Plenary Talk P02: New directions in CNS neuronal migration

Mary B. Hatten
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Over the past decade, it has become clear that a variety of migratory pathways underly vertebrate brain histogenesis. In cortical regions of brain, neuronal migrations along glial fibers provide a primary pathway for the formation of cortical laminae. To examine the mechanisms underlying glial-guided migration, we analyzed the dynamics of cytoskeletal and polarity signaling components in living neurons. Migration involves the coordinated two-stroke movement of a perinuclear tubulin ‘cage’ and the centrosome, with the centrosome moving forward before nuclear translocation. Over-expression of the polarity complex mPar6α disrupts the perinuclear tubulin cage, retargets PKCζ and γ-tubulin away from the centrosome, and inhibits centrosomal motion and neuronal migration. Thus, mPar6α controls the migration of CNS neurons along glial fibers. To search for novel genes that regulate CNS migrations, we have screened for vertebrate genes related to the genes that control neuroblast migration in C. elegans, and carried out a large scale gene expression screen using BAC transgenic mice (the GENSAT PROJECT) to discover novel regulatory pathways for migration. Several important new families of migration genes are described, one related to the C. elegans mig-13 gene, and another to Pde1c and other genes that regulate a novel pathway for migration across the surface of the developing neocortex.
Symposium S03: Wiring the nervous system

Chair: M. Fox

S03-01
Dendritic guidance
A CHIBA
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Like axons, dendrites, too, need guidance. Dendrites from a neuron exhibit outgrowth directionality distinct from its axon. However, molecular mechanisms that support such a directional axodendritic asymmetry remain elusive. My lab uses Drosophila CNS neurons as an in vivo model to study dendritic guidance. Previously our search has identified Slit-Robo and Netrin-Frazzled/DCC as dendritic guidance molecules. These molecules serve a dual role as axodendritic guidance molecules because they also guide axons in the same group of neurons. The finding has led to a new question: How do individual neurons, while using the same receptors, each maintain that its axon and dendrite adopt distinct outgrowth directionality? Our recent evidence favors the model that each neuron has an internal ‘clock’ that controls its expression of guidance receptors. Coupled to the pan-neuronal shift from axogenesis to dendrogenesis, this cell-specific clock biases whether, in an individual neuron, such receptors should enrich in axon or dendrite. My lab also focuses on dendritic targeting of neuropils.

S03-02
Sensory ganglia development
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To understand the formation and function of circuits that are involved in nociception, we focus on the trigeminal sensory ganglion. Trigeminal sensory neurons assemble into precisely positioned, compact clusters, arborize the skin and sense thermal, mechanical and chemical stimuli. We will discuss data that suggests that (1) chemokine signaling assembles trigeminal sensory ganglia, (2) repulsive interactions self-organize the arborization pattern of trigeminal axons, and (3) a member of the TRPA family mediates chemical sensing.

S03-03
FGFs are presynaptic organizing molecules in the mammalian brain
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As the nervous system develops, axons become specialized for neurotransmitter release precisely at sites of contact with their synaptic targets, indicating that target-derived factors organize presynaptic differentiation. We purified such factors from postnatal mouse brain, using the ability to cluster synaptic vesicles in cultured neurons as a bioassay. One factor purified in this way promoted both vesicle clustering and neurite branching; it was identified by mass spectrometry as fibroblast growth factor (FGF) 22. FGF22 is closely related to FGF7 and 10, both of which shared vesicle clustering and neurite branching activities; other FGFs tested had distinct effects in our assay. FGF22 is expressed by cerebellar granule cells when they are receiving synapses; FGF7 and 10 are expressed at low levels by these cells. FGF receptor (FGFR) 2, the major receptor for this subfamily is expressed by the pontine and vestibular neurons, whose axons (mossy fibers) make synapses on granule cells. Since FGF7/10/22 bind selectively to a splice variant of FGFR2 called 2b, we used a soluble extracellular domain of FGFR2b (sFGFR2b) as a blocking reagent. FGF22 and granule cells promoted presynaptic differentiation of pontine and vestibular neurons in vitro. sFGFR2b blocked these effects. Likewise, sFGFR2b inhibited presynaptic differentiation of mossy fibers in cerebellum in vivo. Finally, postnatal deletion of FGFR2, using a conditional mutant, also inhibited mossy fiber differentiation in vivo. These results indicate that FGF22 and its close relatives are presynaptic organizing molecules in the mammalian brain, and identify a novel function for this family of signaling molecules.

