine and immune systems. These clones were characterized by Northern blot analysis, DNA-sequence analysis and putative gene products were identified with peptide antisera. The properties of some of these genes will be discussed.

S 205 GLUCOCORTICOID REGULATION OF THE GENE FOR THE CATECHOLAMINE SYNTHESIZING ENZYME PNMT Marian Evinger and Tong Joh, Cornell Univ. Med. College, New York, NY 10021

Phenylethanolamine N-methyltransferase (PNMT) catalyzes the final step in the catecholamine pathway - biosynthesis of the neurotransmitter-hormone epinephrine. In the adrenal gland, the presence of glucocorticoid hormones is necessary for maintaining physiological levels of PNMT enzymatic activity. In contrast to studies suggesting hormonal stabilization of the enzyme against proteolytic degradation, this study establishes that glucocorticoids enhance levels of PNMT activity by directly stimulating production of PNMT mRNA.

Glucocorticoid modulation of PNMT gene expression in vivo has been demonstrated by comparing PNMT mRNA levels in the adrenal glands of hormonally depleted (hypophysectomized-HPX'd) and of glucocorticoid-treated HPX'd rats. After four days of treatment with dexamethasone (DEX), PNMT mRNA levels in HPX'd animals increased to levels three-fold greater than those in saline-treated HPX'd animals, amounts equivalent to those achieved indirectly by administration of adrenocorticotropin.

Moreover, glucocorticoids increase PNMT mRNA production by stimulating the rate of transcription from the PNMT gene. 18 hr after treatment with DEX, the rate of PNMT transcription was 2.5-fold greater than that in saline-treated animals. These effects are exerted rapidly: an increase in the rate of transcription is observed within 20 min of DEX treatment. Hybridization in situ in dispersed rat adrenal chromaffin cells using PNMT cDNA also confirms the direct stimulatory influence of DEX on the PNMT gene. Therefore, glucocorticoid stimulation of PNMT mRNA transcription constitutes the primary mechanism regulating expression of this important catecholamine synthesizing gene.

S 206 SOMATOSTATIN IN THE DEVELOPING CORTEX, Susan C. Feldman, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

The diversity of neurotransmitters, neuromodulators and neuroactive molecules in the cortex requires a precise arrangement of biochemically related neurons, the organization of which is presumably laid down in development. In our laboratory, we have been studying the maturation of neurons containing the peptide somatostatin (SRIF) in visual cortex. Before birth the somatostatin system consists of immunoreactive neurons and fibers at the top of the cortical plate and a population of round cells in the deepest layers. Studies combining the incorporation of tritiated thymidine with immunocytochemical localization of SRIF show that at birth, the majority of neurons born on embryonic day 16 (E16) lie superficial to SRIF neurons and fibers. In addition, a population of the small SRIF-containing cells born on E16 have not migrated from the deepest layer of the cortex. From day 0 to day 2 the number of immunoreactive round cells increases to a maximum by day 2 then disappears after day 4. After this time the cortex is characterized by both an increasing number of immunoreactive neurons and a migration of these neurons from deeper to more superficial layers. By day 15, the time of eye-opening in the rat, the distribution of SRIF neurons and fibers resembles that seen in the adult. Our results suggest that SRIF neurons are born in two main waves, an early wave whose migration is complete before birth, and a later postnatal one which coincides with the major time of cortical organization. The early appearance of SRIF neurons and fibers suggests that cells containing this peptide are in a position to influence the organization of the visual cortex. The finding that the peptide is apparently present in migrating neurons lends support to the hypothesis that biochemical maturation may precede morphologic maturation.

S 207 CHARACTERIZATION OF THE GENES FOR THE RAT BRAIN VOLTAGE-SENSITIVE SODIUM CHANNEL, A.L. Goldin, T. Snutch, D. Krafte, R. Dunn*, H.A. Lester, W.A. Catterall# and N. Davidson, Caltech, Pasadena, CA 91125; *U. Toronto, Toronto, Canada; #U. Washington, Seattle, WA 98195.

Several cDNA clones coding for the high molecular weight (α) subunit of the voltage-sensitive sodium channel have been selected by immunoscreening a rat brain cDNA library constructed in the expression vector λgt11. These clones hybridize with a low-abundance 9 kb RNA species from rat brain, muscle and heart, along with a series of both larger and smaller RNA species. Sucrose-gradient fractionation of rat brain poly(A) RNA yielded a high molecular weight fraction containing these RNAs, which resulted in functional sodium channels when injected into Xenopus oocytes. The high molecular weight sodium channel RNA was also selected from rat brain poly(A) RNA by hybridization to a single-strand antisense cDNA clone. Translation of this RNA in oocytes resulted in the appearance of tetrodotoxin-sensitive voltage-sensitive sodium channels in the oocyte membrane. These results demonstrate that mRNA encoding the α subunit of the rat brain sodium channel is sufficient for translation to give functional channels in the absence of any β-subunit RNA. The β subunits, may modify the α subunit encoded sodium channels, however. We are currently examining this possibility by using the same expression cloning strategy to isolate clones for the β_1 and β_2 subunits of the rat brain sodium channel.

S 208 THE MULTIFUNCTIONAL Ca^{2+}/CaM-DEPENDENT PROTEIN KINASE MEDIATES DEPOLARIZATION-INDUCED PHOSPHORYLATION AND ACTIVATION OF TYROSINE HYDROXYLASE; Leslie C. Griffith and Howard Schulman, Stanford University, Stanford, CA 94305

Stimulation of PC12 cells with A23187, carbachol, or high K^+ medium, agents which increase intracellular calcium, results in the phosphorylation and activation of tyrosine hydroxylase (TH). We have identified three major protein kinases in PC12 cells and investigated their roles in the Ca^{2+}-dependent phosphorylation of TH and other cytosolic proteins. Distinct sets of PC12 proteins were phosphorylated in response to depolarization (high K^+) and kinase C activators. In addition, there was a set of proteins which responded to both stimuli. The three kinases, the multifunctional Ca^{2+}/calmodulin-dependent kinase (CaM kinase), the cAMP-dependent protein kinase, and protein kinase C all phosphorylate TH in vitro. Neither agents which increase Ca^{2+} nor agents which directly activate kinase C increase cAMP or activate the cAMP-dependent kinase, excluding this pathway as a mediator of these stimuli. The role of kinase C was assessed by long-term treatment of PC12 cells with TPA, which causes its specific desensitization. In cells pretreated in this manner, agents which increase Ca^{2+} influx continued to stimulate TH phosphorylation maximally, while protein kinase C activators were completely ineffective. Comparison of tryptic peptide maps of TH phosphorylated by the three protein kinases in vitro with phosphopeptide maps generated from TH phosphorylated in vivo indicates that phosphorylation by the CaM kinase most closely mirrors the in vivo phosphorylation pattern. Conditions which result in Ca^{2+}-dependent phosphorylation in PC12 also cause a 2-3 fold activation of TH. These results indicate that the multifunctional CaM kinase is the mediator of hormonal and electrical stimuli which elevate Ca^{2+} in PC12 cells.

S 209 STRUCTURE, EXPRESSION AND REGULATION OF RAT PREPROTACHYKININ I GENE IN THE CNS, James E. Krause, Mark S. Carter, Philip E. Dykema, and Margaret R. MacDonald, Washington University School of Medicine, St. Louis, MO 63110.

We have explored molecular genetic and biochemical mechanisms responsible for the production of several tachykinin peptides, including Substance P (SP) and Neurokinin A (NKA; known also as Substance K). A single rat gene, called the PPTI gene, is responsible for the production of three preprotachykinin (PPT) mRNAs that encode various combinations of tachykinin peptides. This gene is comprised of 7 exons and multiple 5' control regions, and is 9kb in length. Two TATA-like promotor sites are used for initiation of PPTI gene transcription. Three PPT mRNAs (α-, β- and γ-PPT mRNA), which differ in protein coding sequence, are derived from this gene. α-PPT mRNA encodes a PPT of 112 amino acids and lacks the NKA-coding sequence (exon 6), β-PPT mRNA encodes a PPT of 130 amino acids (exons 1-7) and γ-PPT mRNA encodes a PPT of 115 amino acids and lacks a pentadecapeptide sequence between SP and NKA (exon 4). Both tachykinins SP and NKA can be produced from β- and γ-PPT, as can N-terminal extensions of NKA. The three mRNAs are expressed in various CNS regions, with γ-PPT mRNA > β-PPT mRNA > α-PPT mRNA. Tissue specificity of splicing occurs since some peripheral tissues express β-PPT mRNA in greatest abundance, in contrast to that observed in the CNS. Our current experiments are directed to understand tissue-specific splicing of PPT mRNAs, co- and post-translational processing of the PPTs, and regulation of PPTI gene transcription and processing in discrete CNS systems.

S 210 A CENTRAL NERVOUS SYSTEM α-BUNGAROTOXIN BINDING PROTEIN: COMPARISON WITH THE TORPEDO ACETYLCHOLINE RECEPTOR, James T. McLaughlin and Edward Hawrot, Department of Pharmacology, Yale Medical School, New Haven, Connecticut 06510.

