NEUROBIOLOGY: MOLECULAR BIOLOGICAL APPROACHES TO UNDERSTANDING NEURONAL FUNCTION AND DEVELOPMENT

Paul O'Lague, Organizer
April 1 — 7, 1984

Plenary Sessions

Poster Sessions

Receptors and Growth Factors

1085 ISOLATION OF A cDNA CLONE CODING FOR THE γ SUBUNIT OF MOUSE NERVE GROWTH FACTOR, Axel Ullrich, Department of Molecular Biology, Genentech, Inc., South San Francisco, CA 94080

A new approach was taken to isolate a full length cDNA encoding the gamma subunit of nerve growth factor. An oligonucleotide primer was used to first identify kallikrein-related cDNA clones in a cDNA library made with male mouse submaxillary gland mRNA. 860 out of 10,000 clones were identified as being kallikrein-related and were further analyzed using synthetic oligonucleotides and a hybridization procedure which disregards base composition and enables distinction between partial and perfect sequence complementarity. Nine clones hybridized with a gamma subunit-specific probe at high stringency, and the nucleotide sequence of the longest cDNA insert was determined. Regions of homology and variability within the kallikrein gene family are maintained in this protein, and probably reflect the similar functions yet different substrates for this family of proteins.

1086 TROPHIC FACTORS AND NEUROLOGIC DISEASE, Stanley H. Appel, Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Recent advances in developmental neurobiology have documented the importance of target-cells for the survival, growth, and differentiation of innervating neurons. Within the peripheral sympathetic and sensory systems, target tissue provides diffusible factors such as NGF which subserve such functions. To determine whether other diffusible factors from target tissue exert system-specific retrograde effects, we have employed tissue culture assays to purify morphologic and neurotransmitter enhancing factors from target tissue extracts. Muscle extracts enhance neurite elongation as well as cholinergic activity of cultured dissociated ventral horn cells. The morphologic and cholinergic factors have different biochemical properties, although all are proteins. The morphologic factor is approximately 33,000 to 35,000 daltons and resides only in muscle, while the cholinergic factors are 55,000 daltons and 1,500 daltons and reside in brain, cardiac, and skeletal muscle. Striatal extracts similarly enhance neuritic outgrowth and specific dopaminergic uptake of cultured substantia nigral explants. A peptide smaller than 3,000 daltons appears responsible for this activity. Hippocampal extract enhances morphologic as well as cholinergic activity of cultured medial septal explants. Tissues from other organs are relatively inactive. A peptide of approximately 1,500 daltons appears responsible for the hippocampal effects.

These neurotrophic hormones which enhance morphologic and neurotransmitter activity may well be of therapeutic value in several degenerative diseases of the CNS. Amyotrophic lateral sclerosis, Parkinsonism, and Alzheimer's disease have no known cause. Each involves specific pathways within the peripheral or central nervous system. The availability of purified neurotrophic factors for each of these systems should provide us an opportunity to assess their importance in development of the specific networks, determine a specific mechanism of action, and assess their potential loss in disease.

Appel SH. A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer Disease. Ann Neurol 10:499-505, 1981

Smith RG and Appel SH. Extracts of skeletal muscle increase neurite outgrowth and cholinergic activity of fetal rat spinal motor neurons. Science 219, pp 1079-1081, 1983.

Supported in part by grants from the John A. Hartford Foundation and Robert J. Kleberg and Helen C. Kleberg Foundation.

1087 Early Events in Growth Factor Action by Luis Glaser, Paul Rothenberg, Ying-Xin Zhuang, Paul Schlesinger, Tom Deuel, and Dan Cassel, Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, MO 63110.

One of the earliest events that can be observed following addition of mitogenic polypeptides to quiescent cells is an increase in the rate of sodium entry into cells. Our electrophysiological studies demonstrate that this sodium entry is electroneutral. Since the sodium entry is also to a large extent sensitive to inhibition by amiloride or amiloride analogues, it is presumed to reflect an increase in the rate of sodium/hydrogen exchange. We have developed methods for the measurement of cytoplasmic pH by introduction into cells of a fluorescent dye linked to dextran using the osmotic shock method of Okada and Rechsteiner (Cell 29, 33-41, 1982). Using dimethylfluorescein linked to dextran we have been able to show an increases in cytoplasmic pH following addition to appropriate cells of EGF (A431 cells), PDGF (in NR6 cells, a Swiss 3T3 variant) as well as by the addition of vanadate at micromolar concentrations.

In all cases, changes in pH follow a stereotyped response whereby after a lag period of approximately 1.5 minutes there is an alkalinization of the cytoplasm of 0.15 to 0.2 pH units, which is sustained for at least 1 hour. The cytoplasmic alkalinization requires the presence of external Na^+, is independent of external Ca^{++} and is sensitive to inhibition by amiloride. All of these characteristics are consistent with the assumption that this alkalinization is a consequence of activation of Na^+/H^+ exchange by each of these mitogenic compounds.

The possible relation of such alkalinization to the mitogenic response will be discussed. The fluorescent method described allows not only study of the actions of mitogens on cells, but also allows a study of the factors involved in pH homeostasis in cultured cells with very high sensitivity. Supported by Grant GM18405 and by funds from the Monsanto Company.

1088 THE ABILITY OF CELLS TO SYNTHESIZE AND RESPOND TO PLATELET-DERIVED GROWTH FACTOR: POSSIBLE INVOLVEMENT IN SEVERAL FORMS OF GROWTH REGULATION, Daniel F. Bowen-Pope, Ronald A. Seifert, Arthur Vogel and Russell Ross, University of Washington, Seattle, WA 98195; and Angie Rizzino, Epply Institute, Omaha, NE 68105

Platelet-derived growth factor (PDGF) is a potent mitogen for many connective tissue cell types, including vascular smooth muscle cells (SMC), fibroblasts, and glial cells. PDGF was discovered in, and purified from, blood platelets, where it is highly concentrated within secretory granules. Its release from platelets has been postulated to play a beneficial role in wound healing and a pernicious role in the development of atherosclerotic lesions (1). Recently, however, PDGF-like molecules (PDGF-c) which bind to the PDGF receptor and are at least partially recognized by antiserum against human PDGF, have been shown to be produced by several cell types and may play a role in regulating normal and pathological growth under other conditions: **1)** Aortic SMC from 2-week-old rats synthesize and secrete mitogenic levels of PDGF-c. SMC from 3-month-old rats secrete less than 1% as much PDGF-c. **2)** Embryonal carcinoma cells F9 and PC-13 secrete PDGF-c while growing as undifferentiated stem cells, but stop secreting PDGF-c when induced by retinoic acid to differentiate into endoderm-like cells. **3)** Cultured aortic endothelial cells secrete high levels of PDGF-c (2). Regulators of synthesis have not been found. **4)** The oncogene product of simian sarcoma virus (SSV), P28 sis, is homologous to at least a portion of the PDGF molecule (3,4) and SSV-transformed 3T3 cells contain a PDGF-like mitogen (5). We have found that PDGF-c is also secreted by cells transformed by agents (Moloney and Kirsten murine sarcoma viruses, adenovirus, SV40, and "spontaneous" transformation) which do not themselves code for PDGF-like sequences. In these instances, the transformed cells seem to be expressing the cellular PDGF gene. It is possible that the synthesis of PDGF-c by embryonal and neonatal cells, and the cessation of synthesis during further development, is involved in regulating the growth of these cells and that induction of synthesis of PDGF in many transformed cells may help account for their reduced requirement for exogenous mitogens.

1. Ross, R, and Glomset, J.A. (1976) New Engl. J. Med. **295**:369-377 and 420-425.
2. DiCorleto, P.E. and Bowen-Pope, D.F. (1983) Proc. Natl. Acad. Sci. **80**:1919-1923.
3. Dolittle, et al. (1983) Science **221**:275-277.
4. Waterfield, et al. (1983) **304**:35-39.
5. Deuel, et al. (1983) Science **221**:1348-1350.

Gene Expression and Multigene Families

1089 DEVELOPMENTAL REGULATION OF GENES IN MAMMALIAN CENTRAL NERVOUS SYSTEM, Ronals M. Evans*, Susan Amara+, and Michael G. Rosenfeld+, *Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, CA 92138, +School of Medicine, University of California, San Diego, CA 92093

The complexity of the central nervous system function is attributable to the extraordinarly intricate pattern of neuro-interconnections, the large number of regulatory molecules which are produced and secreted by neurones, and the diversity of the neuronal receptor systems. The challenge, then, is to define the developmental mechanisms determining neuronal migration and interconnection patterns, neural transmission, and neuron-specific patterns of gene expression. We will present an approach used to identify novel regulatory neuropeptides in the absence of prior structural or functional information. The analysis of Calcitonin gene expression in the brain revealed the operation of a new developmental mechanism involved in the generation of diversity of brain regulatory peptides. As a consequence of these studies, we have discovered a novel neuropeptide (CGRP) exhibiting a unique pattern of production and distribution in the central and periphial nervous systems. Histochemical analysis of CGRP producing cells and pathways in the brain and other tissues suggest functions for the peptide in ingestive behavior, nocioception, and modulation of the automonic and endocrine systems. We will present evidence using DNA mediated gene transfer studies that transacting factors specifically produced in the brain interact with the Calcitonin-CGRP gene to determine specific utilization of alternative polyadenylation sites which ultimately result in selective RNA processing pathways.

We have begun to address the potential function of neuropeptides as regulators of gene expression in target cells. Utilizing the expression of growth hormone and prolactin genes as models, we are able to demonstrate rapid and specific transcriptional regulatory effects by the hypothalamic peptides TRH and GRF as well as by EGF. Evidence for the molecular basis of peptide regulation of gene expression as mediated by specific membrane receptor systems will be presented.

Finally, utilizing transgenic animal technology, we will present recent studies on the genetic manipulation of the neuroendocrine system. Specifically, we will describe the generation of a pedigree of mice expressing growth hormone fusion genes in the motor neurones of the vagus nerve and in the magnocellular secretory cells of the hypothalamus. We will describe the consequences of the expression of growth hormone genes in these animals and how this approach might be used to study the nature of complex neuropathways in the adult brain as well as provide novel markers from the study of early developmental events.

1090 CELLULAR AND MOLECULAR STUDIES OF THE NEUROPEPTIDES USED BY APLYSIA ABDOMINAL GANGLION NEURONS R3-14. Richard H. Scheller, Rashad-Rudolf Kaldany, Thane E. Kreiner and Mark Schaefer, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Aplysia abdominal ganglion neurons R3-14 are thought to modulate cardiovascular physiology. These cells takeup, package into vesicles, and transport free glycine 20-40 times more efficiently than other cells. Glycine also modulates excitatory inputs to the heart strongly suggesting that it is used as an extracellular messenger by these cells. The R3-14 neurons also use neuropeptides as chemical messengers. Using differential screening techniques we have cloned the gene encoding the precursor for these products. The gene is interrupted by two intervening sequences and encodes a 1.25 KB mRNA. This RNA is translated into a 108 amino acid preproprecursor which contains a 20 amino acid signal sequence. Synthetic peptides to various regions of the precursor have been generated. These peptides are being used to raise antibodies and for physiological studies. In addition to the R3-14 cells, two symmetric clusters in the cerebral ganglion, each consisting of about 10 cells, were found to contain immunoreactive material. Pulse chase studies using tritiated amino acids are being conducted to determine the proteolytic processing pathway. At least 5 peptides, ranging in size from 9 to about 40 amino acids are cleaved from the precursor. The physiological significance of using multiple extracellular messengers is being investigated.

1091 BRAIN SPECIFIC mRNAs: CONTROL OF THEIR EXPRESSION AND LOCALIZATION OF THE PROTEINS THEY ENCODE, J. Gregor Sutcliffe, Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Of the 30,000 distinct mRNAs expressed in the rat brain, 90% are specific to the brain and most are probably specific to small subsets of cells (1). Brain specific, rare mRNAs average 5000 nucleotides in length, thus are considerably larger than the 1700 nucleotide number average length of total brain mRNA. We have determined the nucleotide sequences of several cDNA clones corresponding to rat brain specific mRNAs. From these sequences, we have deduced the amino acid sequences of the proteins encoded by these brain specific mRNAs, raised antisera to chemically synthesized peptides mimicking short regions of the putative brain specific proteins, and used the sera in immunocytochemical studies to localize each protein in a subset of neurons (2). One target is a brain specific glycoprotein of 100K which is synthesized in cell bodies in brain stem, hypothalamus and other regions. The protein is found in vesicles in axons and terminal fields ramifying in cortex, hippocampus, cerebellum and other regions. Both the sequence of the protein and HPLC fractionation experiments suggest that 18236 is proteolytically processed to small fragments, and neurophysiological and behavioral studies with the synthetic peptides suggest that at least one of these fragments has biological activity. The protein is likely to be a precursor for neurotransmittor peptides.

Brain specific genes contain an 82 nucleotide "ID" sequence in some of their introns (3). ID sequences are transcribed specifically in neurons by RNA polymerase III, thereby activating the surrounding region for Pol II transcription. ID sequences probably respond to lineage specific transcription factors.

1 Milner, R.J. and Sutcliffe, J.G. (1983) Nucleic Acids Res. 11:5497-5520.
2 Sutcliffe, J.G., Milner, R.J., Shinnick, T.M. and Bloom, F.E. (1983) Cell 33:671-682.
3 Sutcliffe, J.G., Milner, R.J., Bloom, F.E. and Lerner, R.A. (1982) Proc. Natl. Acad. Sci. USA 79:4942-4946.

Membrane Channels: Structure, Function and What Next?

1092 STRUCTURE AND FUNCTION IN PURIFIED SODIUM ION CHANNELS. Robert L. Barchi, Institute for Neuroscience, Univ. of Penna. Med. School, Philadelphia, PA 19104.