S03-04
Molecular mechanisms of synaptic differentiation
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The normal function of neural networks depends on the accurate formation of excitatory and inhibitory synapses during development. Synapse formation is thought to be regulated by bi-directional signaling between pre-and postsynaptic cells. We have analyzed the molecular machinery that contributes to synapse assembly in the central nervous system. We demonstrate that the neurexin-neuroligin adhesion complex mediates trans-synaptic interactions that direct the differentiation of pre-and postsynaptic membrane domains. Down-regulation of neuroligin isoform expression results in a loss of excitatory and inhibitory synapses. Electrophysiological analysis revealed a predominant reduction of inhibitory synaptic function. Thus neuroligins control the formation and functional balance of excitatory and inhibitory synapses in neurons of the central nervous system.
Symposium S04: Frontiers in embryonic stem cell research

Chair: O. Brustle and S.C. Zhang

S04-01

Improved culture of human ES cells

JA THOMSON

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Human embryonic stem (ES) cells provide an important model for understanding the differentiation and function of human tissues, yet their culture remains labor intensive and technically challenging. Improved culture conditions would facilitate the more widespread research use of human ES cells, and would increase their future utility in ES cell-based transplantation therapies. Human ES cells are generally grown on fibroblasts, or in fibroblast-conditioned medium, in the presence of serum, or serum components. Here I will describe recent progress in the culture of human ES cells in the absence of fibroblasts in defined conditions. The talk will emphasize the importance of bFGF signaling and the suppression of BMP signaling in the self-renewal of human ES cells.

S04-02

Pluripotent human embryonic stem cell line derived from a cloned blastocyst and its potential applications

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The isolation of pluripotent human embryonic stem (ES) cells and breakthroughs in somatic cell nuclear transfer (SCNT) in mammals have raised the possibility of performing human SCNT to generate potentially unlimited sources of undifferentiated cells for research, with potential applications in tissue repair and transplantation medicine. This concept, known as ‘therapeutic cloning,’ refers to the transfer of the nucleus of a somatic cell into an enucleated donor oocyte. ES cells would be isolated from the inner cell mass (ICM) of the cloned preimplantation embryo. When applied in a therapeutic setting, these cells would carry the nuclear genome of the patient; therefore, it is proposed that following directed cell differentiation, the cells could be transplanted without immune rejection for treatment of degenerative disorders such as diabetes, osteoarthritis, and Parkinson’s disease, among others. In this study, we report the derivation of a pluripotent embryonic stem cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells display typical ES cell morphology and cell surface markers and are capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in SCID mice. After continuous proliferation for >100 passages, SCNT-hES-1 cells maintain normal karyotypes and are genetically identical to the somatic nuclear donor cells. The DNA fingerprinting assay along with imprinted gene analysis provides support that cloned human ES cells was derived from somatic cell nuclear transfer of a donor cell.

S04-03

The molecular biology of stem cells

R MCKAY

National Institutes of Health, NINDS, Bethesda, MD, USA

The principle goals of our work are to define the cellular states and signals that control the differentiation and survival of cell types in the central nervous system. The identification of neural stem cells is central to the work in this group. In the past year, we have defined the mechanisms that control the sub-cellular localization of a new nucleolar, p53 interacting protein (nucleostemin, NS) that is specifically found in stem and cancer cells. These data support a model of four different molecular states of nucleostemin in the nucleolus and the nucleoplasm, and provide a molecular basis for understanding the regulatory mechanism controlling the nucleolar and the mitotic activity of stem cells. These and other studies focused on the basic biology of signal pathways are now generating new insight into the control of the number of neural stem cells and the disruption of these mechanisms in cancer cells. The potential use in Parkinson’s disease of functional dopamine neurons derived from embryonic stem cells is a leading example of the clinical value of this new technology. In vitro strategies to generate functional somatic cell types are required to apply human ES cells in medicine. We have used an in vitro cell aggregation system to generate the early somatic precursors and extra-embryonic cells. Large numbers of functional dopaminergic neurons can be generated from human ES cells in vitro. These data add to the evidence suggesting that the developmental potential of human ES cells can be controlled in vitro to generate large numbers of clinically valuable human functional cells.
Neuronal subtype specification from embryonic stem cells