Curaremimetic neurotoxins such as alpha-bungarotoxin (Btx) bind to nicotinic acetylcholine receptors (AChR) from skeletal muscle or electric organ with dissociation constants in the nanomolar range. This high affinity interaction has been successfully exploited in the biochemical characterization of the muscle receptor; a similar approach to the characterization of nicotinic AChRs from CNS tissue has not been as successful. Using protein blotting techniques, we recently demonstrated a high molecular weight Btx binding component in lower vertebrate CNS (Hawrot et al., 1986 Brain Res. 373:227-234). We have extended this approach to compare the properties of the CNS binding protein with those of the Torpedo AChR. Binding activity detected in fish brain membranes, as measured by filtration assay, could be quantitatively extracted with lithium dodecylsulfate (LDS); similar treatment of Torpedo electric organ membranes results in loss of 90-98% of toxin binding activity. Using a modified dot-blot assay, we found that the affinity of the brain binding activity for Btx was 50 to 100 times greater than that of Torpedo AChR, again, after treatment with LDS (1 nM vs 50-100 nM). The brain binding site also demonstrated a higher affinity for other cholinergic ligands, as measured by their ability to compete with radiolabeled toxin. Unlike Torpedo AChR, the brain Btx binding component did not bind concanavalin A, but appears to be susceptible to digestion with endoglycosidase H. Finally, the brain binding site appears to be a more basic protein than Torpedo AChR in that it binds weakly to anion exchange columns at neutral pH.

S 211 ARE THE NEUROTRANSMITTERS EXPRESSED IN SENSORY NEURONS SPECIFIED BY THEIR TARGETS? CALCITONIN GENE RELATED PEPTIDE IS ENRICHED IN THE SENSORY INNERVATION OF THE CEREBRAL VESSELS. Tim O'Connor and Derek van der Kooy, Dept. of Anatomy, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Trigeminal innervation of cerebral vessels and the surrounding dura conveys most intracranial head pain. Small diameter fibers containing substance P (sub P) have been observed in the periadventia of cerebral vessels. These fibers have been postulated to be the trigeminal substrate for pain of vascular origin. Calcitonin gene related peptide (CGRP) co-exists with most sub P neurons within the trigeminal ganglion. In addition a population of CGRP only containing neurons has been observed. We now report that the population of trigeminal ganglion cells projecting to the cerebral vasculature is enriched in CGRP only containing neurons.

Approximately 32% of the trigeminal ganglion cells retrogradely labeled after a True Blue application to a cerebral artery were observed to contain CGRP, whereas on 17% and 18% contained sub P and cholecystokinin (CCK) respectively. The 32% of ganglion cells projecting to the cerebral vasculature that contain CGRP stands in contrast to the 10% CGRP positive seen in the population of ganglion cells projecting out to another target (the forehead) and the 23% CGRP positive observed in the entire ganglion. Sub P and CCK are not enriched in the trigeminal innervation of the vasculature. This CGRP enrichment emphasizes the functional importance of CGRP in cerebral vasculature tone and more importantly suggests an underlying developmental process whereby a peripheral target determines the neuropeptide synthesized in its sensory innervation.

S 212 LOCALIZATION OF NEUROTRANSMITTER AND NEUROPEPTIDE RECEPTORS IN HUMAN BRAIN: SELECTIVE ALTERATIONS IN CERTAIN NEUROLOGICAL DISEASES. Rémi Quirion, Yves Robitaille, N.P.V. Nair and Serge Gauthier. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Québec, Canada H4H 1R3.

The quantitative in vitro autoradiographic distribution of multiple neurotransmitter and neuropeptide receptor binding sites has been studied using whole hemisphere thin-sections. The sites investigated included the following: muscarinic (M_1 and M_2), nicotinic, serotonergic (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2), opioid (μ, δ, κ), neurokinin (substance P, neurokinin A), neuropeptide Y, somatostatin, neurotensin, calcium channel antagonists, glutamate (NMDA), phencyclidine, phorbol ester (protein kinase c) and IP_3 sites. This extensive autoradiographic study demonstrates that each population of sites is discretely and selectively distributed in normal human brains. For example, high densities of nicotinic receptor sites are found in the thalamus while high densities of M_1 sites are seen in the striatum. NMDA and phencyclidine binding sites are similarly distributed in cortical and hippocampal areas. Neurotensin binding sites are markedly decreased in the substantia nigra in Parkinsonian patients (n = 6). In senile dementia of the Alzheimer's type (n = 8; SDAT), the cortical density of 5-HT_2 binding sites is markedly decreased especially in layers III and IV of the temporal cortex. Similarly, nicotinic receptor sites are much decreased in various cortical areas. Preliminary results indicated that NMDA, phencyclidine, phorbol ester and IP_3 sites could be decreased in certain brain regions in SDAT. These results show the neurotransmitter and neuropeptide receptor sites are selectively altered in certain neurological diseases.

S 213 NEUROPEPTIDEC Y IN THE RAT AND MOUSE BRAIN AND ADRENAL GLAND: HYBRIDIZATION HISTOCHEMISTRY, IMMUNOHISTOCHEMISTRY AND NORTHERN BLOTTING, Martin Schalling, Kim Seroogy, Thomas Hokfelt, Hakan Persson and Dan Larhammar

Rat and mouse brain and adrenal gland were analyzed by hybridization histochemistry using a nick translated 280bp cDNA probe for rat neuropeptide Y (NPY) (Larhammar et al.,PNAS, in press 1987). The probe was labelled with ^{35}S-dTTP to a specific activity of $4x10^8$ cpm/ g. Immunohistochemistry was performed using antisera for NPY, tyrosine hydroxylase, VIP, acetylcholinesterase and PNMT. Furthermore, in the rat brain and adrenal medulla changes in mRNA levels following reserpine treatment was evaluated using Northern blotting and hybridization histochemistry as well as immunohistochemistry. Male Sprague Dawley rats (with or without a single dose of reserpine 10 mg/kg), and Bald C mice, NZB mice and CBA mice were all anesthetized with nembutal and perfused through the ascending aorta with ice-cold NaCl followed by ice-cold 10% formalin in phosphate buffer. After immersion fixation the tissue was cut on a cryostat and sections treated essentially as described by Hafen and Levine (EMBO J., 2:617-623, 1983) and Siegel and Young (Neuropeptides, 6:573-580, 1986). Hybridization histochemistry was performed at 42°C for 18 h using 2-3 ng of probe section ($0.8-1.2 \times 10^6$ cpm).

Localization of NPY mRNA was demonstrated in the cerebral cortex and in the adrenal medulla of all species examined. Using emulsion coated slides we as in the adrenal medulla. Furthermore, in the locus coeruleus and adrena; medulla an increase in the level of NPY mNRA localization which is in good agreement with previous immunohistochemical studies. Furthermore, reserpine which depletes catecholamines neurons and induces transcription of tyrosine hydroxylase also increase mRNA levels for NPY in cell groups were NPY and catecholamine are known to be costored.

S 214 REGULATION OF PROENKEPHALIN A EXPRESSION IN THE ADRENAL MEDULLA, O. H. Viveros[a], E. J. Diliberto, Jr.[a], J. H. Hong[b], J. S. Kizer[c], R. J. Rigual[a], L. Fricker[d] and C. Auchter[a], Wellcome Research Labs, RTP, NC 27709[a]; NIEHS, RTP, NC 27709[b]; UNC, Chapel Hill, NC 27514[c]; and Albert Einstein College of Medicine, NY 10461[d].

A brief period (2 hr) of increased splanchnic stimulation of the rat adrenal medulla results in a fast and large increase in proenkephalin A (PEA) mRNA followed sequentially by increases in enkephalin containing peptides (cryptic opioid peptides, COP) and receptor-active enkephalins (native opioid peptides, NOP). The ratio of COP/NOP increases during the first 24 hrs after stimulation followed by a progressive decrease, below control values, during the next 3 days, suggesting that neurogenic stimulation enhances both de novo synthesis of PEA and processing. In cultured adrenomedullary cells forskolin (through increased cAMP) and the catecholamine depleting agent tetrabenazine (cAMP-independent) increase PEA mRNA levels and total OP. Tetrabenazine and other catecholamine depleting agents, but not forskolin, increase processing of intermediate size COP to enkephalins. A synergistic increase in NOP is obtained by combining both drug treatments. The activity of the processing enzyme peptidyl-glycine α-amidating monoxygenase is also increased in the adrenal medulla after stimulation. Preliminary data also shows an increase in adrenomedullary carboxypeptidase E mRNA in culture and in vivo and increases in enzymatic activity in vivo. It is proposed that the large and sustained increase in adrenomedullary enkephalin after neurogenic stimulation result from a coordinated regulation of the expression of PEA and its processing enzymes through multiple intracellular signals.