Successful solubilization and purification of the voltage-dependent sodium channel has now been reported from eel electroplax, rat and rabbit sarcolemma, and rat brain.[1] The emerging picture of the physical and chemical properties of these channels suggests that they are remarkably similar in their basic characteristics. Typically, these sodium channels in mixed micellar form behave comparably to a ~ 9S globular protein on sucrose gradients, and exhibit an apparent Stokes radius of 80-90 Å. Each contains a large glycoprotein of > 200,000 apparent MW having anomalous migratory characteristics on SDS-PAGE.[2,3] In the mammalian channels one or more smaller subunits of MW near 38,000 are seen.

Several of these purified channels have now been reconstituted in a functional form. After incorporation into egg PC vesicles, the rat sarcolemmal channel can gate the influx of monovalent cations in response to activation by batrachotoxin (BTX), veratridine or aconitine; these fluxes are specifically blocked by TTX or STX.[4] Similar results have been reported for the channel from rat brain.[5] Cation selectivity through the open (BTX-activated) channel has also been determined for the rat sarcolemmal channel; the purified channel preserves the relative selectivity amongst Na^+, K^+, Rb^+, and Cs^+ characteristic of the native channel.[6] Purified sarcolemmal channels reconstituted into PS/PE vesicles are now being used for biophysical measurements with patch clamp or BLM methods. Preliminary results indicate the presence of single channel conductances of 15-20pS; voltage dependent activation has been observed.

Polyclonal and monoclonal antibodies have been raised to the eel and the rat sarcolemmal channel. These antibodies are providing new insight into channel localization in situ and are being used to further probe channel function and structure at the molecular level. Physical, chemical and immunological approaches, combined with new initiatives in gene cloning, offer hope for successful structure-function correlation in this unusual and complex channel protein.

Ref. (1) Barchi (1982) Int. Rev. Neurobiol. 23:69-101; (2) Barchi (1983) J. Neurochem. 40:1377-1385; (3) Miller et al. (1983) Biochemistry 22:462-470; (4) Weigele and Barchi (1982) Proc. Natl. Acad. Sci. 79:3651-3655; (5) Talvenheimo et al. (1982) J. Biol. Chem. 257:11868-11871; (6) Tanaka et al. (1983) J. Biol. Chem. 258:7519-7526.

1093 CLONING OF POTASSIUM ION CHANNEL GENES, Mark A. Tanouye, Division of Biology, Calif. Inst. Tech., Pasadena, CA 91125

A combination of electrophysiological, genetic, and molecular genetic methodologies have been used to examine the Shaker (Sh) gene complex in Drosophila. Electrophysiology experiments have suggested that Sh mutants encode abnormal K^+ channels or channel subunits. For example, we have recorded action potentials in normal and Sh mutant axons using intracellular microelectrodes (1). The mutants showed abnormally long delays in repolarization. The K^+ channel blocker 4-aminopyridine (4-AP) mimicked the mutant defect when applied to normal animals but had little effect when applied to mutants. These results are in good agreement with other studies which showed that 4-AP mimics all major Sh^{KS133} behavioral and physiological defects (2,3). Experiments using other drugs have eliminated other likely explanations of Sh. Thus, Sh is not likely due to a Na^+ or Ca^{++} channel defect (1,2). Voltage clamp experiments have identified fast, transient, voltage-sensitive K^+ current which is specifically altered by Sh mutations (3,4).

Sh has been well-defined genetically (1). It is located on the X chromosome between salivary gland chromosome bands 16F1-6. The region contains about 100 Kb of DNA. Sh appears to be a complex genetic locus organized into three regions. Mutations in two flanking regions give rise to animals with Sh abnormalities. A central region, when deleted, results in haplolethality. The left flanking region has been characterized in the greatest detail genetically (Ferrus & Tanouye, unpublished). The limits of the region are clearly defined by bracketing chromosomal breakpoints. It contains about 50 Kb of DNA. Five putative Sh genes, defined by mutation, have been identified and mapped by recombination.

Sh genetics provides that basis for its analysis by recombinant DNA methodologies. A cDNA clone has been mapped to the left flanking region of Sh by Southern and in situ hybridization analyses of intact and broken Sh chromosomes. The cDNA clone was used as an hybridization probe to screen libraries of cloned Drosophila DNA segments. About 30 Kb of DNA in the Sh region have been cloned and restriction mapped. Several Sh mutations have been mapped in the cloned region.

1. Tanouye, et al. (1981) PNAS 78:6548-6552.
2. Jan, et al. (1977) Proc. R. Soc. Lond. B. 198:87-108.
3. Salkoff and Wyman (1981) Nature 293:228-230.
4. Wu, et al. (1983) Science 220:1076-1078.

Intracellular Messengers

1094 DEVELOPMENTAL STUDIES OF PHOSPHOLIPID/Ca^{2+}-DEPENDENT AND S-100 PROTEIN-MODULATED PROTEIN PHOSPHORYLATION SYSTEMS IN BRAIN. J.F. Kuo, R. Scott Turner and De-Fang Qi, Department of Pharmacology, Emory University, Atlanta, GA 30322

Phospholipid-sensitive Ca^{2+}-dependent protein kinase (PL-Ca-PK) is particularly rich in brain tissues (1) and phosphorylates a number of proteins in the cytosolic and particulate fractions of rat and guinea pig brain (2). The major endogenous substrate protein for PL-Ca-PK in the particulate fraction of rat brain appears to be myelin basic protein (MBP) (3). The activity level of PL-Ca-PK in the particulate fraction of rat grey and white matter and the soluble fraction of grey matter increased rapidly and markedly after birth, reaching the highest level at day-30, and declined slightly or remained unchanged thereafter. The enzyme level in the soluble fraction of white matter, in contrast, remained constant throughout the development and maturation of brain.

Various ontogenetic changes in the substrate proteins for PL-Ca-PK were also noted. The levels of MBP and other substrates (notably of M_r 87,000, 58,000, 54,000 and 50,000 in grey matter) progressively increased during development, reaching the highest levels in the adult. These proteins, termed "adult proteins", may be involved in brain maturation. The level of an M_r 66,000 protein from the particulate fraction of white and grey matter, on the other hand, increased rapidly after birth, reached a peak at day-18 and then declined to the initial neonatal level in the adult. This protein, a major substrate for PL-Ca-PK and termed "neonatal protein", may be crucial for brain development.

We have reported the presence in brain of an enzyme (tentatively named protein kinase X) which catalyzes protamine phosphorylation modulated by S-100 protein (4). An M_r 19,000 endogenous substrate protein of protein kinase X was present at a much higher level in brain from adult than neonatal rats (2-day old), a developmental change similar to that seen for the enzyme. The activity levels of phosphoprotein phosphatases (assayed using ^{32}P-labeled MBP, histone and protamine-SO_4) were found to only slightly (up to 60%) increase or decrease in certain fractions from different brain regions during development, suggesting that phosphorylation, but not dephosphorylation, may be the rate-limiting step in determining the phosphorylation state of cellular proteins. It is concluded that phospholipid/Ca^{2+}-dependent and S-100 protein-modulated phosphorylation systems and the sequential replacement of substrate proteins are involved in the development and maturation of brain.

(1) Kuo, J.F., Andersson, R.G.G., Wise, B.C., Mackerlova, L., Salomonsson, I., Brackett,N.L., Katoh, N., Shoji, M. and R.W. Wrenn. Proc. Natl. Acad. Sci. USA 77:7039-7043, 1980.
(2) Wrenn, R.W., Katoh, N., Wise, B.C. and J.F. Kuo. J. Biol. Chem. 255:12042-12046, 1980.
(3) Turner, R.S., Chou, C.-H.J., Kibler, R.F. and J.F. Kuo. J. Neurochem. 39:1397-1404, 1982.
(4) Qi, D.-F., Turner, R.S. and J.F. Kuo. J. Neurochem., in press.

1095 STRUCTURE, EXPRESSION AND EVOLUTION OF CALMODULIN GENES, A.R. Means, R. Simmen, J.P. Stein and J.A. Putkey, Dept. Cell Biology, Baylor College of Medicine, Houston, TX

Calmodulin (CaM) is a Ca^{++} receptor present in all eukaryotic cells. It is abundant in nervous tissue and has been found associated with virtually all intracellular membranes in the Purkinje cells of the cerebellum (1). The CaM gene is single copy in a variety of vertebrate organisms (2). The chicken gene CL-1 contains a 5' nontranslated region of 100 NT, the coding region for CaM and 860 NT of 3' nontranslated region (3). The coding region of CL-1 is composed of 7 exons interrupted by 6 introns. The first intron separates the ATG from the first NT of the codon which for the N-terminal amino acid (aa) of CaM (ala). Introns II, V and VI interrupt the 12 aa Ca^{++} binding subdomains I, II and IV whereas intron IV occurs in the linker region between the highly homologous N- and C-terminal halves of CaM. A second gene CM-1 has been cloned that encodes a 148 aa protein that bears an 87% sequence homology with chicken CaM (4). This gene does not contain introns but possesses a TATA box, is in open reading frame, and has a single polyadenylation signal 486 NT from the TGA. This gene is flanked both 5' and 3' by a 9 bp direct repeat. These data suggest that CM-1 was originally derived as a processed copy of CL-1. This copy, lacking introns, was inserted back into the genome. Northern analysis reveals the presence of a mRNA in several chicken tissues that hybridizes to CM-1. Such experiments are compatible with the tissue specific expression of a processed CaM gene and predict the presence of a CaM-like protein that differs in 19 of the 148 aa from CaM. A strategy was devised to construct bacterial expression vectors harboring the chicken CaM cDNA or the CM-1 gene. Properties of bacterially synthesized CaM and CM-1 protein have been compared to those of rat testis CaM. Testis CaM and bacterially synthesized CaM are identical with respect to all properties examined. The CM-1 protein is similar to CaM in that it is heat stable, can be purified on phenothiazine-Sepharose, cross-reacts in a CaM RIA and, in the presence of EGTA, migrates identically to CaM on 1D SDS gels. CM-1 protein can be distinguished from CaM in that it undergoes an aberrant Ca^{++}-dependent mobility shift on SDS gels and is more basic than CaM upon analysis by 2D gel electrophoresis. Finally CM-1 protein can be separated from CaM by chromatography on thiol-Sepharose since it contains 2 cys residues and CaM does not. This property has been utilized to demonstrate the presence of CM-1 protein in chicken breast muscle. Thus the chicken genome contains 2 CaM genes: one that encodes the CaM found in all tissues (CL-1) and one that appears to be a processed gene (CM-1) that is expressed in a tissue specific manner and encodes a novel CaM-like protein.

1. Lin, C.T., Dedman, J.R., Brinkley, B.R. and Means, A.R. (1980) J. Cell Biol. 85:473-480.
2. Lagace, L., Chandra, T., Woo, S.L.C. and Means, A.R. (1983) J. Biol. Chem. 258:1684-1688.
3. Putkey, J.A. et al. (1983) J. Biol. Chem. 258:11864-11870.
4. Stein, J.P. et al. Proc. Natl. Acad. Sci. U.S.A. 80:6485-6489.

1096 ROLE OF cAMP-DEPENDENT PROTEIN PHOSPHORYLATION IN MODULATION OF POTASSIUM CHANNELS IN *APLYSIA* NEURONS, S.A. Siegelbaum, J.S. Camardo, M.J. Shuster, and E.R. Kandel, Dept. of Pharmacology and Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, New York, NY 10032.

Serotonin (5-HT) produces a slow EPSP associated with a decrease in membrane conductance in *Aplysia* sensory neurons by closing a specific class of background K^+ channels (1). This action of 5-HT is simulated by intracellular injection of cAMP and has previously been shown to be mediated by cAMP-dependent protein kinase (cAMP P-K) (2). However, the nature of the link between protein phosphorylation and closure of the serotonin-sensitive (or "S") K^+ channels remains unknown. As a first step towards localizing the site of kinase action, we have used single channel recording techniques to study the effects of cAMP P-K on K^+ channel currents in a simplified *in vitro* system.

Single S channel currents were recorded from cell-free membrane patches in which the normal cytoplasmic surface of the membrane faces the bathing solution (inside-out patch). Purified catalytic subunit of cAMP P-K ($\sim 10^{-7}$ M) was added to the bath solution in the presence or absence of MgATP. Addition of protein kinase, in the presence of ATP, caused a significant increase in S channel closure in 68% of our experiments (n=25). The rate of kinase action, however, was slow. The mean latency from the time kinase was added to the bath to the closure of the first channel was 6.0 $\pm$ 5.2 minutes. In the absence of ATP, kinase was much less effective - causing closure in only one out of eight experiments.

The effect of protein kinase on channel activity in cell-free patches is qualitatively similar to the effects of 5-HT on S channel activity in cell-attached patches. The action of serotonin is somewhat more effective, however, causing closure in 76% of our experiments (n=33) with a mean latency of 1.7 $\pm$ 1.3 minutes.

Such quantitative discrepancies could reflect: (1) restricted access of the enzyme to the membrane in cell-free patches, (2) an effect of patch isolation on channel or membrane structure that impedes the action of protein kinase, or (3) a role of some intermediate accessory protein involved in channel closure. These possibilities are currently being investigated.

(1) Siegelbaum, S.A., Camardo, J.S. and Kandel, E.R. (1982) Nature 299, 413-417.
(2) Kandel, E.R. and Schwartz, J.H. (1982) Science 218, 433-443.