SC ZHANG

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Embryonic stem cells (ESCs) offer a tool for dissecting molecular pathways underlying cell lineage diversification in mammals. Using a chemically defined culture system established in our lab, we have found that ESCs from human, monkey, and mouse generate Sox1+ neuroepithelial cells that organize into neural tube-like rosettes at a time that corresponds to formation of the neural tube in vivo, suggesting the preservation of the intrinsic program of neuroectodermal specification in our in vitro system. Further analyses revealed that the generation of the Sox1+ neuroectodermal cells was preceded by a more primitive stage at which the precursor cells express early neural transcription factors Pax6 and Otx2. These primitive neuroectodermal cells progress to Sox1-expressing definitive neuroectodermal cells with concomitant expression of positional markers characteristic of rostral-caudal and dorsal-ventral domains of the brain and spinal cord depending upon the presence of morphogens. These region-specific neuroepithelial cells further differentiate to electrophysiologically active neurons such as spinal cord motor neurons and midbrain dopamine neurons in the presence of neurotrophic factors. Our finding points to the critical need to apply morphogens to the primitive neuroectodermal cells in order to specify neuronal types with a particular positional and transmitter identity. It suggests that the primitive neuroectodermal cells are multipotent and that the generation of definitive neuroectodermal cells is accompanied regional specialization.

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From ES cells to functional neurons and glia

O BRÜSTLE

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In vitro differentiation of embryonic stem (ES) cells permits the derivation of a large variety of tissue-specific cell types for disease modeling, drug development and cell therapy. We have developed differentiation and lineage selection strategies for the generation of highly purified CNS neurons and glia. BMP-mediated promotion of neural crest fates extends the potential applications of ES cell-derived precursors to the peripheral nervous system. Transplanted ES cell-derived neurons, astrocytes and oligodendrocytes integrate into the host nervous system and acquire cell type-specific functional properties. Upon incorporation into the developing, adult and lesioned CNS ES cell-derived neurons develop mature functional phenotypes and receive glutamatergic and GABAergic synaptic input. Oligodendrocytes derived from ES cells generate new myelin when introduced into the CNS of dysmyelinating mutants or into foci of inflammatory demyelination. Newly developed technologies for automated ES cell culture and efficient genetic modification of human ES cells provide perspectives to translate these technologies to pre-clinical applications.
Colloquium C04: Toll-like receptors in the nervous system

Chair: G. Konat

C04-01
Differential roles for TLR2 in CNS bacterial infection and glial activation
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Toll-like receptor 2 (TLR2) is a pattern recognition receptor that plays an important role in enabling cells of the innate immune system to recognize conserved structural motifs on a wide array of pathogens including gram-positive bacteria. Although microglia and astrocytes express TLR2, the functional significance of this receptor in mediating glial activation remains unknown. To ascertain the significance of TLR2 in astrocyte and microglial responses to *S. aureus* and its cell wall product peptidoglycan (PGN), we evaluated primary glia from TLR2 knockout (KO) and wild type (WT) mice. In astrocytes, TLR2 was pivotal for both *S. aureus* and PGN recognition. In contrast, TLR2 was only required for maximal responses to PGN in microglia, whereas alternative receptors were responsible for distinguishing intact bacteria. To assess the importance of TLR2 in the context of CNS infection, we evaluated the pathogenesis of *S. aureus*-induced experimental brain abscess in TLR2 KO mice. Surprisingly, brain abscess severity was similar between TLR2 KO and WT mice, with comparable mortality rates, bacterial titers, and blood-brain barrier permeability observed between TLR2 KO and WT strains. Despite these similarities, the expression of two proinflammatory mediators, IL-1β and macrophage inflammatory protein-2 (MIP-2/CXCL1), were differentially regulated in brain abscesses of TLR2 KO and WT mice. These findings reveal the complex nature of gram-positive bacterial recognition by glia which occurs, in part, through engagement of TLR2 and highlight the importance of receptor redundancy for *S. aureus* recognition in the CNS.