S 215 ACTIVATION OF PROTEIN KINASE C PREVENTS NATURAL MOTONEURON CELL DEATH, Cheryl L. Weill, Louisiana State University Medical Center, New Orleans, LA 70112.
Experiments were undertaken to explore the possibility that the prevention of natural motoneuron cell death by dibutyryl cyclic GMP is mediated by protein kinase C. To this end, cultured spinal cord neurons were treated with the known protein kinase C activators 12-O-tetradecanoylphorbol-13-acetate (TPA) and 1-oleoyl-2acetyl-glycerol (OAG), a diacylglycerol analog; the cyclic nucleotide content assessed by radioimmunoassay. Treatment of 6-day old chick spinal cord neuronal cultures with either 10 ng/ml TPA or 30 ug/ml OAG at 37°C caused a nearly 2-fold increase in neuronal cGMP levels within 15 min. Continued exposure of cultures to TPA resulted in the maintenance of elevated cGMP levels, while continued exposure to OAG resulted in a decline in cGMP levels to initial levels within 1 hr. During this period the cAMP levels declined. Treatment of developing embryos with TPA (5 ug/day) on embryonic days 5-9 resulted in the survival on day 10 of 17,032 +/- 740 (n=6) cells per lateral motor column, which is a statistically significant increase, as compared with 14,740 +/- 497 (n=5) cells for vehicle treated controls (P=0.010, assessed by the Mann-Whitney U test). This represents a 21% increase in the number of surviving motoneurons, or TPA treatment prevents the death of 34% of those motoneurons that would normally die by day 10.

Thus, the known activators of protein kinase C, TPA and OAG, cause an increase in neuronal cGMP levels. Dibutyryl-cyclic GMP and TPA prevent natural motoneuron cell death in vivo. Taken together, these data suggest an involvement of protein kinase C in the elevation of neuronal cGMP levels and motoneuron survival during development.

S 216 SEIZURES INDUCE MULTIPLE CHANGES IN HIPPOCAMPAL GENE ACTIVITY, Jeffrey D. White and Christine M. Gall, SUNY, Stony Brook, NY 11794 and U. Cal., Irvine, CA 92717.
Previous studies have demonstrated that bilateral recurrent seizures which are induced via a unilateral lesion of the dentate gyrus hilus lead to an increase in enkephalin peptide biosynthesis and in the content of preproenkephalin mRNA in the granule cells of the hippocampus. This rise in enkephalin peptide and mRNA begins during seizure activity, continues to rise beyond the cessation of seizures but returns to normal values after 3-4 days. In the current study, we sought to investigate the generality of the effect of seizures on the synthesis of other potential neuropeptide transmitters used by the excitatory pathways of the hippocampus and to investigate the regulation of the synthesis of possible intracellular proteins which might act as mediators of the effects of seizures. Thus, total RNA was isolated from entorhinal cortex tissue samples taken from hilus-lesioned animals at various times post-lesion (in all cases the tissue contralateral to the lesion was used). Following separation of the RNA on denaturing agarose gels and transfer to nylon membrane, the samples were analyzed for changes in the content of preproenkephalin, preprocholecystokinin, and preproneuropeptide Y mRNA. In all cases, the mRNA content was increased; however, this increase was not uniform, i.e. preproenkephalin mRNA increased to the greatest extent. Similarly, we evaluated changes in the mRNA content for three cellular protooncogenes, c-fos, c-H-ras and c-myc, and for ornithine decarboxylase in the hippocampus. Again, both the time course of effect and the extent of change in mRNA content for these genes was differentially regulated with c-fos mRNA demonstrating the earliest and most dramatic rise. Additional studies demonstrated that inhibition of seizure activity also inhibited the changes in mRNA content for the various genes.

S 217 EVIDENCE FOR EXISTENCE OF MONOAMINERGIC-CHOLINERGIC INTERACTIONS IN THE BASAL FOREBRAIN, L. Zaborszky and Victoria N. Luine, University of Virginia, Charlottesville, VA 22908 and Rockefeller University, New York, NY 10021.
Two days after a large knife cut at the meso-diencephalic border, which interrupts all ascending brainstem fibers, degenerating terminals were found on choline acetyltransferase (ChAT)-positive dendrites in the forebrain. This large brainstem cut transects several ascending brainstem systems, among them the monoaminergic fibers. 6-OHDA or 5,7-DHT were injected into the ascending monoaminergic pathways (4.3mm behind the bregma). Two weeks later, tissue samples of the forebrain were removed for ChAT measurement. In order to test the efficacy of the lesions, NE or 5-HT were measured in the preoptic area. 6-OHDA-treatment resulted in a 21-35% decrease of ChAT in the vertical and horizontal limbs of the diagonal band, respectively. 5,7-DHT caused a 25% reduction of ChAT level in the ventral pallidum-substantia innominata area. Light microscopic data using intensified and non-intensified DAB technique for the simultaneous visualization of ChAT and TH or 5-HT immunireactivities strongly suggest that monoaminergic fibers may contact the forebrain cholinergic neurons. EM studies, using double antigen-detection techniques, are in progress to determine whether monoaminergic axons indeed make synaptic contacts with the cholinergic neurons.
Supported by NINCDS Grant NS 23945 and a Grant from the American Health Assistance Foundation.

Receptors and Growth

S 300 DEVELOPMENTAL ANALYSIS OF THE XENOPUS VOLTAGE-GATED SODIUM CHANNEL Vanessa Auld and Robert J. Dunn, University of Toronto, Toronto, Canada.

Using polyclonal antibodies to purified rat brain sodium channels our group has successfully isolated and sequenced the complete cDNA coding for the rat brain voltage-gated sodium channel. To study the expression of the sodium channel in the developing nervous system of Xenopus laevis it was necessary to obtain probes specific to the Xenopus sodium channels. A Xenopus genomic library was constructed using the EMBL4 lambda vector and a lambda gt-11 cDNA library was constructed from tadpole mRNA. These libraries were screened at reduced stringency with rat sodium channel probes from regions known to be conserved between the rat and the eel electroplax sodium channels. A number of clones corresponding to the voltage-gated channels of Xenopus were isolated. Some of the clones were analyzed in greater detail to determine the number of different channels obtained and their tissue localization. These clones have been used to analyze sodium channel expression during various stages of Xenopus development.

S 301 DEVELOPMENTALLY REGULATED EXPRESSION OF THE NERVE GROWTH FACTOR RECEPTOR GENE IN THE PERIPHERY AND BRAIN. C.R. Buck, H.J. Martinez, I.B. Black, and M.V. Chao, CUMC, New York, NY 10021.

Nerve Growth Factor (NGF) is required for survival and maintenance of function by sympathetic and some sensory neurons. A potential role for NGF in selected brain populations has been detected only recently. Sensitivity to NGF is conferred by expression of specific receptors on responsive neurons. To study regulation of NGF receptor expression, we have developed sensitive NGF receptor cDNA probes. Receptor cDNAs were isolated from a human GT10 library and subcloned into plasmid vectors containing bacteriophage RNA polymerase promoter sequences. Antisense NGF receptor cRNA was synthesized in vitro in the presence of ^{32}P-labeled UTP. NGF receptor cRNA detects a single 3.8kb human receptor message in Northern blot analysis of human melanoma and neuroblastoma cell lines, and also detects a 3.8kb rat receptor message in PC12 cells. Northern analysis of RNA extracted from rat sympathetic (SCG) and sensory (DRG) peripheral ganglia and poly A+ RNA from basal forebrain, also demonstrate this receptor mRNA in vivo. No receptor mRNA was detected in liver, a tissue unresponsive to NGF. Northern analysis of RNA from various ages revealed a differential profile of steady state receptor message expression. NGF receptor mRNA levels increase steadily from neonate to adult in both the SCG peripherally, and the basal forebrain centrally. In contrast, receptor message levels decline drastically postnatally in the DRG. These findings correlate well with the known requirements for NGF in these ganglia. The presence of NGF receptor message in the basal forebrain supports the notion that NGF has some role in the central nervous system and the developmental profile of expression argues that NGF continues to act in the brain throughout life.

S 302 OLIGONUCLEOTIDE cDNA PROBES USED TO DIFFERENTIALLY LOCALISE PUTATIVE NICOTINIC RECEPTOR a-SUBUNIT mRNAs IN BRAIN, Noel J. Buckley and W. Scott Young, III, NIMH, Bethesda, MD 20892. Nicotinic receptors in the CNS show great diversity with respect to their ligand binding properties and their susceptibility to blockade by a-toxins. This heterogeneity has recently been reflected by the isolation and sequenceing of several cDNAs coding for nicotinic receptor a-subunits. It is becoming increasingly clear that many receptors are composed of a number of discrete domains, some of which may be shared by families of related receptors. In the light of the apparent heterogeneity of the nicotinic receptor we were interested to see if selected short oligonucleotide cDNA probes could be used to differentially localise nicotinic receptor mRNA species using in situ hybridisation histochemistry. Since oligonucleotide probes recognise only short stretches of mRNA, they may be used to hybridise to sequences unique to a particular receptor or to detect families of receptor subtypes that share come common sequences but not others. Such families are difficult to resolve using full length cDNA clones which may hybridise to a number of related mRNA species even after high stringency washing conditions. We have generated two 48mer oligonucleotide probes corresponding to nuc 67-114 (probe G) and to nuc 457-504 (probe B) of a cDNA clone coding for an a-subunit of a nicotinic receptor (a3) cloned from PC12 cells (Boulter et al. Nat. 319,368) and subsequently these probes were used to map brain areas expressing homologous RNAs using in situ hybridisation histochemistry. Areas that bound both probes included the habenula and pineal gland. This discrete localisation is in marked contrast to the widespread pattern seen when the full length a3 clone was used as a probe B. Interestingly, these latter areas show little nicotine binding although they are rich in a-bungarotoxin binding sites. We propose that the mRNA present in the PVN and SON that hybridises to only probe B may correspond to a novel nicotinic receptor a-subunit that is part of the nicotinic receptor that mediates the release of vasopressin and oxytocin from the hypothalamus of a number of species including man. Isolation, expression and sequence analysis of this mRNA are being used to test this hypothesis.