Transmitter Plasticity at the Molecular Level

1097 GENERATION OF DIVERSITY IN EXPRESSION OF GENES THAT CODE FOR NEUROENDOCRINE PEPTIDES, Edward Herbert, James Douglass, Gerard Martens, Lloyd Fricker, Michael Comb, Haim Rosen, and Olivier Civelli, Department of Chemistry, University of Oregon, Eugene OR 97403

In the part two decades a great variety of small peptides have been discovered that mediate specific behavioral responses in aminals. Many of these peptides are synthesized from large precursor proteins that contain more than one kind of neuroactive peptide. The peptides must be cleaved out of these precursors (called polyproteins) and undergo post-translational modifications to acquire activity. Use of the polyprotein mechanism for generating diverse bioactive substances is particularly evident in the expression of the opioid family of peptides. More than 16 opioid peptides have been shown to be derived from 3 polyproteins; pro-opiomelanocortin (POMC), pro-enkephalin and pro-dynorphin. These polyproteins are very similar in length, arrangement of domains of bioactive peptides and sequences flanking the bioactive domains (potential proteolytic cleavage sites). Sequencing of the genes that code for these precursors reveals a very similar arrangement of exons and introns. Sequencing of pro-enkephalin genes from human, rat and Xenopus laevis reveals a very similar organization of exons and introns in these genes. The major exon in the genes from each species codes for 7 copies of enkephalin. However, there is a very important difference. The amphibian pro-enkephalin genes (a closely related family of 3 genes), code for 7 copies of met-enkephalin whereas the human and rat genes code for 6 copies of met-enkephalin and one copy of leu-enkephalin.

Production of bioactive peptides from polyproteins involves endoproteolytic cleavage at pairs of basic amino acid residues flanking the peptides followed by trimming of basic amino acids from the peptides with carboxypeptidases. Very little is known about these enzymes. We have attempted to identify mRNA species that code for processing enzymes by using recombinant DNA techniques. We have used genomic clones that code for a family of proteolytic enzymes known as kallikreins (kindly provided by John Shine). The kallikrein enzymes are known to be involved in processing of growth factors. We have been able to identify a cDNA clone (a cDNA library) that codes for a kallikrein enzyme in AtT-20-D_{16v} cells (mouse pituitary tumor cells that produce large quantitites of pro-opiomelanocortin). Northern blot analysis reveals that the mRNA that codes for the species of kallikrein is present at very high levels in these cells (20% the level of POMC mRNA). Furthermore, sequencing of a number of cDNA clones in the library indicates that there is only one species of kallikrein enzyme in these cells. A carboxypeptidase enzyme has also been identified in AtT-20-D_{16v} cells and its relationship to processing of POMC peptides is being studied.

1098 REGULATION OF NEUROPEPTIDE BIOSYNTHESIS IN THE CENTRAL NERVOUS SYSTEM, Jeffrey F. McKelvy, Jeffrey D. White, James E. Krause, Kim Stewart and Norbert Kremer, Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794

Peptide secreting neurons are widely distributed throughout nervous systems of both vertebrates and invertebrates and are increasingly perceived to be of importance to neural information processing. Because neuropeptides are encoded in polyprotein precursors, possibilities exist for the generation of diverse chemical signals for a given precursor by differential protein processing and by RNA splicing. Such diversity may contribute to the complexity of chemical signalling in nervous systems and to transmitter plasticity at the molecular level. We have developed approaches to the study of the levels of regulation of neuropeptide biosynthesis in defined neuronal projection systems in rat brain. This involves the following: (a) in vivo isotopic labeling studies of the neuropeptides by stereotaxic cannulation of sites of cell bodies of origin of a neuropeptide projection of interest; (b) measurement of mRNA levels in individual projection nuclei by cell-free translation and by hybridization to cDNA probes and (c) determination of peptide species released in vitro after in vivo labeling. Systems which have been subjected to study in this way and which will be discussed, include the projection of oxytocin, vasopressin, enkephalin, dynorphin and somatostatin neurons from the paraventricular nucleus of the hypothalamus; the projection of proopiomelanocortin neurons from the arcuate nucleus of the hypothalamus and the striatonigral projection of Substance P neurons.

1099 PLASTICITY OF NEUROTRANSMITTER PHENOTYPIC CHARACTERISTICS, Ira B. Black, Joshua E. Adler, Cheryl F. Dreyfus, G. Miller Jonakait, David M. Katz and Keith A. Markey, Division of Developmental Neurology, Cornell University Medical College, New York, New York 10021.

A variety of neuronal systems exhibit striking plasticity in the expression and metabolism of transmitter characteristics throughout life. For example, during development a population of cells in the embryonic rat gut transiently expresses tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, and dopamine-β-hydroxylase, which converts dopamine to norepinephrine. Although these characters are lost by E14.0 (day of gestation), the high-affinity uptake system for catecholamines (CA's) persists. Consequently, these neuronal precursors are capable of differentially regulating different phenotypic characters associated with the catecholaminergic phenotype. Transient CA expression also occurs in cranial sensory ganglia of the embryo, TH appearing in trigeminal bipolar cells and nodose unipolar cells. Since some trigeminal neurons are derived from the neural crest, while nodose neurons are placodal derivatives, populations differing embryologically, anatomically and functionally transiently express transmitter traits during development. Moreover, in the neonatal rat, sympathetic neurons may also express the putative peptide transmitter substance P (SP), in addition to CA traits. The expression of SP appears to be regulated, at least in part, by trans-synaptic nerve impulse activity. Additionally, we have detected TH and CA's in heretofore unrecognized populations of the adult rat. Primary sensory neurons of the nodose and petrosal ganglia express these traits. Neurotransmitter plasticity is not restricted to the peripheral nervous system: we have recently succeeded in culturing the pontine nucleus locus coeruleus, and have been able to manipulate transmitter characters with depolarizing agents. The implications of these observations for the regulation of neurotransmitter phenotypic expression will be discussed.

Organizational Principles in the Development, Structure and Behavior of Neural Systems

1100 MOLECULAR MECHANISM OF AVIAN NEURAL CREST MIGRATION AND LOCALIZATION, Jean-Paul Thiéry*, Michel Vincent°, Gordon C. Tucker* and Hirohiko Aoyama*.
*Institut d'Embryologie CNRS et Collège de France, 49bis Av. de la Belle Gabrielle 94130 NOGENT sur Marne, FRANCE and °Centre Hospitalier de l'Université Laval, Ste Foy, Québec, CANADA. During early vertebrate embryogenesis, the neural crest forms as a transient structure which lies at the dorsal aspect of the neural tube. Cells of the neural crest soon emigrate in different directions, before giving rise to most of the peripheral nervous system, melanocytes, some endocrine and paraendocrine cells, and many craniofacial elements(1). Using antibodies to fibronectin, an ubiquitous basement membrane component of embryonic tissues and a monoclonal antibody specific for crest cells, migration pathways were reconstructed from serially sectioned embryos(2,3). At each level of the neural axis, crest cells encounter discrete but transient pathways which lead them to their site of final localization. Most, if not all, crest cells migrate along or between fibronectin-rich basement membranes of adjacent tissues. Both physical obstacles and the local milieu were found to play a critical role in the arrest of crest cells. In vitro, crest cells bind much more efficiently to fibronectin or to fibronectin containing extracellular matrices than to collagen type I or to laminin. Crest cells also migrate preferentially in fibronectin rich areas. Monovalent antibodies to fibronectin inhibit crest cell adhesion and migration on fibronectin coated substrata (4). A decrease in the binding to fibronectin was observed concommitantly with an increased adhesivity to laminin while crest cells formed two and three dimensional aggregates. The neural cell adhesion molecule (N-CAM) is directly involved in their calcium independant process of adhesion. In vivo, both N-CAM and the liver cell adhesion molecule (L-CAM) are detected in the blastoderm prior to gastrulation(5). During early stages of neurulation, L-CAM is lost from the presumptive territory of crest cells. L-CAM is then restricted to the ectoderm while the neural epithelium including crest cells contains increasing amounts of N-CAM. During migration, N-CAM disappears transiently from the surface of crest cells, and reappears at the onset of aggregation into ganglion rudiments. A model for the oriented migration, specific localization and aggregation of crest cells will be discussed(6) particularly with respect to the cell surface modulation hypothesis(7).

1. Le Douarin, N.M. (1982) The neural crest. Cambridge University Press.
2. Thiéry, J-P. et al (1982) Develop. Biol. 93 324-343
3. Vincent, M. and Thiéry,J-P. (1983) Develop. Brain Res. 9 235-238
4. Rovasio, R.A. et al (1983) J. Cell. Biol. 96 462-473
5. Edelman, G.M.et al (1983) Proc.Natl.Acad.Sci. 80 4384-4388
6. Thiéry, J-P. et al (1982) Proc.Natl.Acad.Sci. 79 6737-6741
7. Edelman, G.M. (1983) Science 219 450-457

1101 GUIDANCE OF NEURONAL GROWTH CONES DURING EMBRYONIC DEVELOPMENT, Corey S. Goodman, Michael J. Bastiani, Sascha du Lac, John B. Thomas, Department of Biological Sciences, Stanford University, Stanford, CA 94305

During embryonic development, neurons find other neurons or muscle cells in a remarkably precise way, often traveling long distances along stereotyped routes that involve a series of cell-specific choices. Recent evidence in the grasshopper embryo suggests that growth cones are guided by selective adhesion of their filopodia to particular cell surfaces. We have focused on the cell interactions mediating specific growth cone choices at two different stages of development: (i) the specific choices made by the first growth cones in the CNS as they fasciculate into three distinct axon bundles; and (ii) the specific choice made by the G growth cone when later in development it is confronted with a scaffold of axon bundles and selectively fasciculates upon the A/P axon bundle. Although numerous axon bundles are within filopodial grasp, each of these growth cones, according to its lineage and previous interactions, selectively fasciculates with a particular bundle of axons. Extensive observations of individual growth cones, and ablations of particular neurons, has confirmed the notion that individual neurons have a high affinity for small subsets of other neurons within their filopodial environment. Our results in the grasshopper embryo suggest that growth cone guidance is likely to involve many different molecular specificities that mediate the differential adhesion of growth cones and their filopodia to particular cell surfaces. Furthermore, monoclonal antibodies have revealed cell surface antigens whose temporal and spatial distribution in the embryo correlate with the predictions of the cellular studies, namely, neurons whose axons fasciculate together share common surface antigens. In order to isolate and characterize the surface molecules implicated by our cellular and immunological studies using the grasshopper embryo, we have begun studying growth cone guidance in the CNS of the Drosophila embryo. Fortunately, our recent cellular studies show that the embryonic development of the fly CNS is identical, albeit in a miniature form, to the hopper CNS in terms of the identified neurons and their selective fasciculation. We have begun using a variety of molecular genetic and immunological approaches in the fly in an attempt to isolate and study genes expressed in small subsets of embryonic neurons during these stages.

1102 DYNAMIC SYNAPTIC INTERACTIONS IN THE FORMATION OF A RETINOTOPIC MAP, M.Constantine-Paton and Thomas A. Reh, Dept. of Biology,Princeton University Princeton NJ 08544.

Recent studies in our laboratory using the developing visual projection of the leopard frog Rana pipiens have shown that axon terminals from the ganglion cells of the retina continuously change the tectal neurons with which they are in functional synaptic contact. Throughout this process the projection continues to exhibit a properly aligned map of the retinal surface and a highly refined ordering of the retinal terminal array.

Mechanisms involved in maintaining this dynamic map have been explored using animals in which a third eye primordia is implanted in the presumptive forebrain region of the early embryo. These animals develop with two retinas innervating one tectal lobe. Both retinas form normally aligned maps in the tectal lobe but each map is fractionated into a periodic series of alternating eye-specific stripes in a pattern of afferent segregation reminiscent of that in visual cortex. This competitive exclusion has a component that is dependent on the relative topographic positions of the retinal axons and the tectal cells as evidenced by the fact that partial projections from a third retina can only form stripes in the appropriate region of the host tectum. However the continued maintenance of eye-specific stripes is dependent on action potential activity in the retinal axons. Blockage of the voltage dependent sodium channel in retinal axons by continuous infusion of tetrodotoxin either onto the optic nerves or into the posterior eye chamber for 4 weeks results in the complete overlap of the two doubly innervating retinal projections. Quite similar effects also occur following intravitreous injection of kainic acid. Neither of these procedures affects ganglion cell numbers or morphology. We conclude that a minimum of two independent sets of interactions underly the establishment and maintenance of the topographic projections between the frog's eyes and its tectal lobes. One involves some form of positional matching between regions of the retina and the developing midbrain. This aligns the normal projection. However position matching must utilize gradients rather than absolute pre- to post-synaptic interactions, otherwise synaptic associations could never change throughout development. A second, activity-dependent mechanism probably serves, during normal development, to maintain the relative ordering of moving axon terminals whose cell bodies are neighbors in the retina. When two retinas coinnervate one tectal lobe the activity-dependent mechanism tends to maximize the number of contacts that are made adjacent to contacts from a neighboring ganglion cell while the positional matching mechanism tends to spread each retinal projection throughout the doubly innervated tectal lobe. The resulting periodically alternating stripes represent the best possible solution to the two mechanistic requirements. The wide distribution of striped termination zones in the vertebrate brain suggests that similar mechanisms are common to all topographic projections.

1103 NEURONAL SYSTEMS AND BEHAVIOR, Masakazu Konishi, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

The brain evolved to control behavior. Its design and operating principles for the control of behavior are embodied in the modes of connections and the means of communication between neurons. Of the many forms of signal used by the brain, action potentials are a rapid means of signalling between distant neurons. The tenet of single-unit neurophysiology states that recording action potentials from a single neuron at a time should uncover the brain's operating rules. This tenet is overshadowed today by the view that simultaneous recording of many neurons are necessary for the study of those rules. Each of these views makes certain assumptions about the encoding of information by the brain. I shall use the behavioral and neurophysiological study of sound localization in the owl as an example to illustrate how the single-unit approach can address the problems of the neural codes.

The owl uses interaural phase and intensity differences to localize sound, respectively, in azimuth and in elevation. These two acoustic cues are processed by two parallel neural pathways. Each of the owl's auditory nerve fibers carries both phase and intensity codes to the cochlear nucleus, which consists of two divisions: the phase code is channelled via one type of synapse to one division and the intensity code via another type of synapse to the other division. Each division sends its code to a series of binaural stations in which neurons are sensitive either to interaural phase or intensity differences but not to both. The two pathways converge in the inferior colliculus where certain neurons (space-specific neurons) are sensitive to a particular combination of the two interaural cues, which codes for a specific location in the owl's auditory space.