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C04-02
Astrocytes as sentinel cells for CNS pathogens
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Astrocytes, the major glial cell type in the CNS, have been recognized as potentially important contributors to inflammatory immune responses within the brain. The ability of microbial products to initiate astrocyte immune responses has implicated the involvement of pattern recognition receptors in the perception of CNS pathogens. We have recently demonstrated the functional presence of Toll-like receptors (TLRs), in isolated cultures of primary murine astrocytes. We described the low level constitutive expression of mRNA encoding TLR2, TLR4, TLR5, and TLR9 in resting cultures of these cells. Importantly, the level of expression of mRNA for each of these receptors is markedly elevated following exposure to specific bacteria-derived ligands for these receptors. The functional expression of these receptor proteins is further supported by the ability of known ligands for each TLR to induce both message expression and protein secretion of the pro-inflammatory cytokine, IL-6. In addition, the availability of antibodies to TLR4 has enabled us to directly demonstrate the presence of this receptor on astrocytes by immunofluorescence analysis. More recently, we have found that primary murine astrocytes express robust constitutive levels of mRNA encoding the viral pattern recognition receptors, TLR3 and TLR7. Furthermore, specific ligands for these receptors can upregulate such mRNA expression and can induce inflammatory cytokine production. The demonstration of Toll-like pattern recognition receptors on primary astrocytes provides a mechanistic link between microbial challenge and inflammatory immune responses that may play an important role in the initiation of pathogen-induced inflammatory CNS disorders.

C04-03
Role of Toll-like receptors in microglial activation
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One of the common pathological hallmarks of different neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and HIV-associated dementia (HAD), is microglial activation. Increased expression of Cd11b, the beta-integrin marker of microglia, represents microglial activation during neurodegenerative inflammation. However, molecular mechanisms leading to increased microglial expression of Cd11b are poorly understood. Etiological reagents of AD (Ab1-42), PD (MPP+) and HAD (HIV-1 gp120) increased the expression of TLR2, TLR7 and TLR9 in microglia. Interestingly, knockdown of TLR2 by siRNA and antisense oligonucleotides inhibited Ab-, MPP+ and gp120-induced microglial expression of Cd11b. Because impairment of TLR2 also inhibited the expression of inducible nitric oxide synthase (iNOS) in activated microglia, we investigated the role of NO in microglial expression of Cd11b. Inhibition of Ab-, MPP+ and gp120-induced microglial expression of Cd11b by either PTIO (a scavenger of NO) or L-NIL (an inhibitor of iNOS) and increase in microglial Cd11b expression by a NO donor alone suggest an important role of NO in Cd11b expression. Furthermore, we show that NO induces microglial expression of Cd11b via GC-cGMP-PKG. This study illustrates a novel biological role of TLR2 in regulating the expression of Cd11b in neurotoxin-activated microglia through NO-GC-cGMP-PKG pathway that may participate in the pathogenesis of devastating neurodegenerative disorders.

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Double stranded RNA triggers proinflammatory response in astrocytes: implications for MS etiology

GW KONAT

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A preponderance of epidemiological, histopathological and immunological evidence implicates a latent virus(es) in the etiology of MS. Demyelination is likely to result not from direct viral destruction of oligodendrocytes (OL), but rather from bystander injury of OL that succumb to immune and inflammatory mediators. A potential mechanism for such a bystander demyelination entails focal inflammation triggered by viral components shed from cells undergoing virus replication. Double stranded RNA (dsRNA) is of particular interest, because it is a replication intermediate of most viruses and a very potent proinflammatory agent. dsRNA acts through the Toll-like receptor 3 (TLR3) that is profoundly upregulated by glial cells within MS lesions. In the present study we characterized damaging potential of dsRNA in CNS glial cells in vitro. The administration of dsRNA to rat brain mixed glial cultures profoundly downregulated the expression of proteolipid protein gene indicating OL degeneration. In purified astrocytes cultures dsRNA triggered strong proinflammatory response as seen form the generation of nitric oxide (NO) and the upregulation of proinflammatory cytokines. dsRNA-induced NO generation and upregulation of IL-1\(\alpha\) and IL-1\(\beta\) was similar to that induced by LPS, a potent proinflammatory agent. However, dsRNA induced over two fold greater upregulation of IL-6 and TNF\(\alpha\) that did LPS. Moreover, dsRNA induced approximately 15 fold increase in the expression of \(\alpha\)B-crystallin, a heat shock protein implicated in the pathogenesis of MS lesions. In conclusion, dsRNA is a putative viral component that through its robust proinflammatory activity can instigate bystander OL damage.