S 303 EXPRESSION AND SECRETION OF IMMUNOGLOBULINS IN CELLS OF THE NERVOUS SYSTEM BY DNA TRANSFECTION, Antonino Cattaneo, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH (England).
The possibility of expressing specific immunoglobulins (Ig) into cells of the nervous system has been investigated. DNA sequences coding for the heavy and light chain of an IgM directed against the (4-hydroxy-3-nitrophenyl)acetyl (NP) hapten were cloned into the pSV2-gpt and pSV2-neo expression vectors respectively (Cattaneo and Neuberger,1987). Expression of Ig genes transfected into non-lymphoid cells has been recently obtained (Cattaneo and Neuberger, 1987) by substituting the tissue specific Ig promoter in the Ig genes with the promoter of the *Drosophila gene coding for the heat shock* induced protein hsp70. The plasmid DNA was introduced into C6 glioma and PC12 pheochromocytoma cells by transfection and stable transfectants were isolated and screened for expression of light and/or heavy chain. Double transfectants were also derived and screened for expression of IgM antibodies recognizing the NP antigen. The results obtained show that upon incubation of cells at 43°C both C6 and PC12 cells express intracellular heavy and light chain at high levels. In the double transfectants the IgM is correctly glycosylated and is secreted in the extracellular medium at a concentration of 0.5 μg/ml after a single inducting heat shock. The efficient secretion of functional antibodies from cells of the nervous system makes it conceivable to utilize the expression of antibodies in the brain to block selected pathways or functions.

S 304 SYNTHESIS OF CHICKEN NERVE GROWTH FACTOR BY PERIPHERAL NERVES, Ian A. Ferguson, Reg Williams and Robert A. Rush, Department of Physiology, Flinders University of South Australia, Bedford Park, South Australia, Australia 5042

Chicken NGF production by peripheral nerves in culture was studied using an ELISA (immunoassay) and a dissociated sympathetic neuron bioassay, with antibodies which react with chicken NGF as demonstrated by Western Blots.

Similar amounts of trophic activity were released by chicken sciatic nerves cultured in the presence of whole or a dialysate of foetal calf serum. Nerves cultured in the latter medium for two days contained only relatively low amounts of trophic activity whereas the conditioned medium was rich in trophic activity. Much more trophic activity was released by live than metabolically poisoned sciatic nerves in culture. Antibody to chicken NGF inhibited about 17% of the total amount of trophic activity in homogenates of freshly removed nerves, but about 40% of the trophic activity present in either day 1 rat or chicken sciatic conditioned media. This level of inhibition was maintained in media cultured over rat nerves for 2 to 6 days. In the chicken however, the level of inhibition fell rapidly to near zero by the third day in culture. These studies were confirmed by immunoassay.

These studied demonstrate that (1) cells within peripheral nerve tissue synthesize and release NGF and at least one other trophic factor active on sympathetic neurons, and (2) tissue culture conditions modulate differently NGF production and release from rat or chicken nerves.

S 305 DIFFERENTIAL DISTRIBUTION OF PROTEIN KINASE C ISOZYMES IN THE CEREBRAL CORTEX: LOCALIZATION OF TYPE I ENZYME IN THE OCCIPITOTEMPORAL VISUAL PROCESSING PATHWAY, Freesia L. Huang, Yasuyoshi Yoshida, David P. Friedman, Leslie G. Ungerleider, and Kuo-Ping Huang, NICHD, NIH, and NIMH, Bethesda, MD 20892, and NIDA, Rockville, MD 20857

Protein kinase C (PKC) has been implicated in the control of neurotransmitter release and neuroplasticity. Activation of PKC in neuronal tissues is thought to couple to the inositol phospholipid signalling pathway. Increased phosphorylation of one of the PKC substrates, protein F1, has been related to the induction of long-term potentiation and, perhaps, the information storage. We examined the regional distribution of PKC (F1 kinase) along the visual information processing pathway of the monkey. Three types of PKC isozymes, designated type I, II, and III, were purified from monkey brain and identified with monospecific antibodies against rat brain PKC isozymes. Using immunoblot analysis we found that the type I PKC formed a gradient of increasing concentration rostrally along the cerebral cortex of occipital to temporal and then to entorhinal areas, a finding which parallels that observed for the endogenous phosphorylation of protein F1 (Nelson, R.B. et al., Soc. Neurosci. Abstr., 12, 1168, 1986). Unlike the type I isozyme, type II PKC was evenly distributed and the type III enzyme was relatively low along this visual information processing pathway. The type I PKC was also found to be highly enriched in hippocampus and amygdala regions, which are both important in information processing. Since neurobehavioral evidence indicates that temporal lobe areas participate more than occipital areas in the storage of visual representations, our results suggest that the type I PKC may be important for the memory formation.

S 306 DEVELOPMENTAL ORGANIZATION OF SYNAPTIC VESICLE PROTEIN (P38) IN THE SOMATOSENSORY NEOCORTEX OF THE RAT. K. Jensen, and J. O'Callaghan, Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

P-38 is an intrinsic protein of the membrane of small synaptic vesicles (Jahn et al., 1985, PNAS 82:4137-4141). We have examined the distribution of p38 in the developing somatosensory neocortex of the rat using immunohistochemistry in conjunction with radioimmunoassay. Thalamocortical projections to the rat somatosensory cortex have a chacteristic organization that mimics the pattern of whiskers on the face. We have previously reported that the pattern of p38 immunoreactivity clearly demonstrates the vibrissae related pattern of thalamocortical afferents on postnatal day 5 (pnd-5). Radioimmunoassays indicated that there is a several fold increase in the relative abundance of p38 from the day of birth to pnd-5. From pnd-5 to pnd-21 there was an even more dramatic increase in the relative abundance of p38 in the somatosensory neocortex. From pnd-5 to pnd-21 vibrissae associated clusters of immunoreactivity became obscured by higher levels of staining in the adjacent tissue. The increase in p38 occurring from pnd-5 to pnd-21 may reflect the development of intracortical projections which are known to be organized in manner complimentary to thalamocortical afferents.

S 307 $pp60^{c\text{-}src}$ INDUCTION DURING PERIPHERAL NERVE REGENERATION, Jean M. Le Beau and Gernot Walter, Dept. of Pathology, University of California San Diego, La Jolla, CA 92093.

$pp60^{c\text{-}src}$, the cellular homolog of the transforming protein of Rous sarcoma virus, $pp60^{v\text{-}src}$, is a tyrosine-specific protein kinase that is highly expressed in the brain. Recent studies have demonstrated that the appearance of $pp60^{c\text{-}src}$ during neuronal development occurred with the onset of neuronal differentiation and that this expression was maintained in mature, differentiated neurons. We asked if $pp60^{c\text{-}src}$ might also play a role in cellular differentiation during nerve regeneration. To examine this, a model of peripheral nerve regeneration was used in which the proximal and distal stumps of a severed rat sciatic nerve were sutured into a 14mm long silicone tube. Within six weeks, complete regeneration of the sciatic nerve occurred across the length of the tube. The proximal regenerated nerve segment was extracted at various days following nerve transection and analyzed for c-src kinase activity by *in vitro* kinase assays. We report here on an induction of c-src kinase activity at 7 days following nerve transection. From then on this activity increased, and reached a level that was 6-fold greater than the control (normal nerve) at approximately 25 days following nerve transection. This expression of kinase activity occurred at the same time as axonal sprouting and myelination. Therefore, $pp60^{c\text{-}src}$ may play a role in these events during nerve regeneration.

S 308 EXPRESSION OF NEURAL CELL ADHESION MOLECULE DURING NEURITE EXTENSION IN CEREBRAL CORTICAL NEURONS, Daniel R. Marshak, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

The establishment of specific, functional connections among embryonic neurons requires their morphological and biochemical differentiation. Two of the necessary processes of such differentiation are neurite extension and cell adhesion. In previous work, we described the purification and structural analysis of neurite extension factor (NEF), an acidic protein that stimulates neurite outgrowth from cerebral cortical neurons of the chicken embryo cultured in serum-free, defined media (Kligman and Marshak, Proc. Natl. Acad. Sci. USA 82, 7136-7139). Using this model system of neurite extension, we have examined the expression of neural cell adhesion molecule (N-CAM) on the surfaces of neurons treated with NEF. Cells were cultured with or without the addition of NEF for 12-96 hours. Following incubation, the cells were stained by immunoflourescence for N-CAM without permeabilization of the cell membrane. The primary antiserum employed was prepared using a synthetic peptide that corresponds to the highly conserved, amino-terminal domain of vertebrate N-CAM previously described (Rougon and Marshak, J. Biol. Chem. 261, 3396-3401). The results show patches of intense fluorescence on undifferentiated cells, while cells extending neurites show some fluorescence localized only to the perikaryon. The growing neurites showed no detectable reaction with the antiserum in several, morphologically distinct neuronal subtypes. These findings are consistent with the hypothesis that N-CAM expression is inversely related to cell motility events in developing neurons.