Role of Cytoskeleton

1104 TRANSPORT OF STRUCTURAL GENE PRODUCTS SPECIFIES REGIONAL DIFFERENCES IN NERVE CELLS
Monica M. Oblinger, Case Western Reserve University, Cleveland, OH 44106

Aspects of structure, function and biochemical properties of the cytoskeleton in a neuron's various peripheral extensions can differ. The proteins comprising the cytoskeleton in processes such as axons and dendrites are synthesized in the cell body and subsequently transported into these peripheral regions. Therefore, selective regulation of synthesis and the selective routing of cytoskeletal elements into a neuron's extensions plays a central role in generating the anisotropy found in a neuron. In addition, since cytoskeletal elements in axons are dynamic structures that exhibit continual flux through axons from the soma to the terminal regions of the axon, other factors can participate in the regulation of the axonal cytoskeleton after its synthesis and export.

The dorsal root ganglion cell is well suited as a model for studying various parameters involved in the generation of regionally differentiated cytoskeletons since it has two functionally distinct axons that can be separately analyzed. Both structural and dynamic aspects of the cytoskeleton of the peripheral and central branch axon of the DRG cell differ. For example, the amount of cytoskeletal proteins and their rate of transport in the peripheral axonal branch of this cell is much greater than in the central branch. Studies of cytoskeletal transport in the DRG cell have suggested that the cytoskeletal networks of the peripheral and central axon may be separately organized in the neuron. This issue has been examined by stimulating the DRG cell by axotomizing one of its two axonal branches. The neuron responds by selectively altering the cytoskeletal proteins it synthesizes and exports, reducing its synthesis and export of neurofilament protein and increasing tubulin and actin. However, the changes in cytoskeletal output are not equally expressed in both axons, suggesting that the cytoskeletal networks of each axonal branch can be selectively regulated at the level of the cell body. Such regulation has a functional correlate since the regenerative properties of the two axons of the DRG cell are also selectively affected.

1105 TOPOGENESIS OF DISTINCT MEMBRANE-CYTOSKELETAL DOMAINS IN NEURONS: SPECTRIN AS A MODEL SYSTEM, E. Lazarides, W. J. Nelson and T. Kasamatsu, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The chicken cerebellum expresses a polypeptide antigenically and biochemically related to the α subunit of spectrin, an erythrocyte membrane-cytoskeletal protein. Most of this polypeptide is associated with a brain specific spectrin subunit, γ-spectrin, and is localized in virtually all neuronal cell bodies and processes. Cerebellum also expresses polypeptides antigenically related to the β subunits of erythrocyte spectrin and these are also found in association with cerebellar α-spectrin but are confined to the plasmalemma of the neuronal cell bodies. This suggests that there is a mechanism for segregating different spectrin complexes into distinct membrane domains within a single cell. The developing chicken cerebellum contains also two forms of the plasma membrane-associated actin-binding protein spectrin. The brain form, αγ-spectrin (fodrin), is expressed constitutively in all neuronal cell bodies and processes during all stages of cerebellar morphogenesis. On the other hand, the erythrocyte form, αβ'β-spectrin, accumulates exclusively at the plasma membrane of the cell bodies of Purkinje and granule cells and of neurons in cerebellar nuclei, but only after these cells have become postmitotic and have completed their migration to their final positions in the cerebellum. The appearance of αβ'β-spectrin coincides temporally with the establishment of axosomatic contacts on these three neuronal cell types, which suggests that αβ'β-spectrin accumulates in response to the formation of functional synaptic connections during cerebellar ontogeny. Similarly, the chicken optic system contains the brain-specific form of spectrin (αγ-spectrin or fodrin) as a major membrane-associated, axonally transported cytoskeletal protein. The chicken optic system also contains the erythrocyte-specific form of spectrin (αβ'β-spectrin), which has a more restricted distribution; it is confined to the plasma membrane of dendrites and cell bodies of retinal ganglion cells, is absent from the optic nerve fibers, and is not axonally transported from the retina into the optic nerve. During development of the optic system, the expression of αγ-spectrin is constitutive in all cell types. On the other hand, the accumulation of αβ'β-spectrin is detected in only the ganglion cells, and at a time in development which coincides with the phase of synaptogenesis. These results indicate the existence of a developmentally regulated mechanism which topologically segregates the erythroid and brain forms of spectrin from each other and the former from axonal transport, and suggest that erythroid spectrin may be involved in establishing restricted membrane-cytoskeletal domains in neurons during synaptogenesis, and maintaining them in the adult cell.

Probing Nervous System Molecules: Growth Factors, Neuropeptides, Channels, Cell Markers

1106 **A SIMPLE MICROTECHNIQUE FOR THE RAPID ISOLATION OF TISSUE RNAS: USE IN THE QUANTITATION OF SPECIFIC MESSENGER RNAS IN HUMAN BRAIN TISSUE AND BIOPSY SPECIMENS,** **Marcelle R. Morrison, W. Sue T. Griffin, and Scott Jamison, University of Texas Health Science Center at Dallas, Dallas, TX, 75235.**

We have used tubulin and actin recombinant DNA probes to quantitate these specific mRNA levels in different brain areas of rat and human. In both cortex and cerebellum, tubulin and actin mRNAs decrease during development, although the time course of these changes is unique for each brain area. Rapid assay of mRNA levels in a large number of samples has been limited by the lack of appropriate isolation techniques as traditional methods of isolating undegraded RNA require large amounts of tissue and are both lengthy and difficult. In order to determine specific mRNA levels in discrete brain nuclei and cell populations, we have developed a technique for rapidly isolating cytoplasmic RNAs from 1-5 mg of tissue, using a needle punch biopsy procedure. A large number of samples can thus be processed simultaneously. Briefly, tissue is homogenized, nuclei pelleted and the cytoplasm extracted with phenol then chloroform. The RNA concentration is determined by ethidium bromide staining of 1 µl aliquots and specific mRNA levels are determined by slot blot hybridization of the cytoplasmic RNAs to [^{32}P] nick-translated DNA probes. This technique gives results quantitatively similar to those obtained using conventional RNA isolation techniques. We are isolating RNA from micropunches of specific areas of developing cerebellum in order to compare relative tubulin and actin mRNA levels with those obtained by our in situ hybridization analyses. We are also using this technique to compare mRNA levels and to identify possible viral etiologies in various neurological disorders.

1107 IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF NOVEL INSULIN-LIKE SEQUENCES FROM FETAL BRAIN RNA. Lydia Villa-Komaroff, Song Hou-Yan, Antonio Gonzalez, Paul Dobner, David Shine and Bruce Wentworth, Univ. Mass. Medical Center, Worcester, MA 01605.

We have used cloned sequences encoding rat and human insulin to look for sequences homologous to insulin in RNA from human and mouse fetal brain. We have detected several RNAs from human and mouse fetal brain with substantial homology to sequences encoding insulin. These sequences appear to be brain specific and in the mouse are expressed during late fetal development. We enriched for these sequences by attaching the cloned insulin sequence to cellulose and hybridizing the DNA to total polyA$^+$ RNA from E17 mouse brains. The RNA which hybridized to the cloned DNA was eluted and used as template for the synthesis of double-stranded cDNA. The ds-cDNA was inserted into the Pst 1 site of the plasmid vector and used to transform E. coli strain JM83. Using colony hybridization, we have identified several colonies which contain plasmids which hybridize to insulin coding sequences. Analysis by restriction enzymes indicates that at least 2 distinct sequences are present. DNA sequence analysis indicates that one of these sequences encodes a novel insulin-like sequence with substantial homology to the B peptide of insulin. We are analyzing the genomic sequences corresponding to these DNA and studying the expression of these sequences during brain development.

1108 NERVE GROWTH FACTOR FROM MASTOMYS NATALENSIS, AN AFRICAN RAT WITH HIGH LEVELS OF ALPHA AND BETA SUBUNITS, Margaret Fahnestock, Thomas L. Darling, and Eric M. Shooter, Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

Nerve growth factor (NGF) from the submaxillary gland of the male mouse is a 7S complex consisting of three subunits: the β-NGF dimer which contains the nerve growth promoting activity; γ, a trypsin-like enzyme implicated in the biosynthesis of the β subunit; and α, a protein with over 90% homology to γ but with no detectable enzymatic activity and of unknown function. Mastomys natalensis is an African rat with high levels of NGF in both male and female submaxillary glands. However, the NGF isolated from this source is a 5.1S complex which contains α and β but no γ subunits. The absence of γ subunits in Mastomys suggests this animal might contain an altered α or β subunit.

A cDNA clone of an α subunit from the mouse has previously been isolated in this laboratory. Southern analysis using this probe to compare mouse and Mastomys genomic DNA shows hybridization to a single band in both EcoRl and BamHl digests of both samples; furthermore, both the strengths of the hybridization signals and the molecular weights of the bands are the same in both species, indicating a high degree of similarity between the two. Northern analysis using both cloned α and β cDNAs as probes also confirms the similarity, since hybridization patterns are the same between mouse and Mastomys. The mouse clones are currently being used as probes in obtaining clones coding for the Mastomys α and β subunits.

1109 IN SITU mRNA HYBRIDIZATION AND IMMUNOCHEMICAL LOCALIZATION OF SOMATOSTATIN IN RAT HYPOTHALAMUS. Frank Baldino, Jr., Gerald A. Higgins, René Arentzen, Yuan Lin, Betty Wolfson, Robert W. Manning, Leonard G. Davis. E.I. du Pont de Nemours, Central Res. and Develop. Dept., Glenolden, PA.

The distribution of somatostatin mRNA within the peri- and paraventricular hypothalamus of rats was assessed using in situ hybridization techniques with synthetic oligonucleotide probes. Synthetic oligonucleotides (22 and 24 mers) were synthesized on a Model 380A DNA synthesizer (ABI) and 5' end labeled using T_4 polynucleotide kinase and ^{32}P ATP. The hybrid probes were either used individually or after ligation to a 46 mer with T_4 DNA ligase. Animals were perfused with 4% paraformaldehyde and serial tissue sections (<10 μm) were taken from the level of the preoptic-anterior hypothalamus through the paraventricular hypothalamus. Somatostatin-immunoreactive neurons were identified using standard immunocytochemical procedures with anti-somatostatin (Immunonuclear). In situ hybridizations were conducted between 4° and 37°C in hybridization buffer. Our results with these oligonucleotide probes have shown that the peri- and paraventricular hypothalamus is particularly rich in transcripts coding for somatostatin. Our immunocytochemical results have shown that somatostatin-like immunoreactive neurons are co-distributed regionally with somatostatin mRNA. Current work in our laboratory is addressing co-localization at the cellular level.

1110 DETECTION OF mRNAs CONTAINING REGULATORY PEPTIDE SEQUENCES USING SYNTHETIC OLIGODEOXYNUCLEOTIDES. Illana Gozes, Mordechai Bodner, Hasia Shwartz, Yael Shani, Clive Dutlow and Mati Fridkin, The Weizmann Institute of Science, 76100 Rehovot, Israel.
To understand the regulation of peptide hormone production, it is vital to identify their biosynthetic pathways. We chose to study two major regulatory peptides, vasoactive intestinal peptide (VIP) and gonadotropin-releasing hormone (GnRH), two peptides of both neurotransmitter and neurohormone actions. To identify the respective peptide mRNA we are using, as hybridization probes, synthetic oligodeoxynucleotides with relatively unambiguous nucleotide sequence complementary to the predicted peptide mRNA sequence. These probes are synthesized, using the deoxynucleoside phosphoramidite approach, to a length of 17 bases, and contain all the possible DNA sequences according to the genetic code. These specific probes are then radioactively labelled using the reaction catalyzed by the enzyme polynucleotide kinase and afterwards hybridized to mRNA, which had been resolved on denaturing agarose gels. Employing this approach, we discovered an as yet unknown enriched source for VIP production, namely, a human buccal tumor. This enabled the successful identification and partial purification of a mRNA (~1600 bases long) containing VIP sequences which can be translated in vitro into VIP immunoreactive peptides. The mRNA which was characterized was detected in brain as well, but was found to differ in size from a known VIP-mRNA identified in human neuroblastoma cells, suggesting the possibility of different VIP-mRNAs in different cell types. As for GnRH, we have tentatively identified a single mRNA band that hybridizes to a synthetic oligodeoxynucleotide specific for this peptide. This mRNA was also detected in the human buccal tumor, suggesting the possible occurrence of VIP and GnRH in the same cell.

1111 THE NORMAL CELLULAR ONCOGENE PP60^{c-src} IS DEVELOPMENTALLY REGULATED IN THE NEURAL RETINA, Patricia F. Maness, Laurie K.Sorge, and Barcey T. Levy, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

We have localized the normal cellular oncogene product pp60^{c-src} in the developing chick neural retina by immunocytochemical staining using antisera raised against bacterially expressed pp60^{v-src}, the src gene product of Rous sarcoma virus. pp60^{c-src} was expressed in developing retinal neurons at the onset of differentiation. Expression of pp60^{c-src} persisted in mature neuronal cells that were postmitotic, fully differentiated, and functional. pp60^{c-src} immunoreactivity was localized within nerve processes and cell bodies of ganglion layer neurons, processes of rods and cones, and in some but not all neurons of the inner nuclear layer of the retina. Protein kinase assays and Western transfer analyses identified the immunoreactive protein as pp60^{c-src}, and confirmed that its expression occurs at the time the first neuronal cells in the retina differentiate. We conclude from these studies that pp60^{c-src} is the product of a developmentally regulated gene that is more important in neuronal cell differentiation or mature function than in cell proliferation.