Acknowledgement: Supported by a WVU School of Medicine Research Development Grant.
**Colloquium C05: Beyond immune privilege: is the CNS an immunologically active organ?**

**Chair: B. Melchior**

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**C05-01**

**Dendritic cells and the initiation of immunity in the central nervous system**  
**ZZ FABRY, J KARMAN, C LING and M SANDOR**  
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The central nervous system (CNS) is an immunoprivileged site based on observations such as the presence of the blood-brain barrier (BBB), graft acceptance, lack of conventional lymphatics, low T cell trafficking into the brain and low MHC expression on brain cells. However, it is clear that brain derived antigens can induce strong systemic immune responses. We propose that dendritic cell migration is a critical requirement for inducing immunity against brain antigens. Since one of the characteristics of differentiated dendritic cells is mobility, brain-derived dendritic cells migrate out of the brain, reach peripheral lymph nodes and induce naïve T cell activation. We provide supporting data for this hypothesis in this presentation. We show that microinjection of protein antigen into the brain leads to accumulation of dendritic cells in the CNS. Next, we demonstrate that antigen-pulsed, CD205, CD11c and MHC class II expressing dendritic cells traffic from the brain to draining cervical lymph nodes and induce the preferential recruitment of antigen specific T cells to the brain. Reverse migration of dendritic cells to the periphery require live dendritic cells that are capable of signaling through G proteins. Our in vitro studies analyzing dendritic cell migration in an in vitro model of blood brain barrier also indicate that these cells are capable of migration across brain endothelium. These studies indicate that T cell mediated immune responses against brain antigens are initiated similarly to other tissues, in a sense that brain emigrant dendritic cells are critical to support T cell activation in the periphery and their accumulation to the brain. These findings can lead to new therapeutic treatments to interfere with brain inflammatory diseases.

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**C05-02**

**Formation of ectopic lymphoid tissue in the inflamed brain**  
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To spare its delicate neuronal networks from inflammatory threats, the central nervous system (CNS) has developed unique anatomical and functional features exemplified by the different ways whereby distinct CNS compartments handle immunity. The neural parenchyma strictly qualifies as an immune privileged site, as it is devoid of dendritic cells and lymphatic vessels and is shielded from circulating immune cells and molecules by the blood-brain barrier. Conversely, the ventricular compartment and the associated choroid plexus and meninges containing macrophages and dendritic cells are the sites where CNS immune surveillance is routinely performed. Multiple sclerosis (MS), a putatively autoimmune CNS disorder, is characterized by an abnormal humoral immune response with intrathecal production of antibodies which are thought to play a role in brain lesion development. We have recently shown that ectopic lymphoid follicles with germinal centers form in the cerebral meninges of a subset of MS patients and that factors favoring B-cell/plasma cell homing and survival are produced within MS lesions. These findings indicate that the meningeal compartment may favour local B-cell maturation and perpetuation of humoral autoimmunity, while the CNS parenchyma can provide a favourable niche for autoantibody-producing plasma cells. Lymphoid follicle-like structures were also detected in the meninges of mice with experimental autoimmune encephalomyelitis, an animal model for MS. Using this model, we began to evaluate whether local targeting of molecules regulating B-cell responses and lymphoid organogenesis might represent a valid therapeutic strategy to inhibit intracerebral immune reactivity and possibly disease progression.