S 309 CELLULAR PROTO-ONCOGENE EXPRESSION ACCOMPANYING ACTIVITY AND PLASTIC CHANGE IN THE RAT CNS, Jeffrey F. McKelvy and Laura Berkowitz, State University of New York, Stony Brook, New York 11794.

We are investigating the hypothesis that the expression of cellular proto-oncogenes may contribute to the plastic changes which mature neuron systems exhibit in response to environmental change. As model systems for such phenomena, we are studying the neuroendocrine projections of vasopressin and oxytocin-secreting neurons which arise from the supraoptic and paraventricular nuclei of the hypothalamus and project to the posterior pituitary gland. These neuronal systems are known to undergo reversible activity-driven plastic change in maintaining osmotic and body fluid homeostasis, and during lactation in the female. These plastic changes include dramatic re-arrangements of neuronal dendrite-glial process relationships, electrical coupling between adjacent neuronal cell bodies and the interactions between nerve terminals, glial cells and capillary endothelial cells in the posterior pituitary gland. In a second experimental model, we are studying long-term potentiation (LTP) in the hippocampus. In both of these model systems we have established by Northern blot analysis and in situ hybridization that c-ras and c-fos are expressed in neural pathways mediating plastic changes. Studies are in progress to correlate the expression of the proto-oncogene mRNAs, and the proteins for which they code, with specific cellular interactions believed to be occurring. In addition, the expression of other proto-oncogenes is also being studied in these models.

S 310 NGF-REGULATED GENE EXPRESSION IN PC12 CELLS. J. Milbrandt. Depts. of Pathology & Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110

The information required to initiate the basic program of neuronal development resides in the genetic complement. To further our understanding of these developmental processes, we have begun to examine the changes in gene expression elicited by the trophic agent, nerve growth factor (NGF). To identify genes whose expression is induced by NGF, cDNA libraries have been constructed from PC12 cells that have been treated with NGF (50 ng/mg) and cycloheximide (10 µg/ml) for 3 hours. Clones corresponding to mRNAs which are present in increased amounts in NGF-stimulated cells were identified by differential hybridization. Two NGF-induced cDNA clones, A20 and B8, correspond to mRNAs which are present at very low levels in naive PC12 cells, but are rapidly induced by treating the cells with NGF. The A20 clone hybridizes to a single mRNA species that is 3.5 kb in length. Significant amounts of this mRNA have accumulated after 15 min. of NGF treatment, but the highest concentration (approximately 100-fold over the basal level) is reached in 30-45 min. This transcript persists at an elevated level for up to 6 days in NGF-treated cells. The B8 cDNA hybridizes to a 2.4 kb mRNA whose level is also rapidly increased after NGF treatment, but it returns to the level observed in naive PC12 cells within 4 hours after the initial NGF stimulus. To determine if these genes are transcribed in vivo, a Northern analysis was performed on RNAs isolated from various tissues of the adult rat. Both A20 and B8 transcripts were detected in the adrenal gland and brain of the adult rat; in addition, A20 mRNA was also found in the lung.

S 311 IL-2 BINDING TO AN OLIGODENDROGLIA CELL LINE. Mizrachi, Y,. Merrill, J,. Department of Neurology, UCLA, Los Angeles CA. 90024.

There is increasing evidence that the immune response lymphocytes and their lymphokines may play a role in the central nervous system during development and following trauma and disease. Recently it was reported by this laboratory that glial growth factors secreted by human activated T cells enhance the proliferation of primary rat oligodendroglia and cloned human oligodendroglioma derived cell lines. The lymphokines described were the Glial Growth Promoting Factor (GGPF) and Interleukin 2 (IL-2). In the current study we demonstrate that the biological activity of IL-2 is mediated via direct binding of the IL-2 to membrane receptors. Using membrane preparations from the subcloned 5D7 cell line, 125I-IL-2 specifically bind in the nM range (Kd 2-6 nM), a range equivalent to that of low affinity binding to the T cell IL-2 receptors. IL-2 proliferation enhancement of these oligodendroglial cells occurs over the same range. In addition the receptor on the oligodendroglia cell line is much more sensitive to protease activity in contrast to the receptor on T cells. It appears by biological and biochemical criteria that the IL-2 receptor on the oligodendroglial cells may be different from that on T cells. Understanding the regulation of the proliferation of oligodendroglial cells by lymphokines might have important thearpeutic implications fo demyelinating diseases such as multiple sclerosis

S 312 PURIFICATION OF A NEURITE-PROMOTING FACTOR FROM SKELETAL AND HEART MUSCLE, Tae H. Oh and George J. Markelonis, Dept Anat, Univ Maryland Sch Med, Baltimore, MD 21201.
In vitro studies indicate that survival and neurite outgrowth of spinal cord neurons are influenced by trophic factors derived from the muscle tissue they innervate. In this study, we used cultures of chick embryo spinal cord neurons to help isolate a neurite-promoting factor from bovine muscle tissues. Soluble extracts of bovine skeletal and cardiac muscles stimulated neurite outgrowth from cultured spinal cord neurons. The active component in these extracts was a protein that was labile to papain and protease but not to trypsin or chymotrypsin. The active component stimulated neurite outgrowth only after absorption to a polycationic culture substratum. The neurite-promoting protein was ultimately purified from bovine muscle tissues by gel filtration on Sephadex G-200 followed by ion-exchange on DE-52 cellulose. SDS gel electrophoresis demonstrated that the protein eluted from DE-52 was apparently pure, with a native molecular weight of 180 Kd but a mol. wt. under denaturing conditions of 50-Kd. The purified 50-Kd protein stimulated neurite outgrowth from spinal cord neurons, cerebral cortical neurons, ciliary ganglion neurons and DRG neurons in culture. Western blot analysis revealed that 50-Kd protein was not related to laminin, NGF or fibronectin. Furthermore, absorption of the protein with antibodies to laminin, NGF or fibronectin did not inhibit the neurite-promoting activity of the 50-Kd protein in cultured neurons. Our results demonstrate that the 50-Kd protein purified from muscle tissues stimulates neurite outgrowth from a variety of avian neurons from both central and peripheral neural tissues.
Supported by NIH grants (NS 15013 and NS 20490).

S 313 EXPRESSION OF NERVE GROWTH FACTOR (NGF) mRNA, Hakan Persson[1], Christiane Ayer-LeLievre[2], Ted Ebendal[3] and Lars Olson[2], Department of Medial Genetics[1] and Zoology[3], Uppsala University, Department of Histology[2], Karolinska Institute, Stockholm, Sweden. NGF producing cells were identified in the rat and mouse central nervous system (CNS) and peripheral tissues by in situ hybridization. Male mouse submandibular and sublingual glands showed high levels of NGF mRNA in the granulated cells of the convoluted tubules and excretory ducts, respectively. In the mouse testis, spermatocytes and to a lesser extent spermatides were producing NGF mRNA. The pseudostratified columnar epithelium lining the lumen of the mouse epididymis contained NGF producing cells. In the rat CNS, NGF synthesizing cells included pyramidal and granular neurons of the hippocampus as well as cortical neurons, particularly in the frontal and parietal cortex. A chicken NGF gene was cloned in a mammalian expression vector and used for transfection in Cos-cells. Conditioned medium from transfected cells stimulted fibre outgrowth of several chick ganglia prepared at different stages of development. The period of maximal responsivness to recombinant chicken NGF protein correlated with high levels of NGF mRNA in the chicken embryo.

S 314 BETA-ADRENERGIC REGULATION OF NERVE GROWTH FACTOR mRNA CONTENT IN C6 GLIOMA CELLS, Joan P. Schwartz and Willie Turner, LPP, NIMH (current address LNRI, NINCDS), NIH, Bethesda, MD 20892.
Numerous studies suggest that glial cells can synthesize and release nerve growth factor (NGF). We present evidence that the synthesis of NGF can be regulated by adrenergic neurotransmitters via the beta-adrenergic receptor present on the plasma membrane of the C6 glioma cell: the second messenger for this induction appears to be cyclic AMP. Treatment of the cells with 100 nM isoproterenol for 20 hours leads to a 3-fold increase in NGF mRNA, which is accompanied by a corresponding increase in NGF protein, both in the cells and released to the medium. A significant increase in NGF mRNA is detected within one hour, with a maximal increase at 4 hours. The rapidity of the response, combined with the ability of actinomycin to block the increase in NGF protein, suggests that regulation is occurring at the level of gene transcription. The Ka for isoproterenol stimulation of NGF mRNA is $\sim$200 nM, comparable to that determined previously for activation of adenylate cyclase in C6 cells. The response is blocked by the beta-adrenergic antagonist propranalol but not by the alpha-antagonist phentolamine. Northern blot analysis reveals a single hybridizing mRNA, 1.35 kb in size. These results support the hypothesis that neurons may be able to regulate their own supply of NGF via neurotransmitter receptors on glia.