1112 SUBCELLULAR LOCALIZATION OF PP60SRC IN ADULT RAT BRAIN. Wendy B. Macklin, Gail M. Clinton and Tina Q. Tan, Louisiana State University Medical Center, New Orleans, LA 70112.

It has been demonstrated that pp60src, the normal cellular homolog of the transforming protein of Rous sarcoma virus, is elevated in the chicken nervous system. The present studies were undertaken to investigate whether pp60src is also elevated in the rat nervous system and, if so, when during development pp60src activity is most elevated and, more importantly, where in the nervous system pp60src is localized. The pp60src activity was measured using src - specific IgG as a substrate for phosphorylation. The pp60src is a tyrosine kinase which phosphorylates src - specific IgG only in tyrosine. The data indicated that pp60src activity was elevated in rat brain relative to rat spleen and in adult rat brain relative to 10 day rat brain. In subcellular fractionation studies, low pp60src activity was found in synaptosomes, axolemma and axons, indicating the pp60src is not enriched in neurons. The pp60src activity was most enriched in a fraction that initially purified with myelin but which was not part of compact myelin since it was removed from myelin by osmotic shock. The pp60src enriched fraction was isolated by sedimentation at 100,000 xg x 60 minutes of the membranes released from myelin by osmotic shock. These data suggest that pp60src is enriched in a unique brain membrane and is not evenly distributed amongst all brain cells. It may be localized in a specialized region of myelin which is lost by classical myelin preparation procedures or it might be localized in a nonmyelin membrane which initially copurifies with myelin. Studies are currently in progress to distinguish these possibilities. Supported by PHS Grants #NS18732 and CA34517.

1113 Detection of a low-abundance mRNA for a membrane protein associated with intercellular channels using synthetic oligonucleotides.Hope J, Zervos A Smith AS & Evans WH. NIMR, Mill Hill, LONDON, ENGLAND.
Gap junctions, intercellular communication channels, are formed by protein sub-units and are found in most tissues but the major polypeptide of hepatocyte channels is the only proteinfor which a partial amino-acid sequence is available (Nicholson et al. PNAS 78 7594-7598). Only one of two 11-base-long oligonucleotides (11'mers) can be complementary in sequence to the mRNA structure predicted for the amino-terminal tetrapeptide of this protein while a mixture of 64 17'mers is needed to ensure that one of them is complementary to the predicted mRNA coding for amino-acid residues 43-48 of this protein.
We have used these 32P-labelled oligonucleotides as hybridisation probes to screen for complementary sequences in mRNA purified from various rat tissues and covalently bound to diazothiophenol paper. Heart, liver and pregnant uterus contain more hybridisation-positive mRNA than other tissues, and paradoxically the 11'mers appeared better for this screening procedure than the 17'mers. This methodology provides a general means of assay for specific mRNA provided such oligonucleotides are available and in this specific case facilitates enrichment of mRNA populations in mRNA coding for the Mr28000 gap junction polypeptide prior to cDNA cloning.

1114 Molecular Analysis of the Shaker gene complex in Drosophila melanogaster, Alexander Kamb and Linda Iverson, Caltech, Pasadena, CA 91125
The focus of our research is on the Shaker (Sh) gene complex of Drosophila melanogaster. Electrophysiological and genetic evidence indicates that Sh mutants have altered K^+ conductance and suggest that Sh may encode at least one class of K^+ channels. Using a cDNA probe that hybridizes to the Sh region we have isolated several genomic DNA clones. These clones have been mapped using restriction enzymes and form an overlapping set that contains approximately 30-40 kb of genomic DNA sequences. The cloned region is being extended using the technique of chromosome walking. Genomic DNA clones from one Sh mutant, Sh^{KS133}, have also been isolated. Because Sh^{KS133} is a dominant mutation, these Sh^{KS133} clones are being used to transform wild-type flies to Sh phenotype. These experiments are being performed using the technique of P-element mediated transformation. Successful transformation will provide unequivocal identification of the sequences that constitute the Sh gene(s). As a second approach towards identifying the Sh gene we are currently mapping several X-ray induced Sh mutations using the techniques of in situ hybridization and Southern analysis. The results of these experiments indicate that at least two of these mutations are contained within the cloned region. The sites of mRNA transcription have been identified in normal flies by RNA blot hybridization and a similar analysis of Sh mutant transcripts is in progress.

1115 MOLECULAR GENETIC ANALYSIS OF A MUTATION IN DROSOPHILA WITH ALTERED POTASSIUM CURRENTS, Barry Ganetzky, University of Wisconsin, Madison, Wis. 53706 and Chun-Fang Wu, University of Iowa, Iowa City, Iowa 52242
Mutations of the eag locus in Drosophila alter the electrical excitability of axons, neuromuscular junctions, and muscles. Voltage-clamp analysis of larval muscles fibers demonstrate that eag specifically disrupts potassium currents. Different mutant alleles preferentially affect either the fast transient K^+ current (I_A) or the delayed rectification K^+ current (I_K). Abnormalities in current amplitude and in the instantaneous current-voltage (I-V) relationship have been found in the eag alleles examined. These results suggest that eag^+ takes part in controlling the synthesis of two types of potassium channels and might even represent a structural gene for a component shared by these channels.
To facilitate the molecular analysis of this gene, its product, and its role in membrane excitability it is desirable to obtain DNA clones of this locus. For this purpose, we have used the phenomenon of hybrid dysgenesis to generate eag mutations resulting from the insertion of a transposable element (the p factor) into the gene thus providing a molecular tag. Over 15 candidates have been isolated from appropriate crosses and confirmed as eag mutations by genetic and physiological criteria. That some of these mutations involve the insertion of a p factor is suggested by their high frequency of occurrence and back mutation to normal under the influence of hybrid dysgenesis and by the appearance of a p factor in the appropriate salivary chromosome region. If the nature of these mutations is confirmed by subsequent analysis, they will provide the material to isolate the eag gene and to correlate properties of its product with specific sites of mutation within the gene.

1116 VARIABILITY IN THE SOMA CURRENTS OF IDENTIFIED MOLLUSCAN NEURONS, Elba Elisa Serrano, Department of Biological Sciences, Stanford University, Stanford, CA 94305.
Identified neurons from the brain of Archidoris montereyensis and Anisodoris nobilis were isolated from their ganglia and voltage clamped with a two microelectrode voltage clamp at 11°C. Variability in I_A parameters was measured by comparing data from the same cell in different animals. Comparison of the means between cell types was then possible. I_A was isolated with voltage paradigms in the absence of pharmacological blocks. Some experiments focused on other outward currents under voltage clamp. Experiments were designed to permit comparison between identified Archidoris neurons of: (a) magnitude of I_A during a standard pulse paradigm (b) current density (c) steady state voltage dependence of activation and inactivation of I_A (d) kinetics of I_A (e) total outward current during a set pulse to Ø mV. In Archidoris, the biophysical properties of the ionic conductance I_A are highly conserved within cell types. For any given cell type, the steady state voltage dependence and time course of inactivation varied by 10% about the mean or less. The steady state voltage dependence of activation and inactivation did not vary significantly between cell types. The primary difference between cell types resided in the time course of conductance changes and the amount of I_A (current density). The data indicate that cells also vary in the relative contribution of different ionic currents to the total current. The time course and voltage dependence of I_A are similar in Archidoris and Anisodoris; the species differed on the basis of current magnitude. On the average, I_A current density and peak outward current density were significantly larger in Anisodoris than in Archidoris.

1117 SINGLE CHANNEL PROPERTIES OF A VOLTAGE-ACTIVATED POTASSIUM CONDUCTANCE IN CULTURED HIPPOCAMPAL NEURONS, Michael A. Rogawski, Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bldg. 36, Room 2C-02, Bethesda, MD 20205.
Single-channel currents were recorded from cell-attached membrane patches of dissociated rat hippocampal neurons. The cells were grown in culture for 4 days to 3 weeks. Gigaseal recordings were made at room temperature in Hank's balanced salt solution containing 2-4 mM Ca^{2+}, 2-4 mM Mg^{2+}, 0.2 mM Cd^{2+} and 1-3 μM tetrodotoxin using fire-polished, Sylgard-coated pipettes. Infrequent channel openings were observed with the patch pipette at bath potential. However, upon step depolarization of the patch by 10 or more millivolts, outward-directed elementary currents of about 1 pA amplitude were always observed. The conductance of these single channels was 35 pS and the extrapolated reversal potential was about 5-10 mV negative to resting potential. The probability of channel opening reached a plateau 25 msec following the onset of the voltage command. The open times of the channels were exponentially distributed with a mean of 30 msec. Often the individual channel currents flickered rapidly many times before closing. When repeated voltage steps were applied at a frequency of 0.3-1 Hz, there were usually prolonged pauses during which no channel openings occurred. The channels do not seem to be activated by Ca^{2+} entry as they were recorded with patch pipettes filled with Ca^{2+}-free medium containing EGTA (75 μM). The single channel currents were absent when the cells were exposed to tetraethylammonium (20 mM). This evidence suggests that these channels represent a voltage-sensitive potassium conductance, possibly the delayed rectifier.

1118 IMMUNOCYTOCHEMICAL LOCALIZATION OF A SYNAPTIC VESICLE-ASSOCIATED ANTIGEN IN THE DEVELOPING RAT RETINA. P. Vijay Sarthy and William Bacon, Department of Ophthalmology University of Washington, Seattle, Washington 98195

In order to examine the appearance of synaptic vesicles and to correlate it with the formation of the synaptic layers, we have determined the staining pattern of a murine monoclonal antibody (Serum 48) to a synaptic vesicle-associated protein (Matthew and Reichardt, J. Cell Biol. 91: 257, 1981) in developing rat retina. The antigen was detected by the indirect immunofluorescence technique of Coons, using 16 μm cryostat sections of paraformaldehyde (4%)-fixed retinas. In the adult rat retina, the antiserum stained both the outer plexiform (OPL) and the inner plexiform layers (IPL). The nuclear layers and the nerve fiber layer (NFL) were devoid of any staining. In order to establish the association of the antigen with synapses, we examined localization of the antigen in a 70 day-old RCS-rdy rat retina in which the OPL is lost due to degeneration of the photoreceptors. We found no staining in the outer retina although staining in the IPL was unaffected. In fetal animals, E-15 and later, two bands of staining were observed in the inner retina. These bands corresponded to the IPL and NFL, respectively. This pattern of labeling persisted until postnatal day-8, when staining of the NFL was lost, and only IPL was labeled in the inner retina. In the outer retina, a band of fluorescence, corresponding to the OPL, was first seen in postnatal day-4 retina, and the intensity of the band increased until day-9. As expected from the developmental pattern of retina, all the bands appeared in the central retina first and subsequently in the peripheral retina.
Supported by NIH grants EY-03664 and EY-01730.

1119 E/C8-MONOCLONAL ANTIBODY AS A NEURONAL CELL LINEAGE-SPECIFIC MARKER, Gary Ciment and James A. Weston, University of Oregon, Eugene, OR 97403

The vertebrate neural crest gives rise to a wide variety of cell types, including neurons and glial cells of the peripheral nervous system, glandular tissues, pigment cells, and some connective tissue. One likely hypothesis to explain the generation of such diversity is that a sequence of restrictions in developmental potentiality occurs during crest development, defining the lineage of a particular derivative. One prediction of this hypothesis is that cells exist as "intermediates" in the lineage, defined by their ability (a) to proliferate, (b) to give rise to some crest derivatives, but (c) NOT to others.

We have produced a monoclonal antibody ("E/C8") which binds to neurons, a subpopulation of cultured neural crest cells, as well as the crest-derived mesenchymal cells of the posterior branchial arches. The E/C8-positive cells of the branchial arches are highly proliferative in vivo and express other neuronal markers, such as the A2B5-antigen, in culture. Moreover, using various in vivo tests of developmental potentiality, we have found that these cells can give rise to neurons, as well as glial cells and glandular tissue--but not to melanocytes.

Thus, mesenchymal cells of the branchial arches appear to be a partially-restricted population of neural crest-derived cells, and may represent an intermediate cell type in the crest lineage leading to neurogenesis.

1120 MONOCLONAL ANTIBODIES SPECIFIC FOR GLIA IN THE DEVELOPING CHICK NERVOUS SYSTEM, Vance Lemmon, University of Pittsburgh, Pittsburgh PA 15261

Two monoclonal antibodies that bind to glia in the chick have been developed. Mice were immunized with primary cultures of retina enriched for "flat cells" that are thought to be derived from Muller cells. Hybridoma supernatants were screened using an immunohistochemical method on sections of embryonic chick retina and optic tectum. Hybridomas that stained Muller cells in the retina and radial glia in the tectum were cloned. EM-immunohistochemistry and SDS-PAGE-TRANSBLOT analysis were conducted using these two antibodies. Antibody 25-5E10 binds to an intracellular antigen with an apparent molecular weight of about 230KD. This antibody also binds to vascular endothelia and cartilage cells but not to neurons, muscle cells, or intestinal epithelia. Antibody 25-3A7 also binds to an intracellular antigen but does not transblot. In addition to Muller cells and radial glia, this antibody binds to the axons of some retinal ganglion cells. These antibodies should be useful in characterizing cells in tissue culture of embryonic chick retina and tectum.

1121 INVERTEBRATE NEURONS SHARE A COMMON ANTIGENIC DETERMINANT RECOGNIZED BY ANTIBODIES TO HRP, Shahid Siddiqui and Joseph Culotti, Northwestern University, Dept. of BMBCB, Evanston, IL 60201.