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**C05-03**

**CNS dendritic cells drive naive T cell proliferation and epitope spreading in relapsing EAE**  
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Chronic progression of relapsing EAE (R-EAE) in the SJL mouse is dependent on the activation of T cells to endogenous myelin epitopes, i.e. epitope spreading which plays a major pathologic role in disease progression. Using transfer of naïve CFSE-labeled TCR transgenic T cells and mixed BM chimeras, we show that activation of naïve PLP139-151-specific T cells in SJL mice undergoing PLP178-191-induced R-EAE occurs in the CNS and not in the cervical lymph nodes or spleen. Flow cytometric and histologic examination of the CNS during R-EAE revealed the infiltration of significant numbers of CD11c+ dendritic cells (DCs) (including myeloid, lymphoid and plasmacytoid subsets) which are not seen in the healthy CNS. Functional examination of the antigen presentation capacity of CNS APC populations purified from mice with established PLP178-191-induced R-EAE shows that only F4/80-CD11c+ CD45hi DCs efficiently present endogenous antigen resulting in the activation of naïve PLP139-151-specific Tg T cells. In contrast, DCs as well as F4/80+CD45lo microglia have the capacity to activate memory PLP139-151-specific Th1 cells. These results indicate that naïve T cells can gain access to the inflamed CNS, bypassing the need for activation in peripheral lymphoid sites, and that epitope spreading initiates within the CNS target organ. Further, activation of naïve T cells involved in chronic R-EAE is mediated by CNS DCs, not infiltrating macrophages or resident microglia. Consequently, blocking the recruitment or differentiation of DCs may be a viable target for inhibiting relapse and disease progression in murine MS models and possibly MS patients themselves.
The healthy CNS actively redirects autoreactive T cell responses toward protective effector functions

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To identify CNS-specific mechanisms regulating T cell function, we compared two transgenic models of autoimmunity, in which the target-antigen (influenza hemagglutinin [HA]) and the responding T-cell population (HA-specific CD4+ T cells) were identical. Only the site of antigen expression differed: HA was expressed by pancreatic islet beta cells (Ins-HA), or CNS astrocytes (GFAP-HA). HA-T cells underwent antigen-independent (homeostatic) and antigen-induced proliferation and caused autoimmune destruction of islet tissue when transferred into lymphopenic Ins-HA mice. In contrast, upon transfer into lymphopenic GFAP-HA mice, both the homeostatic and antigen-induced T cell proliferation/activation was reduced and no CNS autoimmunity occurred. Strikingly, upon transfer into double GFAP-HA/Ins-HA transgenic mice, there was a 60% reduction in the incidence of T-cell induced diabetes. Protection from autoimmunity correlated with an increased ratio of IL-10/IFNγ produced by antigen-triggered T cells. These CNS protective mechanisms were overridden by pertussis toxin and revealed that CNS integrity cannot be maintained during an effective autoimmune attack on astrocytes. These results indicate that (1) CNS immune privilege is in part a consequence of continual presentation of CNS antigens to instruct the immune system of privileged targets and that (2) dysfunction in these CNS intrinsic mechanisms of controlling immune responses is likely to contribute to CNS autoimmune pathogenesis.
C06-01
Ependymal primary cultures as a model for studying ependymal functions
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The ependyma is a single-layered epithelium covering the inner ventricle surfaces of the mammalian central nervous system. Although known for more than 150 years, there is a distinct lack of knowledge regarding ependymal biochemistry and function. A cell culture model will be presented which facilitates studying ependymal biochemistry, including energy metabolism and response to hormones. Thrombin acts on freshly seeded cells from rat brains via protease-activated receptor 1 and Rho to yield a culture consisting of 70% kinocilia-bearing cells. These cells build up glycogen to 990 ± 112 nmol glucosyl residues/mg protein and degrade it with a half-life of 21 min upon glucose withdrawal. The neurotransmitter, serotonin, elicits glycogen degradation by a short transient increase in intracellular cyclic AMP. Serotonin itself is rapidly taken up into the cultured ependymal cells by two different transport systems, one of which is the neuronal serotonin transporter SERT, exhibiting a Vmax value of 3.8 ± 0.1 pmol/min (mg protein)−1 and an apparent Michaelis-Menten constant of 0.41 ± 0.03 μM. The main product of serotonin metabolism is 5-hydroxyindole acetic acid, which results from the action of monoamine oxidase A. Glycogen buildup in ependymal cell cultures occurs from glucose. GLUT1, the predominant glucose transporter in these cultures, is apparently regulated by insulin-like growth factor 1, which causes a nearly twofold increase in 2-deoxyglucose uptake. Ependymal cells in primary culture express proteins which have been identified by screening a subtractive cDNA library made from bovine ependyma minus bovine ependyma-free brain. Such proteins may be developed into specific marker proteins for ependymal cells.