S 315 DEVELOPMENTAL REGULATION OF $pp60^{c\text{-}src}$ PROTEIN-TYROSINE KINASE ACTIVITY IN THE CENTRAL NERVOUS SYSTEM. Otmar D. Wiestler and Gernot Walter. Department of Pathology, University of California, San Diego, La Jolla, CA 92093.

The proto-oncogene c-src, the cellular homolog of the transforming gene of Rous sarcoma virus, encodes a protein, $pp60^{c\text{-}src}$, with protein-tyrosine kinase activity. There is increasing evidence that the src protein serves a special function in the brain. We studied $pp60^{c\text{-}src}$ during CNS development and found two forms of $pp60^{c\text{-}src}$ tyrosine kinase activity differentially expressed in distinct areas of the developing mouse brain. One form, pp60, was present at a low level at embryonic day E9. In midbrain and forebrain, its activity increased 50-fold until E18 and remained constant from then on. The other form, $pp60^{+}$ was first dectected at E10. Until E18 it increased 200-fold in forebrain and midbrain and subsequently declined 2-fold. pp60 was the predominant form between E9 and E11 whereas $pp60^{+}$ was more abundant at later stages. The time course of expression was similar in forebrain and midbrain, whereas in the cerebellum, both forms were found at a constant level throughout development. Low levels of pp60 were also dectectable in the liver. $pp60^{c\text{-}src}$ kinase activity was also assayed in the two mutant mouse strains *staggerer* and *weaver* with postnatal degeneration of cerebellar granule neurons. Most of the $pp60^{+}$ protein was lost postnatally in both strains. This indicates that granular cells are the main source for $pp60^{+}$ in the cerebellum. We are currently trying to determine the cellular and subcellular localization of $pp60^{c\text{-}src}$ in other regions of the mammalian brain.

S 316 ACETYLCHOLINE RECEPTOR: ASSEMBLY, FUNCTION, AND SPECIES SPECIFICITY, Lei Yu, Kiyonori Yoshii, Reid J. Leonard, Katharine Mixter-Mayne, Norman Davidson and Henry A. Lester, Division of Biology, California Institute of Technology, Pasadena CA 91125.

Messenger RNAs were transcribed in vitro from mouse and Torpedo acetylcholine receptor (AChR) subunit cDNA clones and injected into Xenopus oocytes. After 2-3 days incubation at room temperature, the AChR molecules appeared on the oocyte surface as monitored by alpha-bungarotoxin binding assay and by voltage clamp analysis. The assembly and function of the mouse AChR were compared to those of the Torpedo. Hybrid AChRs were also studied by substituting individual subunits from mouse AChR with those from Torpedo. These studies indicate that the AChR mRNAs synthesized in vitro from mouse and from Torpedo cDNA clones give rise to the AChRs which differ in their single channel properties. Furthermore, the ability to assemble into the cell membrane, channel kinetics, and voltage sensitivity all appear to be controlled in a subunit specific fashion.

Dementia and Aging

S 400 IDENTIFICATION OF cDNA CLONES USING ANTIBODIES TO ALZHEIMER'S DISEASE AMYLOID, Carmela R. Abraham, Dennis J. Selkoe and Huntington Potter, Harvard Medical School, Boston, MA 02115.

Amyloid deposits in the neuropil, cerebral and meningeal vessels are a hallmark of Alzheimer's Disease, and to a much lesser extent occur in aged normal humans and other mammals. We are interested in characterizing the protein(s) that precipitate into amyloid fibrils. Several amyloid antisera were made by us and used to stain different human tissues in the hope of finding an organ that will produce the protein, which will then be carried by blood to the brain. The liver showed the most specific staining. We then screened a liver λ gt 11 expression library and found three positive clones that proved to be similar in size $\sim$ 1.3 Kb and cross-hybridized. Absorption of the anti-amyloid antiserum with purified amyloid cores abolished the staining of the clones, while a control absorption did not. Sequence analysis of one representative clone is underway and will be presented. To what extent these liver-derived protein(s) actually contribute to amyloid deposits in brain remains to be determined. This approach should allow analysis of the sequence and tissue expression of amyloid-encoding genes in AD and normal aged humans, as well as in aged animals.

S 401 THE STRUCTURE AND REGULATION OF NEUROFILAMENT GENES. D. Flavell, F. Grosveld, K. Yazdanbakhsh and L. Wall. National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. U.K.

The three mammalian neurofilament genes are neuron specific intermediate filament genes coding for the light (NF-L), medium (NF-M) and heavy (NF-H) chains of neurofilaments. We have cloned the human NF-L gene and all three mouse genes, two of which (NF-L and NF-M) are linked on a single cosmid. We are characterizing the mouse NF-H gene, examining neurofilament gene expression in Alzheimers disease affected braom and studying the DNA sequences and protein factors responsible for the tissue-specific expression of the human NF-L gene. Sequence comparisons between mouse and human NF-L genes have indicated regions of homology in the promoter of the gene which we are studying by deletion mapping and expression in neuronal cell lines. We have also initiated in vitro transcription experiments in order to isolate the factor(s) responsible for the neuronally specific expression.

S 402 IMMUNOCYTOCHEMICAL STUDIES OF NEUROFIBRILLARY TANGLES IN AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSONISM-DEMENTIA OF GUAM, R.M. Garruto, S.K.Shankar, R. Yanagihara, D.C. Gajdusek, I. Grundke-Iqbal, and K.S. Kosik, National Institutes of Health, Bethesda, MD 20892; New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314; and Center for Neurological Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115. We studied the antigenic nature of the neurofibrillary tangles (NFT) in amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD) of Guam using monoclonal antibodies to phosphorylated and nonphosphorylated epitopes of the 200 kDa neurofilament subunit, to paired helical filaments (PHF) and to microtubule-associated protein tau (MAP-tau). Cryostat-cut serial sections (10 um) of formalin-fixed and unfixed frozen hippocampus from 28 clinically and neuropathologically confirmed Guamanian patients with ALS (9) and PD (19) and from 12 neurologically normal Guamanians (5 with and 7 without NFT) were used for immunocytochemical and histochemical staining. Aberrant accumulation of the phosphorylated 200 kDa neurofilament subunit was found in the neuronal perikarya and dendritic processes of pyramidal neurons in the hippocampus of ALS and PD patients. Prominent labeling of these structures occurred with antibodies to PHF and MAP-tau. NFT-bearing hippocampal neurons in neurologically normal Guamanians were also labeled by these monoclonal antibodies, while neurons without NFT showed no immunoreactivity. A strong positive correlation was found between the immunoreactivity of pyramidal neurons and congophilic birefringence in adjacent sections of the same cellular areas. Our data indicate that the major antigenic components of the NFT in Guamanian ALS and PD are similar to those reported for Alzheimer disease. Recent studies demonstrate that the amino acid sequence of purified NFT in PD is identical to that of the NFT and amyloid plaque cores in Alzheimer disease.

S 403 POLYPEPTIDE SEQUENCE OF THE NEUROFIBRILLARY TANGLES IN GUAMANIAN PARKINSONISM-DEMENTIA. Don C. Guiroy, Gerd Multhaup, Peter Fischer, Ralph Garruto, Masayuki Miya zaki, Konrad Beyreuther, Colin Masters, Gail Simms, Clarence J. Gibbs, D. Carleton Gajdusek, NIH, Bethesda, MD 20892; Univ of Cologne, W. Germany; Univ of Western Australia, Australia. Guamanian parkinsonism-dementia is characterized by the presence of abundant intraneuronal neurofibrillary tangles(NFT) in the substantia nigra and other subcortical nuclei, hippocampus, temporal and frontal cortex. These NFTs are composed of paired helical filaments, similar to Alzheimer disease(AD) and aging brain, as well as, triplets and other amyloid filaments more pleomorphic than those seen in AD. There is absence of extracellular amyloids in form of vascular amyloid deposits or amyloid plaques. Thus, we have isolated, purified and aminoacid sequenced the polypeptide subunit of the amyloid fibrils in these NFT. It is a 4-4.5 kDa polypeptide of 42 residues in its full sequence, similar in molecular weight and amino acid composition to the subunit polypeptide of NFT-AD and Down syndrome(DS). The first 15 amino acids of the longest polypeptide are identical to the amyloid polypeptide of the intra cellular and extracellular amyloids of AD and DS. The N-terminal heterogeneity is the same as in NFT-AD, but most of the polypeptide are of the full lenght chains, rather than the predominantly shorter forms seen in NFT and amyloid plaque cores of AD and DS. The cDNA coding for this amyloid has recently been isolated, cloned and sequenced by Goldgaber, Lerman et al; Beyreuther et al and mapped on chromosome 21 by Golgaber, Lerman et al. It specifies for the amyloid of the NFT which appear at an early age in the people of the high incidence foci of Parkinsonism-Dementia and Amyotrophic Lateral Sclerosis in the Western Pacific, as well as, for the brain amyloid in AD, DS and aging.