Immunological probes that identify specific molecules within the nervous system are useful in studying neural development by molecular approaches. Jan and Jan (PNAS 79, 2700, 1982) have reported that antibodies to horseradish peroxidase (anti-HRP) stain all neurons in Drosophila and grasshopper embryos. We have found that in the nematode Caenorhabditis elegans affinity purified polyclonal antibodies to HRP recognize specific sensory neurons and support cells. To characterize the neural antigen recognized in these three invertebrates, we have studied the molecular specificity of anti-HRP by immunoblotting proteins from crude cell homogenates electrophoresed on an SDS/polyacrylamide gel. The major antigen recognized in homogenates from C. elegans corresponds to a molecular weight of 34 k daltons and is present throughout both late embryonic and postembryonic development. In addition, minor bands are also present. Significantly, anti-HRP recognizes major antigens in homogenates from Drosphila and grasshopper embryos which are similar in size to the nematode antigen. Neuronal staining was compared in C. elegans by competition experiments between different carbohydrates including N-acetyl glucosamine, mannose, mannan, and galactose, and anti-HRP. In the presence of these sugars the binding of antibodies is reduced indicating that the neural antigen is a glycoprotein. Whether the 34 k dalton glycoprotein is the neural antigen remains to be proven, but our results indicate a common antigenic determinant on neurons in three different invertebrates.

1122 MORPHOLOGY OF IDENTIFIED NEURONS IN C. ELEGANS AS REVEALED BY ANTI-HRP ANTIBODIES. J. Culotti, S. Siddiqui, and L. Gremke, Dept. of BMBCB, Northwestern University, Evanston, IL 60201.

Antibodies to neural components can be used to study the morphology of identified neurons in simple nervous systems without resorting to serial section electron microscopy. Such markers could be very useful for the characterization of neuronal growth abnormalities in mutants of the nematode Caenhorabditis elegans, since the structure and wiring of every one of its 302 neurons is known. Using indirect immunofluorescence, we have identified several antibodies that bind to neurons in C. elegans, including monoclonal antibodies made against Ascaris nervous tissue (A.O.W. Stretton, C.D. Johnson, J. Culotti, and S. Siddiqui, unpublished results) and polyclonal antibodies to horseradish peroxidase (anti-HRP antibodies). The anti-HRP antibodies recognize identifiable chemosensory and mechanosensory neurons, some interneurons and neuron-like sensory support cells in nematode whole mounts. However, unlike Drosophila (Jan & Jan, PNAS 79: 2700-2704, 1982), anti-HRP antibodies do not appear to recognize motor neurons in C. elegans following staining conditions that readily reveal other neuron types. We have used these antibodies to characterize axon growth defects known to exist in one of the uncoordinated nematode mutants (see abstract by Perkins et al), and have found that among the cells in the tail that are stained by antibodies to HRP, the axon growth defects are limited to a small subset of sensory neurons that send fibers through the pre-anal ganglion.

1123 PROLIFERATIVE CAPACITY OF DIFFERENTIATED NG108 CELLS. Alphonse Krystosek, University of Colorado Health Sciences Center, Denver, CO 80262

Neuroblastoma-glioma hybrid 108 cells expressed differentiated properties, including neurite formation, when treated with dibutyrylcyclic AMP in serum-free N2 medium. The extent to which induction of differentiation extinguished the capacity for proliferation was examined in the present experiments. Cells cultured for 1 to 7 days in the induction medium were washed and challenged by the addition of growth medium containing 10% fetal calf serum. Single cells marked for position on the plate and neurite length were followed daily by microscopic observation. The majority (89%) of process-bearing cells retracted their neurites within the first 24 hour of the challenge. The time required for cells to divide (doublet formation) was variable but followed a trend. The time required to enter the proliferative pool increased with increasing times of the previous exposure to the inducing medium. Furthermore, the probability that a cell would re-extend a neurite (without cell division) was also larger for cells previously exposed for longer times to dibutyrylcyclic AMP. These results are consistent with a previously proposed model (Krystosek, A. Proc. 13th Internat. Cancer Congress, abstract p. 46, 1982), in which cells exposed to inducing conditions stochasticly enter the differentiated state. Neurite extension does not irreversibly eliminate proliferative capacity. Rather, the probability of cell division decreases with extent of differentiation. (Supported by USPHS grant CA 32260).

1124 BETA-ADRENERGIC RECEPTORS AND MONOAMINE UPTAKE SITES IN CULTURED CELLS, R.C. Henneberry and P. G. Lysko, N.I.H., Bethesda,MD 20205.
We have previously shown that HeLa cells contain functional beta-adrenergic receptors as determined by the binding of tritiated dihydroalprenolol (DHA) and isoproterenol stimulation of adenylate cyclase. Receptor expression can be increased 3- to 5-fold by sodium butyrate and related fatty acids; other inducers, such as 5- or 6-azacytidine, together with butyrate show synergistic effects. We now report studies with several mammalian cell lines in which we find that DHA is rapidly taken up by intact cells. There is a striking effect of pH on uptake and competition at this site does not show stereospecificity; saturable binding of DHA cannot be obtained under standard assay conditions in intact cells. We have, however, used CGP-12177 to differentiate between uptake sites and binding sites in several cell lines. Our results show that uptake of DHA and other monoamines occurs at a specific site distinct from the beta-adrenergic receptor; uptake is driven by an electrochemical proton gradient dependent on Mg++ and ATP, similar to transporters described in platelets, synaptic vescicles, and chromaffin granules. However, the uptake site we describe has a unique specificity which discriminates markedly among monoamines. For example, imipramine and other tricyclic antidepressants are taken up as well as DHA, propranolol and alprenolol; isoproterenol, epinephrine, norepinephrine, serotonin and dopamine are not taken up at this site.

Probing Gene Expression, Neural Function, Development and Plasticity

1125 DEVELOPMENTAL REGULATION OF GROWTH HORMONE AND PRO-OPIOMELANOCORTIN IN THE NEUROENDOCRINE SYSTEM OF NORMAL AND TRANSGENIC ANIMALS, Neal C. Birnberg, Olivier Civelli, Edward Herbert, Richard D. Palmiter, Robert Hammer, Ralph Brinster, Michael G. Rosenfeld and Ronald M. Evans

What are the features of a gene that confer its correct developmental program for expression? We approach this broad question using two gene products from the neuroendocrine system as a model, Growth Hormone (GH) and Pro-opiomelanocortin (POMC). GH is a polypeptide hormone and POMC is a precursor protein to several peptide hormones including ACTH and β-endorphin. Both are expressed in the anterior pituitary. POMC is, in addition, expressed in the neurointermediate lobe (NIL) of the pituitary and in the hypothalamus and amygdala. Both gene products are regulated by glucocorticoids in the anterior pituitary but in opposite directions. GH is induced by glucocorticoids and POMC is repressed. POMC, however, is not under glucocorticoid regulation in the NIL or hypothalamus. A fusion gene constructed from the coding region of the rat growth hormone gene and the mouse metallothionein promotor and introduced into the germline of mice by microinjection was found to be expressed in an ectopic fashion in many tissues. Due to the juxtaposition of the metallothionein promotor, expression of the fusion gene at the levels of transcription, GH mRNA and serum GH concentrations were found to be inducible by heavy metals. The fusion gene was also expressed at high levels in the brains of several siblings in the F1 generation. This heritibility of tissue specificity suggests that position in the genome plays a significant role in determining developmental patterns of gene expression but clearly, associated sequences near a gene and specific properties of various cell types, as in the case of POMC gene expression, are also involved.

1126 TRANSCRIPTIONAL REGULATION OF PREPROENKEPHALIN mRNA EXPRESSION IN STRIATUM AND ADRENAL MEDULLA, Joan P. Schwartz, Italo Mocchetti, Osvaldo Giorgi and Tam Thanh Quach, Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032

In previous studies we have demonstrated that the content of preproenkephalin mRNA (PE mRNA) in rat brain striatum is increased by chronic blockade of nigral dopamine transmission with haloperidol. In addition we have shown that treatment of bovine adrenal chromaffin cells with 8-Br-cAMP increases the content of PE mRNA. In both tissues RNA gel hybridization analysis using a 918 bp probe for human proenkephalin (Comb et al., Nature 1982) reveals the presence of a 1400 base mRNA, in agreement with other laboratories, but also two larger species, 3800 and 7300 bases. Two weeks of daily haloperidol treatment increased the content of all three species in striatum, in a dose-dependent manner. In adrenal chromaffin cells, one day of treatment with 1 mM 8-Br-cAMP resulted in an increase in all three species with the greatest increase in the largest (7300 b) and the least in the smallest (1400 b). Preliminary results suggest that the two larger species can be isolated from nuclei. These results are consistent with a precursor-product relationship among these three RNA species and suggest that chronic blockade by haloperidol of dopaminergic transmission in rat striatum or a long-term increase of chromaffin cell cyclic AMP content regulate PE mRNA at the level of gene transcription.

1127 BACTERIAL EXPRESSION OF THE NATURAL AND PROCESSED GENES FOR CHICKEN CALMODULIN, J.A. Putkey & A.R. Means, Dept. Cell Biol., Baylor Coll. Med., Houston, TX 77030

We have reported the isolation of two genomic clones for chicken calmodulin (CaM). One clone (CL-1) contains multiple introns and encodes authentic chicken CaM with 148 amino acids (JBC 258, 11869-11870). The second gene (CM-1) also encodes a protein with 148 amino acids, 19 of which are different than the corresponding amino acids in chicken CaM (PNAS 80, 6985-6989). CM-1 has no introns, is in open reading frame and seems to be expressed in a tissue specific manner. To investigate the characteristics of the protein product of CM-1 (CM-1 protein) we have constructed bacterial expression plasmids which harbour either the CaM cDNA or CM-1. The properties of bacterially synthesized CM-1 protein were compared to bacterially synthesized chicken CaM and purified rat testis CaM. CM-1 protein comigrates with CaM on SDS gels in the absence of Ca^{++} and is recognized by a monospecific CaM antibody. In the presence of Ca^{++}, the electrophoretic mobility of CM-1 protein in an SDS gel is increased. However, this increased mobility is only half that observed for CaM in the presence of Ca^{++}. Similar to CaM, the CM-1 protein will bind to phenothiozine-sepharose in the presence of Ca^{++} and can be eluted with EGTA. The presence of two cys residues in CM-1 allow it to bind to thiol-sepharose while CaM will not. These results predict the existence of a novel CaM-like protein which, although similar to CaM, exhibits altered Ca^{++}-binding characteristics and therefore may have limited CaM-like regulatory functions. The physicochemical properties of the bacterially-produced CM-1 protein are currently being exploited to demonstrate the presence of this protein in chicken tissues.

1128 MOLECULAR CLONING OF THE CA^{2+} BINDING PROTEIN PARVALBUMIN, M.W. Berchtold and A.R. Means, Dept. Cell Biol., Baylor Coll. of Med., Houston, TX 77030

Parvalbumin (PV) is a high affinity Ca^{2+} binding protein which is predominantly localized in fast twitch muscles. It functions as a soluble relaxing factor and its expression is dependent on innervation. In the brain this protein was found exclusively in a distinct subpopulation of neurons in both cerebrum and cerebellum. Although PV is preferentially found in GABAergic neurons its physiological significance in the central nervous system is unknown. In order to study the expression of the PV gene during development we have cloned a nucleic acid probe. Poly RNA from the rat M. gastrocnemius served as a template for the synthesis of first strand cDNA. Double stranded cDNA was ligated to Eco RI linkers and subsequently cut with S1 nuclease. After the addition of Sal I linkers the products were digested with Eco RI and Sal I endonucleases and inserted into the vector PUC8 cut with Eco RI and Sal I. A 30,000 member cDNA plasmid library was prepared using E. coli JM103 as the host. The library was screened with a 17mer oligonucleotide containing the codons for amino acids 28-33 of rat PV. 3 recombinants were obtained that hybridized specifically to the probe. Sequence analysis confirmed their identity as parvalbumin clones. One of these clones is being utilized as a probe to compare the expression of the specialized Ca^{2+} binding protein PV with the multifunctional Ca^{2+} receptor calmodulin during rat brain and muscle development.

1129 Ca^{++}/CALMODULIN DEPENDENT MYOSIN LIGHT CHAIN PHOSPHORYLATING ACTIVITY: NERVE ENDING LOCALIZATION IN RAT CEREBRAL CORTEX. Arthur M. Edelman, Dale D. Hunter, Anita E. Hendrickson and Edwin G. Krebs, Howard Hughes Med. Inst. and Univ. of Washington, Seattle, WA 98195 We have studied the localization of Ca^{++} and calmodulin dependent myosin light chain phosphorylating activity in rat cerebral cortex using classical subcellular fractionation techniques. The activity found was specific for the smooth (as opposed to skeletal) muscle phosphorylatable myosin light chain. Within the primary subcellular fractions 52% of the activity was found in the crude synaptosome containing fraction (P_2), 35% in the nuclear fraction (largely due to undisrupted tissue), 6% in microsomes and 7% in the cytosol. After fractionation of P_2 by sucrose density gradient centrifugation 77% of the recovered activity was found in the 1.0-1.2 M sucrose interface containing large nerve endings as verified by electron microscopy. The subsynaptosomal distribution of the activity was studied after hypoosmotic lysis. Most of the activity (65%) was in the synaptosomal membrane fraction with 15% and 20% in crude synaptic vesicles and cytosol, respectively. After rehomogenizing the membrane fraction in 75 mM NaCl, 95% of the activity resedimented indicating that there had been no artifactual redistribution of the activity due to low ionic strength during osmotic lysis. Also the activity could not be released from membranes by 4 mM EDTA suggesting that membrane association was not divalent cation dependent. The high degree of localization of Ca^{++}/calmodulin dependent myosin light chain phosphorylating activity to synaptosomal membranes taken with previous reports of the presence of actin, myosin and calmodulin in synaptic junctional areas, suggests that a Ca^{++}-dependent chemo-mechanical system exists in nerve endings which may be involved in events underlying synaptic transmission.

1130 THE MAJOR PROTEIN KINASE ACTIVITY FOR ENDOGENOUS SUBSTRATES IN NEURONAL NUCLEI IS CA^{+2}-CALMODULIN(CAM)-DEPENDENT, Harry Le Vine III, Naji E. Sahyoun, Duane Bronson and Pedro Cuatrecasas, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

In contrast to nuclei isolated from glia or liver, cerebral cortical neuronal nuclei display a major Ca^{+2}-CAM-dependent protein kinase activity which acts on a variety of endogenous substrates. Moreover, several low m.w. proteins are specifically phosphorylated in the presence of Ca^{+2}-phosphatidylserine (PS), implying the presence of protein kinase C. Brain cytosol and the non-nuclear particulate fraction manifest Ca^{+2}-CAM-dependent activity on a different spectrum of substrates. Initial characterization of nuclear substrates by 0.4 N H_2SO_4 extraction and SDS-PAGE yields phosphorylated proteins comigrating with authentic H1, H3, H2A and H2B histones. In the presence of 30 μM $CaCl_2$, the EC_{50} for CAM is 15 nM. The enzyme is partially extracted by 0.5 M NaCl, which removes many non-histone chromosomal proteins. Ca^{+2}-CAM-dependent kinase activity is also developmentally regulated in that neonatal neuronal nuclei contain less activity per unit protein than adult nuclei. Thus, the neuronal nucleus is a possible target for Ca^{+2}-CAM-dependent phosphorylation modulated by Ca^{+2} fluxes induced by neuronal depolarization with consequences that may relate to neuronal development and long-term plasticity.

1131 GENETIC ANALYSIS OF SEGMENT DETERMINATION IN DROSOPHILA MELANOGASTER, Mary Ellen Digan, Susan R. Haynes and Igor B. Dawid, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md. 20205

We have cloned approximately 100 kilobases from 7D3-6 of the X-chromosome of Drosophila melanogaster. As shown by deletion mapping, in situ hybridization and genomic Southern blots, this chromosomal region is the proximal end of the deletion, Df(1)sn and contains the genes female sterile(1)homeotic (fs(1)h) and lethal (1)myospheroid (l(1)mys). Both fs(1)h and Df(1)sn havebeen shown to cause homeotic transformation of appendages derived from segments more posterior than the mid-thorax when present in the mothers of the affected embryos and when in combination with other homeotic mutations in the zygote (Gans, M. (1980) Genetics 96: 887; Forquignon, F. (1981) Wilh. Roux's Arch. 190: 132). There is a strong interaction between Df(1)sn and $Df(3)red^{P52}$; $Df(3)red^{P52}$is deleted for the gene Regulator-of-bithorax (Rg-bx; also called trithorax), a gene which is thought to affect the expression of both the Antennapedia and the bithorax gene clusters. We are particularly interested in the maternal effect caused by an insufficiency of the $fs(1)h^{+}$ gene product and in the interaction of this product with Rg-bx. We are currently constructing a transcription map of the cloned region, including l(1)mys, which is allelic to two non-jumping mutants isolated by R. Wyman (personal communication). We plan to examine the spatial distribution of expression of fs(1)h and l(1)mys using in situ hybridization to sectioned embryos and later stages.

1132 A MOLECULAR APPROACH TO DROSOPHILA EYE DEVELOPMENT, S.L.Zipursky, T.R. Venkatesh, D.B. Teplow and S. Benzer, Caltech, Pasadena, California, 91125

The molecular and cellular mechanisms which give rise to neuronal patterns are unknown. To approach the problem we have used monoclonal antibodies(MAbs) to detect structural, cellular and molecular events in the developing retina of Drosophila. The reiterative structure of photoreceptor cell bundles in the retina forms via a non-clonal mechanism in a spatiotemporal manner. In the eye imaginal disc of the third instar larva, a wave of morphogenesis, marked by a furrow in the disc surface, sweeps anteriorly, leaving in its wake a highly ordered array of photoreceptor cell clusters, each sending a bundle of axons into the developing optic lobes. These bundles transduce the formationof the underlying optic lobes, resulting in a similarly reiterative structure. Changes in the extracellular matrix associated with the furrow are highlighted by MAbs 3E1 and 22G8. MAb 22C10 stains developing photoreceptor cell neurons, as well as neurons in the embryo, larva, and adult, whereas MAb 6D6 stains cells surrounding the developing clusters. At a later stage, MAb24B10 exclusively stains photoreceptor cell bodies and axons providing the first indication that these cells are highly specialized neurons. As a step towards understanding the molecular genetic control of photoreceptor cell development, we have purified the polypeptide recognized by MAb 24B10 and determined a partial amino acid sequence at its N-terminus. Using synthetic oligonucleotide probes corresponding to a portion of the N-terminal amino acid sequence, putative genomic DNA clones encoding this antigen have been isolated.

1133 CHOLINE ACETYLTRANSFERASE FROM THE NEMATODE C. ELEGANS: A COMPLEX GENE CODES FOR A MULTI-FUNCTIONAL PROTEIN. James B. Rand and Richard L. Russell, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

In C. elegans, cha-1 is the structural gene for choline acetyltransferase (ChAT). Severe cha-1 mutant alleles lead to >99% reduction in ChAT as well as a characteristic uncoordination, slow growth, small size, and resistance to cholinesterase inhibitors. Mutations at the very closely linked unc-17 locus lead to the same group of phenotypes (including the same type of uncoordinated behavior and drug resistance), except that unc-17 strains have approximately normal levels of ChAT activity. Genetic analysis shows that, while in general, cha-1 and unc-17 mutations appear to represent two distinct complementation groups, there are three alleles which are members of both complementation groups. It is noteworthy that two of these "overlap" alleles contain significant ChAT activity, yet they fail to complement the enzyme-deficient cha-1 mutations. Through fine-structure genetic mapping and recombination analysis, we have now shown that cha-1 and unc-17 are both part of the same complex gene; this conclusion is supported by biochemical studies of ChAT from unc-17 strains. We suggest that the ChAT protein is composed of two structurally discrete and functionally independent domains, one of which contains the catalytic site of the enzyme, and is disrupted by most cha-1 mutations. The other domain we believe to be involved in the proper subcellular localization of ChAT; most unc-17 mutations impair this function without much effect on the catalytic domain. The fact that most cha-1 mutations complement most of the unc-17 mutations suggest that the enzyme functions in vivo as a multimer.

1134 NEMATODE MUTANTS DEFECTIVE IN SENSORY NERVE GROWTH AND DIFFERENTIATION, Lizabeth A. Perkins[1], Edward M. Hedgecock[2], Joseph G. Culotti[1], and Nichol Thomson[2], [1]Dept. of BMBCB, Northwestern University, Evanston, IL., 60201, [2] MRC LMB, Cambridge, England.

The nematode, Caenorhabditis elegans, is an ideal organism in which to study genes that are involved in neuron growth and development of sensory specializations. Six pairs of sensory neurons in the head and 2 pairs in the tail concentrate fluorescein isothiocyanate (FITC) when live animals are placed in a solution of dye making them visible by fluorescence microscopy. Twenty-nine mutants, representing 19 genes, alter the pattern of FITC uptake. These fall into 2 classes. I. Mutants in 5 genes are uncoordinated in movement, show normal or partial dye uptake, and exhibit axon growth and guidance defects. In 4 of these, axon outgrowth occurs but is blocked at a position where the axons would normally change direction and start forming synapses with other neurons. The anterior tips of neurons and sensory support cells in one mutant are abnormally filled with short microtubules as determined by serial electron micrographs. In the fifth mutant, the cell bodies of the neurons in the tail are occassionally mispositioned, in which case misdirected axons are seen. II. Mutants in 14 genes exhibit normal coordination, either totally or partially fail in dye uptake, and are defective in one or more chemosensory behaviors. Sensory neurons in 10 of these have been analyzed by serial electron microscopy and all show defects in ciliary specializations of one or more major head sensilla. One mutant is lacking ciliary rootlets normally present in 2 types of sensory neurons. The mutants described here will be useful for identifying gene products involved in axon growth and sensory ciliogenesis in this simple metazoan.

1135 EARLY INTERACTIONS DETERMINE THE FATES OF TWO SIBLING NEURONS IN THE EMBRYONIC GRASSHOPPER CNS. J.Y. Kuwada & C.S. Goodman, Stanford Univ., Stanford, CA 94305

We are studying the roles of cell lineage and interactions in the determination of identifiable neurons in the CNS of the grasshopper embryo by assessing the effects of specific ablations. Two morphologically distinct sibling neurons, the H cell and H cell sib, are the progeny from the symmetric division of midline precursor 3 (Goodman, et.al., J. Neurosci., 1981). The progeny are identified by their unique location, can be filled intracellularly with dye, and are easily manipulated. Initially the two progeny are indistinguishable. However, 36 hr following their birth they are spatially and morphologically distinct: one progeny is dorsal and is the H cell while the other is ventral and is the H cell sib. This pattern of development is reproduced in control ganglia when embryos are allowed to develop in a culture system.

When one of the progeny was ablated within 5 hr of birth and the embryo allowed to develop in culture, only one progeny was present and it was the H cell sib in 22 of 26 cases and the H cell in 4 cases. Thus at this time the embryo cannot compensate for the loss of one progeny and restore the original number of progeny,and the progeny are yet individually determined for the most part. Instead the remaining progeny appears to regulate its fate in response to the ablation of its sibling. However, when one progeny is ablated 10-15 hr after birth, the remaining progeny can be either the H cell or the H cell sib with equal facility. Thus as little as 5 hr later the progeny are determined and act autonomously in response to ablation of one of the siblings. This suggests that a hierarchy of cell fates governed by early interactions exists for these sibling neurons.

1136 A BIOELECTRICAL THEORY OF AXONAL GUIDANCE, Geoffrey Q. Fox, Dept. Neurochemistry, Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen, Fed. Rep. GERMANY.

A theory of axonal guidance is advanced based on a bioelectric paradigm. The theory derives from studies investigating bioelectrical properties of growing cell systems which show a prior extracellular manifestation of bioelectric current flow eminating from sites that later host growth related changes at macromolecular and higher levels of organization. It is proposed that axonal growth cones and target cells exhibit the same sequence of properties insofar as they both generate extracellular ionic currents. The first sensory domain that axonal growth cones find themselves in is, therefore, bioelectric in form. As this domain is generated by the neuron's own growth mechanism and is localized around the tip of the growing process it is regarded as a bioelectric sensing system analogous to the highly evolved electroreceptive systems of weakly electric fish. Axons orient and grow in electric fields and filopodia are not restricted to substrates as are growth cones. Filopodia thus sweep the forward area for target generated electrical information arriving in the form of ionic perturbations. A chemical environment is also present but information from this realm is not primarily intended for orientational purposes though it may be secondarily used as such. In any case, it must first pass through the electrical domain indicating that any directional influence it might play must of necessity follow already processed electrical information. Axonal guidance is thus seen as a sequence of electrical, biochemical and mechanical directional information processing events, the final decision being made after all relevant information has been considered.

1137 FAST AXONAL TRANSPORT IS NOT AFFECTED BY DIMETHYL SULFOXIDE (DMSO) USED TO FACILITATE GLYCERINATION AND/OR GLUTARALDEHYDE FIXATION OF SQUID AXONS, William J. Adelman, Jr. and Alan J. Hodge, Laboratory of Biophysics, NINCDS, NIH at the MBL, Woods Hole, MA 02543

Squid fin and stellate nerves were examined with video-enhanced differential interference contrast (VEDIC) optics during application of DMSO-containing solutions designed to facilitate (a) formation of a glycerinated model axon, and (b) rapid fixation using glutaraldehyde as the cross-linking agent. Fresh preparations in oxygenated seawater showed vigorous fast axonal transport (FAT). Irrigation with seawater containing 15% DMSO caused no change in rate or character of FAT over several hours. Glutaraldehyde fixative (Hodge and Adelman, 1980, J. Ultrastr. Res. 70: 220-241) containing 15% DMSO caused rapid fixation as judged by cessation of transport deep in the giant axon axoplasm within a minute with no discernible change in optical properties. Electron microscopy showed preservation of axoplasmic structure, including microtubules, comparable with that obtained by cannulation/irrigation fixation (above ref.). Irrigation with neutral solution containing 15% DMSO and glycerol caused blebbing and vacuolization within a few minutes, but with no apparent effect on FAT. Blebbing receded and disappeared within ten minutes, axons returned to their original appearance, and FAT continued for some time. One nerve fiber frozen for three weeks in 50% glycerol following 15% DMSO and glycerol treatment was thawed and bathed in isotonic isethionate solution containing 0.1 mM Ca^{+}, 1 mM Mg^{++} and 3 mM ATP. In VEDIC this fiber had particle movements similar to FAT. These results suggest that a glycerinated model suitable for study of FAT is feasible.

1138 IDENTIFICATION OF AN ANTIGEN IMPLICATED IN THE ADHESION OF NEURONS TO THE EXTRACELLULAR MATRIX, D. Bozyczko, C. Decker, R. Greggs and A.F. Horwitz
Dept. Biochem/Biophys. School of Med. Univ. of Pennsylvania, Phila., PA 19104

We have used an adhesion perturbing monoclonal antibody (MAB) to isolate an antigen that participates in the adhesion of several different cell types to the extracellular matrix. The antigen migrates on SDS-PAGE as a complex of three integral membrane glycoproteins in the molecular weight range of 140 - 160 kdaltons. Both laminin and fibronection have been tentatively identified as its receptors. The antigen is present on embryonic chick ciliary ganglia cells and participates in their adhesive interaction. When ciliary ganglia cells are plated in the presence of monoclonal antibody process formation is inhibited. When monoclonal antibody is added to established neural cultures, containing numerous processes, large neurite bundles form and the cell bodies aggregate. Thus the antigen participates in the adhesion of neurons to the extracellular matrix and appears to be involved in process and fesicle formation.