S 404 EXTENSIVE POST-MORTEM STABILITY OF MAMMALIAN BRAIN RNA, Steven A. Johnson, David G. Morgan and Caleb E. Finch, Univ. So. Cal., L.A., Ca. 90089-0191.
The post-mortem stability of brain RNA was measured in rat and human samples. Whole rat brains, cooled at a rate approximating that of human brains awaiting autopsy, were collected at intervals from 0 to 48 hours after death and frozen. Two potentially independent characteristics of these RNA populations were measured: (1) yield of RNA/gram tissue (quantity) and (2) extent of degradation (quality). Total RNA yields were similar after all post-mortem delays. Hybridization of [^{32}P]labeled cDNA probes to nitrocellulose filter blots of electrophoretically separated rat brain RNA failed to reveal degradation of the specific rat brain mRNAs during the post-mortem period. Similarly, *in vitro* translation of these same rat total RNA samples produced high molecular weight translation products with no differences between long and short post-mortem times. Human cerebral cortex RNA was isolated from a neurosurgical sample and 4 other donors with post-mortem intervals from 7 to 36 hrs was also examined. Typically, the yield of total RNA from human brain was 40-50% of the yield from rat brain. When analyzed by RNA gel blot hybridization studies human cortical RNA appeared slightly degraded. However, the degree of apparent RNA degradation was *not* related to the post-mortem interval. *In vitro* translation products of human cortical RNA revealed high MW peptides at all post-mortem intervals, but slightly less [^{35}S]incorporation into higher MW products at longer post-mortem times. Together, these results demonstrate an extensive stability of brain RNA during the post-mortem period. Supported by the Alzheimer's Disease and Related Diseases Association (SAJ) and the John Douglas French Foundation (DGM).

S 405 TAU PROTEIN STRUCTURE AND HETEROGENEITY. Gloria Lee, Harvard Medical School, Boston, MA 02115. Tau protein is a family of highly related microtubule associated proteins which promote microtubule assembly *in vitro*. It is found primarily in brain and has been identified as the major antigenic determinant in the neurofibrillary tangles of Alzheimer's disease. To more fully understand the structure and function of tau protein, I have determined the structure of two tau proteins from mouse cDNA clones. The DNA sequences are identical with the exception of the carboxy terminus and 3'untranslated regions; both protein sequences contain a 23 residue stretch which is repeated three times. The point at which the cDNA sequences diverge contains the consensus sequence for alternative splicing junctions. This suggests that both mRNAs originate from one gene. Genomic southern blots have also indicated one gene for tau protein. To definitively answer the question of the origin of tau heterogeneity, we have isolated tau genomic clones from a mouse library. We are also investigating the question of functional domains in tau protein. Because tau is postulated to be elongated and oriented longitudinally along microtubules, it is not clear whether a "microtubule binding domain" exists in tau protein. However, preliminary data from human and rat tau cDNAs suggest that such a domain may exist. Southern hybridization analysis shows that some parts of the mouse coding region do not hybridize to the human and rat clones. One possibility is that the limited area of homology between these species represents a conserved domain of tau, perhaps a microtubule binding domain. To explore this possibility, segments of tau protein have been cloned into pUC9, expressing immunoreactive tau products fused to B-galactosidase. The ability of these tau products to coassemble with tubulin will be tested.

S 406 ALZHEIMER'S DISEASE-LIKE FILAMENTS OBTAINED WITH PURIFIED MICROTUBULE-ASSOCIATED PROTEIN TAU FACTOR. Esteban Montejo de Garcini[1,2], Luis Serrano[1] and J. Avila[1]. [1]Centro de Biología Molecular (CSIC-UAM) Cantoblanco 28049 Madrid. [2]C.U. San Pablo, Boadilla del Monte. Madrid. Spain.

Recently, the microtubule-associated protein tau factor has been identified as the main component of the paired helical filaments appearing in Alzheimer's disease histopathology (i.e. 1,2). We have purified this protein to study whether tau was able to polymerize or if any modification of the protein was required for it. Unmodified tau did not polymerize, however upon urea treatment tau forms filaments closely related in appearence to those found in the brain of patients with Alzheimer's disease.

Among the modifications caused by urea, we have found that the deamination of glutamine to glutamic residues may be the responsible for this self-assembly. Deamination was followed by digestion with V8 protease, an enzyme which cleaves glutamic but not glutamine residues. A higher digestion with the protease was found for treated tau compared to the untreated one. The urea-treated protein was also a better substrate compared to the untreated protein for the enzyme transglutaminase.

1.- Kosik, K. et al., (1986). P.N.A.S. USA. 83, 4044, 4048.
2.- Grundke-Iqbal et al., (1986) J.B.C. 261, 6084-6089.

S 407 SELECTIVE MESSENGER RNA REDUCTION IN ALZHEIMER'S DISEASE, D.R. McLachlan, W.J. Lukiw, L. Wong and N.T. Bech-Hansen, Department of Physiology, University of Toronto and Department of Genetics, Hospital for Sick Children, Toronto, Canada M5S 1A8

The relative yields of 7 messenger RNAs extracted from Alzheimer and control neocortex were examined by Northern and quantitative dot-blot analysis. The average yield of mRNA coding for the 68 kD moiety of neurofilaments was reduced to 27% of control when expressed as the percentage of total RNA or 14% when expressed per gram of neocortex. In contrast, the yields of 6 other messenger RNAs fell into two categories: those which were statistically significantly reduced to about 65% of control and those which were not reduced when expressed as percentage of total RNA.

The anomalous low abundance of neurofilament mRNA in cerebral cortex of Alzheimer's disease cannot be adequately accounted for by a non-specific effect of brain damage, neuron cell loss or neurons with neurofibrillary degeneration. We speculate that the decrease is related to a disorder in gene expression in Alzheimer's disease, perhaps related to a non-random increased compaction of chromatin previously reported from this laboratory.

S 408 IMPAIRED INFORMATION TRANSFER AT AN ISOLATED SYNAPSE TREATED BY ALUMINUM: IS IT RELATED TO DEMENTIA? By H. Meiri and E. Banin, Department of Physiology, Hebrew University-Hadassah Medical School, P.O. Box 1172, Jerusalem 91010 ISRAEL

Elevated content of aluminum in human brain has been found to be associated with dementia of the Alzheimer type, Dialysis Encephalopathy and Amyotrophic Lateral Sclerosis of Guam. In experimental animals, cognitive deficits and motor weakness accompanied by neurofibrillary degeneration have been induced by intracerebral administration of aluminum salts. Surprisingly, however, little is known about the influence aluminum has upon the function of nerve cells and particularly of synapses, through which communication in the nervous system is mediated. The aim of this study was to investigate information transfer across a single cholinergic synapse exposed to aluminum. We describe here a biphasic effect of aluminum (6-200 ug/ml) on synaptic transmission at the isolated frog neuromuscular junction: Shortly following the administration of aluminum the release of acetylcholine was enhanced. Few hours later, however, both the spontaneous and evoked synaptic activity stopped rather abruptly and could not be renewed by removal of aluminum from the bathing medium. Neurotransmitter release ceased sooner in continuously stimulated preparations than in unstimulated synapses. These results indicate that aluminum, at the concentration found in the diseased human brain, interferes with the control of synaptic transmission. Nerve terminals with higher rates of activity are presumably more susceptibile to aluminum toxicity.

S 409 DIFFERENCES IN GENE EXPRESSION IN THE CEREBRAL CORTEX OF ALZHEIMER'S DISEASE AND AGE-MATCHED CONTROL BRAINS, David G. Morgan, Steven A. Johnson, and Caleb E. Finch, University of Southern California, Los Angeles, CA 90089-0191.

We have recently measured the yield of RNA from cerebral cortical samples of Alzheimer's disease (AD) and age-matched control (CTL) brains. Two sources of tissue have been compared; source A is the Alzheimer's Disease Research Consortium of Southern California (LA); Source B is the Brain Tissue Research Center operated by Edward Bird (Boston). The total RNA yields form source A did not differ between AD (377 $\pm$ 29 μg/gm brain, n=8) and CTL (403 $\pm$ 26 μg/gm, n=8). The total RNA yields from source B were decreased by 25% in AD (279 $\pm$ 38 μg/gm, n=7) relative to CTL (375 $\pm$ 10 μg/gm, n=7). However, this difference was due primarily to two AD samples which appeared highly degraded on ethidium bromide stained agarose gels; 5 of the 7 AD samples in source B had total RNA yields within the CTL range. PolyA+RNA was prepared from total RNA of source A by 2 hybridization-elution cycles to oligo(dT)cellulose. The yield of polyA+RNA from AD (1.25 $\pm$ .13% of total, n=8) was similar to the CTL yield (1.35 $\pm$ .11% of total, n=8). Total RNA from source A was translated in vitro in a rabbit reticulocyte lysate system. Incorporation of [^{35}S]met into TCA precipitable material was not statistically different between AD (9,5000 cpm/μg RNA) and CTL samples (11,700 cpm/μg RNA). However, autoradiographic analysis of 1-dimensional and 2-dimensional polyacrylamide gels of the translation products revealed several polypeptides whose [^{35}S] incorporation was consistently increased or decreased in AD. Northern blot analyses of these RNA samples with several cloned probes of known brain proteins are being performed. DGM is supported by the French Foundation. SAJ is supported by the ADRDA.

S 410 RIBONUCLEASE AND RNA LEVELS IN ALZHEIMER'S DISEASE AND CONTROL HUMAN BRAIN, Morrison, M.R., Pardue, S., Maschhoff, K., White, C.L., III, Ilaria, R.L., III, Willcutts, M., Roses, A. and Gilbert, J., U.T.H.S.C.D., Dallas, Texas, 75235, and Duke University Medical Center, Durham, North Carolina, 27710.

Definitive correlation of postmortem biochemical measurements with disease state in human tissue is complicated by variable factors that may alter tissue levels of some biochemical components. These variables include extended hypoxia premortem and the length of time between death and tissue freezing or analysis. Likely because of this variability, we have shown that RNA isolated from 20-40% of human brains frozen between 4 and 16 hours postmortem is degraded. RNA isolated from AD brain is even more easily degraded than RNA isolated from control brain, possibly due to hypoxia prior to death in people dying with AD (Roses, unpublished observations). Undegraded RNA isolates from AD brain do not differ significantly in yield of total RNA or in the levels of translationally-active mRNAs either between pathologically affected and unaffected areas of AD brain or between AD and control brains. The levels of alkaline and acidic ribonucleases and their intracellular distribution are also comparable, as is that of the active alkaline ribonuclease inhibitor. There were no statistical correlations with age, postmortem interval (4-16 hr) or degree of neuropathology. RNA isolates from the Duke rapid autopsy brain bank (patients oxygenated prior to death to minimize hypoxia; postmortem interval < 1 hour) are more reproducible in quality and preliminary results show less abundant mRNAs may be more intact than those isolated from the routine autopsy material.

S 411 VINPOCETINE NORMALIZES THE ELECTROCORTICOGRAM (ECoG) OF AGED RATS. R. R. Notvest, J. J. Inserra and T. A. Emrey. Department of Pharmacology Ayerst Laboratories Research, Inc., Princeton, NJ 08543-9990

Vinpocetine (VINPO), an eburnamenine derivative, is being evaluated as a cerebral activator for use in diseases characterized by symptomatic cognitive and attentional deficits. In young rats (3-month-old), VINPO produces an ECoG pattern similar to that seen in states of behavioral arousal (Notvest et al., Soc Neurosci Abstr 12:704, 1986). This report focuses on the effect of VINPO on the ECoG of aged (28-month-old) male Fischer-344 rats. Cortical function of aged rats is impaired relative to young rats, as measured by power spectral analysis of the ECoG (Emrey et al., Soc Neurosci Abstr 12:1315, 1986). Specifically, the aged rats have lower total spectral power (-25%), accounted for predominantly by a decrease in power of the alpha frequency band (-36%; $p<0.001$). The effect of VINPO (ascending series: 0.3, 1, 3, 10, and 30 mg/kg/day x 5 days, p.o.) was examined by comparing the ECoG of vehicle- (n=9) and VINPO-treated (n=9) aged rats after normalizing for differences in baseline. The VINPO group had a significant increase in total spectral power at 3, 10, and 30 mg/kg/day, relative to the VEH group ($p<0.05$). The increase in total power was accounted for predominantly by an increase in the alpha frequency band at the doses of 3, 10, and 30 mg/kg/day ($p<0.05$). These findings indicate that VINPO administered orally to aged rats normalizes age-related differences in brain function as measured by the ECoG.

S 412 ULTRASTRUCTURAL LOCALIZATION OF CALCIUM-ACTIVATED PROTEASE, CALPAIN, IN ALZHEIMER BRAIN, L.S. Perlmutter, C. Gall, M. Baudry, and G. Lynch, Univ. Calif., Irvine, CA.

Calpain hydrolizes several cytoskeletal proteins, and has been implicated in both experimentally-induced and disease-related degeneration. The large neurons that are at risk for various pathologies are highly immunoreactive for calpain. In addition, a strong negative relationship between total calcium-dependent proteolytic activity in brain and species-specific maximal life span has been found. To examine the possibility that calpain may be involved in the cellular changes accompanying age-related pathologies, the enzyme was localized at the ultrastructural level in autopsy brain samples from Alzheimer's patients (post-mortem time +/- 4h). The samples were immersion fixed in a mixed aldehyde solution, and processed with a mouse-anti-rat anti-calpain I antibody and the avidin-biotin system of the VECTASTAIN ABC kit. Immunoreacted tissue was osmicated, dehydrated, then embedded in Epon-Araldite resin.

Calpain immunoreactive intact cell bodies and dendrites were observed. As was seen in the rat, reaction product was scattered throughout the cytoplasm of the cell body, and decorated dendritic microtubules. Postsynaptic densities exhibited heavy accumulations of reaction product. Many immunoreactive cell bodies contained neurofibrillary tangles. Typically, the filament bundle itself was unlabelled, while immunoreaction product was associated with the filaments at the end of the bundle. Often, bundles of filaments, lipofuscin and calpain immunoreactivity were localized in close proximity. These results suggest that calpain may interact with the filaments before they actually join the bundle. (Supported by NIA AG05373 to LSP and AG00538 to GL.)

S 413 RNA LEVELS AND ALZ-50 IMMUNOREACTIVITY IN ALZHEIMER'S DISEASE HIPPOCAMPUS, R.E. Rhoads, J.A. Doebler, W.R. Markesbery, A. Anthony, P. Davies, and S.W. Scheff, Departments of Biochemistry, Neurology, Pathology and Anatomy, University of Kentucky College of Medicine, and Sanders-Brown Research Center on Aging, Lexington, KY 40536; Department of Biology, Pennsylvania State University, University Park, PA 16802; and Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

RNA changes in neurons positive for Alz-50, a protein present in much higher concentrations in Alzheimer's disease (AD) brain than normal (Wolozin et al., Science 232, 648-650), were examined using immunocytochemistry and azure B-RNA microdensitometry. In both the hippocampal endplate (Rose's H_3-H_5 fields) and the subiculum, Alz-50-reactive neurons exhibited approximately 30% reductions in total cell RNA relative to Alz-50-negative neurons of the corresponding region. The RNA content of Alz-50-positive neurons of the AD brain was also reduced in comparison to Alz-50-negative neurons of age-matched, non-demented controls, whereas there were no significant differences between negative neurons of the two groups. These data suggest that Alz-50-positive neurons are metabolically deficient and hence that Alz-50 immunoreactivity represents an early stage of degeneration in AD.

S 414 LOSS OF M1 MUSCARINIC RECEPTORS WITH AGING IN FISHER 344 RATS, Roy D. Schwarz, Adele A. Bernabei, Carolyn J. Spencer, and Thomas A. Pugsley, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Alterations in CNS cholinergic function have been shown to occur as a result of aging in both humans and experimental animals. These observations have gained importance with the more recent findings of marked cholinergic dysfunction in geriatric disorders such as senile dementia of the Alzheimer's type (SDAT). While effects of aging on the total population of cholinergic receptors has been examined, no studies exist reporting selective changes in brain M1/M2 receptor subtypes during aging. The present study directly addresses this question using receptor binding techniques.

Binding experiments with ^{3}H-quinuclidinyl benzilate (QNB) and the selective M1 antagonist, ^{3}H-pirenzepine (PZ) were performed in three brain regions of young (3-6mo), middle-aged (15-17mo), and aged (22-24mo) male Fisher 344 rats. Statistically significant decreases in receptor number (15-20%) were measured in the cortex, but not striatum or hippocampus, of aged rats when compared to young and middle-aged animals with both ^{3}H-QNB and ^{3}H-PZ. No change in affinity (Kd) was seen in any age group in the three brain regions examined. These results are the first to demonstrate that the number of rat cortical M1 receptors are decreased with aging and may be relevant not only to aging, but also neurodegenerative disorders associated with aging.

S 415 MOLECULAR CLONING AND IN VITRO TRANSLATION STUDIES OF GFAP mRNA FROM NORMAL AND ALZHEIMER DISEASE BRAIN TISSUE. S.B. Zain*, S. Rehman*, E.M. Sajdel-Sulkowska+, M. Salim*, R. Majocha+, W. Chou*, and C.A. Marotta+. *Cancer Center, University of Rochester Medical School, Rochester, NY 14642. +McLean Hospital and Mass. General Hospital, Harvard Medical School, Massachusetts.

The present study was conducted to rule out the possibility that Alzheimer's Disease (AD) RNA undergoes exclusively nonspecific losses which may indicate only generalized pre or post mortem processing. If decreased RNA were related to disease process, then the losses will be confined to affected cells. If this were the case, astroglial mRNAs such as the one for GFAP would be expected to remain constant or increase.

In in vitro translational studies Poly A+ RNA from both control and AD samples stimulated protein synthesis. A prominent protein of 50,000 mol. wt. was identified as GFAP by immunoprecipitation. cDNA libraries were generated from normal and AD brain poly A+ RNA isolated from autopsied brains. Positive cDNA clones were identified and characterized by nucleotide sequence analysis. Using a segment of this cDNA as a probe qualtative and quantitative GFAP sp. mRNA analysis was conducted using Northern blots. We detect only one GFAP specific mRNA with a molecular size of 2.7 Kb, with no indications of degradation products. Sizing of the cDNA molecules synthesized from control and AD brain mRNAs revealed a similar size range of 0.5 - 9.5 Kb, comparable with cDNA generated from freshly isolated human placenta and uterine tissues. We conclude that the loss of brain RNA may be associated with affected neurons, and mRNAs that are not exclusively neuron-specific may appear to remain at nearly normal or perhaps increased levels.