1139 Changes in RNA Populations in PC-12 Cells Treated with Nerve Growth Factor (NGF) Susanne L. Huttner, Paul H. O'Lague, and Allan J. Tobin (Dept Biology, Jerry Lewis Neuromuscular Research Center, and Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The neuron-like clone PC-12, isolated from a rat pheochromocytoma, provides an important model system for studying the actions of NGF at the molecular level. In culture PC-12 cells respond to NGF via specific surface receptors with cessation of cell division and the appearance of several neuronal characteristics. Little is known of how NGF mediates these effects at the molecular level. To determine whether changes in mRNA populations play an important role we have isolated total RNA from PC-12 cells grown either in the presence or in the absence of NGF. The RNA was separated into poly(A)$^{+}$ and poly(A)$^{-}$ components and fractionated by electrophoresis in agarose containing methylmercury hydroxide. Blot hybridizations of the fractionated RNAs with β-tubulin, β-actin, and ribosomal DNA probes showed that our isolation procedures yielded intact, undegraded RNA. Fractionated, ethidium bromide-stained RNA gels revealed visible differences between NGF-treated and untreated PC-12 cells. In addition to the major bands corresponding to 18s and 28s rRNAs, bands corresponding to approximately 4000 and 1500 nucleotides were present in mRNA of NGF-treated PC-12 cells. These species were detected consistently in the RNA of cells exposed to NGF for 7-14 days. Such RNAs were never detected in the RNA of untreated cells. The identities of these bands are not yet known, but the ability to see this RNA indicates that it is abundant in NGF-treated cells. The appearance of these species of RNA may be an important effect of NGF. (This work was supported by grants from the Dysautonomia Fndn, NSF, and NIH.)

1140 THE DISTRIBUTION OF BETA TUBULIN MESSENGER RNAS IN INDIVIDUAL MAMMALIAN BRAIN CELLS, W. Sue T. Griffin, M. Alejos, and Marcelle R. Morrison, University of Texas Health Science Center at Dallas, Dallas, TX, 75235.

Several different tubulin subunits are synthesized *in vivo* in brain of 10 day old rats. By *in vitro* translation of polyadenylated mRNAs isolated from fetal and adult rat brain, we have shown that the steady state levels of the mRNAs encoding the beta 1 tubulin isotype and the alpha tubulin isotypes decrease during development, suggesting that different cell types or cells at different developmental stages have different requirements for these isotypes. In order to determine the distribution of the beta tubulin mRNAs between different cell types, we have used the technique of *in situ* hybridization to compare the levels of beta tubulin mRNAs and total mRNAs in the postnatal rat cerebellum, a brain area containing readily identifiable cell populations undergoing a variety of developmental tasks. [^{3}H] complementary cDNAs and [^{3}H]-poly(U) were both used to determine total poly(A) mRNA levels. Grain counts over individual cell types were highly reproducible, allowing quantitation of the relative amount of total poly(A) mRNA in each cell type. Background grains were low, both in RNase-treated sections and over axon terminals. The [^{3}H] tubulin probe (donated by D. Cleveland) also hybridized reproducibly to different cell types and a [^{3}H] Pbr322 control showed few background grains. Our results show that in the 10 day cerebellum there is relatively more of the beta tubulin mRNAs in mitotically active germinal and premigratory granule cells than in the other cell types. Thus, this technique can be used to quantitate mRNA levels in individual cell types during brain development and to correlate these mRNA levels with specific developmental events.

1141 MONOCLONAL ANTIBODIES AS PROBES OF NEUROFILAMENT STRUCTURE, Ruth Hogue Angeletti and Virginia M.Y. Lee, Univ. of Pennsylvania, Philadelphia, PA

A library of several hundred monoclonal antibodies has been developed to both common and unique antigenic determinants of the neurofilament triplet proteins (NF68, NF150, NF200). Analysis of peptide maps of the purified polypeptides reveals several structural features of neurofilaments. While some epitopes reside in the helical "head" region, others are found in the "tail" region of these proteins. In addition, many antibodies recognize epitopes present on multiple peptides. Analysis of these data are consistent with the hypothesis that the NF150 and NF200 contain repeated structural domains within part of their amino acid sequence. Antibody affinity columns are being used to purify these unique, common and repeated epitopes.

1142 SPECIFICITY AND REGULATION OF BRAIN ACTIN DEPOLYMERIZING FACTOR (ADF), James Bamburg, Faisal Khatib and Barbara Bernstein, Colorado State University, Ft. Collins, CO 80523

Addition of brain ADF to solutions of brain F-actin resulted in the depolymerization of the F-actin. This depolymerization was prevented by the binding of brain tropomyosin to the F-actin. A 4.1/1 molar ratio of actin subunits to brain tropomyosin, achieved by incubation of the F-actin in excess tropomyosin, completely protected the F-actin from depolymerization by excess brain ADF at 25°C. Supernatant (10^5xg) obtained from a chick embryo brain homogenate was enriched with ADF and incubated with γ-^{32}P-ATP. Aliquots were made 1% in SDS, boiled and subjected to SDS-PAGE. Fluorographs of these gels demonstrated the presence of a radioactive protein which comigrated with brain ADF and which was much reduced in intensity in identically treated samples to which no purified ADF had been added. The phosphorylation of ADF was inhibited by EGTA in the presence of excess Mg^{+2} but was not affected by 20μM trifluoperazine. The ADF activity from a whole brain homogenate incubated with γ-^{32}P-ATP was partially purified, incubated in excess with a small amount of F-actin, and the ADF-actin complex which formed was separated from the free ADF by gel filtration chromatography. No radioactivity was found in the ADF-actin complex, whereas some radioactivity was detected in the peak corresponding to free ADF. SDS-PAGE of the pooled concentrated column fraction was followed by fluorography of the gel. The radioactivity in the free ADF peak comigrated with pure ADF. Thus, phosphorylation of brain ADF occurs in brain homogenates and the phosphorylated product is unable to form a stable complex with actin suggesting that the ADF has been inactivated. (Supported in part by NIH grant NS10429).

1143 PURIFICATION AND CHARACTERIZATION OF TWO ACIDIC PROTEINS IN EMBRYONIC CHICK BRAIN, T. Shirao and K. Obata, Gunma University, Maebashi, Japan

Two acidic proteins (S5, S6) in chick brain, which appeared at a certain embryonic stage but were absent in adult, have been identified by the use of two-dimensional gel electrophoresis. (T. Shirao and K. Obata, (1983) Neurochem. Res. 8,807) These proteins were not detected in liver at any developmental stages. They were purified from the soluble fraction of embryonic chick brain by the use of isoelectric precipitation, DEAE-Sepharose column chromatography, ammonium sulfate precipitation, and sodium dodecylsulfate-polyacrylamide gel electrophoresis. The molecular weights of S5 and S6 were 95,000 and 100,000 respectively, and their isoelectric points were about 4, though S5 were more acidic than S6. S5 and S6 were compared by gel electrophoresis peptide mapping using Staphylococcus aureus V8 protease. They have 15 common peptides and 6 distinct ones. This indicates the structual homology of these two proteins.

1144 TRANS-SYNAPTIC REGULATION OF NEURONAL CELL SURFACE AND SYNAPTIC VESICLE ANTIGENS IN DEVELOPING RAT SUPERIOR CERVICAL GANGLION, Karen F. Greif and Holly I. Trenchard, Bryn Mawr College, Bryn Mawr, PA 19010

Normal development of a neuronal cell surface heparan sulfate proteoglycan (HeS) and a synaptic vesicle membrane protein (SV) in the rat superior cervical ganglion (SCG) parallels the time course of development of synapses and neurotransmitter synthetic enzymes (Greif and Reichardt, 1982, J. neurosci. 2:843). We sought to determine whether preganglionic input is required for normal maturation of these antigens. The cervical sympathetic trunk was cut bilaterally in neonatal rats, with littermates acting as unoperated controls. At 30 days, SCGs were removed and levels of antigens were determined by RIA. Total protein, as assessed by Amido-schwarz assay, was reduced in deafferented ganglia to 87% of control levels. The core protein of HeS, as measured by binding of a monoclonal antibody (PG 22) directed against an associated determinant, was present in normal levels in deafferented SCG. In contrast, a carbohydrate side-chain determinant recognized by a second antibody (PG 3) was reduced to 60% of control levels, suggesting that processing of newly-synthesized HeS by ganglionic neurons is diminished after neonatal deafferentation. SV levels were reduced to less than 20% of controls. Since SV is found in the cytoplasm of principal ganglion neurons as well as in presynaptic terminals, this result suggests that SV synthesis by postsynaptic cells is suppressed in deafferented SCG. The effects of denervation of adult SCG are now being tested to confirm this hypothesis. Supported by a grant from the Dysautonomia Foundation.

1145 NEURONOTROPHIC ACTIVITY IN TORPEDO ELECTRIC ORGAN EXTRACTS, Guy P. Richardson and G.Q. Fox, Max-Planck-Institut für biophy. Chemie, Göttingen, Fed. Rep. GERMANY

Cultures of embryonic Torpedo electric lobe tissue and 8 day chick ciliary ganglia have been used to assess whether embryonic or adult electric organ tissues contain neuronotrophic activity. Using electric lobe cultures activity can be detected in electric organ extracts prepared from all stages. Activity increases rapidly between the 60 and 75mm stages and then remains at a plateau between 85 and 105mm stages. This rapid increase in activity occurs over a period when electromotor neuronal cell death is ending in the electric lobes. In addition to maintaining nearly theorectical numbers of electromotoneurons for 7 days extracts from 80-105mm stage organs cause a 2 to 3 fold increase in CAT activity. Survival activity is only slightly reduced by heat treatment although the component responsable for causing an increase in CAT is thermolabile. Using the chick ciliary ganglion activity can be detected with 50-80mm stage electric organ extracts, and is developmentally regulated such that it peaks at the 70mm stage. This cross reactive activity is thermolabile. The results suggest electric organ extracts contain at least 2 factors acting on electromotoneuron survival and development.

1146 SWITCH FROM A CHEMICAL TO AN ELECTRICAL SYNAPSE IN A NEURONAL CULTURE, Rolf Bodmer and Irwin B. Levitan, Friedrich Miescher-Institut, CH-4002 Basel, Switzerland

Aplysia neurons in primary culture have been found to express primarily electrotonic synapses. Under certain circumstances unidirectional chemical connections have also been found. Because of the large size of molluscan neurons, it is possible to carry out continuous intracellular recordings from a given pair of cells for long periods of time. We have found a chemical (probably serotonergic) synapse between two abdominal neurons in culture, during the first two days after the switch from serum-containing culture medium to serum-free saline. During continuous recording over three days in saline this chemical synapse disappeared and the two cells exhibited strong electrically mediated transmission by the third day. This switch in synaptic type demonstrates dramatically the plastic potential of these cultured neurons.

1147 AFFERENT ACTIVITY INFLUENCES DEVELOPMENT OF VISUAL CALLOSAL CONNECTIONS IN HAMSTER. Robert W. Rhoades, Richard D. Mooney an Stephen E. Fish, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854.

Afferent activity has been shown by several investigators (eg. Stryker, M.P. Soc. Neurosci. Absts., 7: 842, 1981; Schmidt, J.T. and D.L. Edwards Brain Res., 269: 29-39, 1983; Constantine-Paton, M. and T. Reh Soc. Neurosci. Absts., 9: 760, 1983) to be important in the refinement of retinotectal and geniculocortical connections. Callosal connections undergo considerable postnatal refinement and we (Rhoades, R.W. and S.E. Fish Exp. Brain Res. 51: 451-462, 1983) have suggested that this may depend upon correlated cortical activity. In this experiment, we have asked whether a manipulation which should correlate firing throughout both visual cortices would result in abnormal retention of callosal connections. Hamsters were reared in an environment where the only visual stimulation was a bright strobe flash. Visual callosal connections in these hamsters were compared with those in normal and dark reared animals using autoradiographic and HRP techniques. Data from the normals and dark reared hamsters were identical. Labelled cells and fibers were located primarily at the borders of area 17. In the 17-18a border region labelled neurons were located primarily in layers II, III and V and anterograde labelling was visible in all laminae. Medially, a few labelled cells and light anterograde labelling were visible in layers V and VI. In the strobe reared animals labelling near the 17-18a border was qualitatively normal. Medially, an abnormally large number of labelled cells were visible in lamina V and VI and anterograde labelling was also extremely heavy in these layers. There were also silver grains overlying lamina I throughout the striate cortex. EY03546, EY04170, DE06528.

1148 ANATOMICAL STUDIES OF THE NEONATAL RAT'S TRIGEMINAL SYSTEM, Mark F. Jacquin, William E. Renehan & Robert W. Rhoades, The University of Medicine & Dentistry of New Jersey: School of Osteopathic Medicine & Rutgers Medical School, Piscataway, N.J. 08854

The infraorbital (IO) nerve's innervation of the whiskers and its highly ordered representations throughout the trigeminal (V) neuraxis have been used to examine CNS sequelae to neonatal nerve damage. Surprisingly, little is known regarding the normal organization of the V system at birth, when such experimental lesions are performed. HRP applied to the IO nerve at birth labeled diffusely scattered cell bodies throughout the ophthalmic-maxillary portion of the V ganglion, while their central axon collaterals were organized into radially oriented bands and barrel-like patches within these bands. Cylinders of primary afferent terminals extended throughout the length of the brainstem V complex, and appeared isomorphic with the pattern of the whiskers on the face. A barrel-like pattern in neonates is consistent with the barrel-like appearance of 1) cytochrome-oxidase staining in the neonatal brainstem, 2) HRP-labeled terminals from the adult IO nerve, and 3) terminal boutons from intra-axonally labeled whisker primary afferent fibers in adults. Thus, an adult-like topography and arborization pattern exists in the central processes of the neonatal IO nerve. This is of interest in light of the fact that neonatal (but not adult) IO damage reduces the number of fibers in the adult IO nerve (5260 myelinated, 2747 unmyelinated vs. the normal 19740 myelinated, 13319 unmyelinated), markedly reduces the number of IO ganglion cells, and alters their central projections.