C06-02
Hydrocephalus in a murine intraflagellar transport mutant
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Cilia are motile or immotile (primary cilia) organelles found on most cells of the body that are constructed through a process called intraflagellar transport (IFT). Disruption of IFT, such as in Tg737 mutants, results in multiple phenotypes, one of which is hydrocephalus. While the mechanism by which cilia defects result in hydrocephalus are unknown, it is thought to arise from accumulation of cerebrospinal fluid (CSF) through excess production, lack of reabsorption, or blockage of flow through the ventricles. In the brain ventricular system, cilia are present on ependymal (EP) and choroid plexus (CP) cells. Analysis of the EP and CP cells reveal that they have primary cilia early in development followed by conversion to motile cilia. In Tg737 orpk mutants, the cilia on the EP and CP cells are malformed and beat irregularly leading to reduced CSF movement. However, analyses reveal that the pathology develops prior to formation of motile cilia indicating that loss of these cilia may not be the initiating factor. Thus, we are exploring the effect of cilia defects on the CP. Our data indicate that CP cilia defects result in altered ion transport. As seen in other affected tissues of Tg737 orpk mutants, the hydrocephalus involves excessive chloride secretion into the CSF. This is associated with disturbed function of NKCC1, anion exchanger type 2, and Na/H exchangers. We suspect that the CP cilia defects are leading to excess CSF production that in combination with the altered cilia motility on EP cells result in ventricular expansion. This is being explored further using conditional mutants to disrupt cilia in the CP of EP cells independently.

C06-03
Specialized ependyma: subcommissural organ and adult germinative zones
P FERNÁNDEZ-LLEBREZ*, J PÉREZ*, M CIFUENTES*, JM GRONDONA*, MD LÓPEZ-ÁVALOS*, M PÉREZ-MARTÍN*, G ESTIVILL-TORRUS† and LM RODRÍGUEZ-PÉREZ*
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In the developing brain, radial glia are neural stem cells and provide guidelines for radial migration. After neurogenesis, most radial glia transform into the multiciliated cells (ependyma) lining the brain ventricles and spinal central canal. Yet, some cells remain as resident neural stem cells in the adult brain, retaining a CSF-contacting process and GFAP immunoreactivity. In some regions, specialized ependyma serve distinct functions. The subcommissial organ (SCO), beneath the posterior commissure, is a secretory gland. It produces large glycoproteins that coalesce into a thick fibre (Reissner’s fibre, RF) that extends along the ventricles and central canal. The SCO-RF complex is involved in CSF circulation and homeostasis. Mutant mice lacking SCO develop hydrocephalus. Specialized ependyma also occurs in neurogenic regions of the adult telencephalon. In bovine, it shows basal processes and immunoreactivity to radial glia and ependymal markers such as vimentin, GFAP, BLBP, S-100B protein and nestin. When cultured in media free of trophic support, ependyma of non-neurogenic regions of bovine lateral ventricles gradually acquires features of ependyma in neurogenic regions. Also, an IGF-1 dependent subependymal neurogenesis occurs. Choroid plexus, tanyocytes in the third ventricle, and the so-called circumventricular organs are also specialized ependyma. We are still far for understand the complex roles of the ependyma in the maintenance of brain homeostasis.

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C06-04

Putative linkage of FGF2 with AVP in brain fluid homeostasis: role of the choroid plexus
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The choroid plexus (CP) and ependymal cells are both a source and target for neuropeptides that regulate brain fluid balance. In the choroidal epithelium we have found that basic fibroblast growth factor (FGF2) colocalizes with AVP, a neuropeptide that regulates CSF formation. Also, FGF2 inhibited CSF production by both the in vitro (primary culture) and in vivo choroid plexus. To explore further the role of FGF2 in brain fluid homeostasis, we analyzed the response of CP epithelium and other central fluid-regulating regions to dehydration. Adult Sprague-Dawley rats were deprived of water for 72 h. The choroidal epithelium of the lateral ventricle displayed markedly enhanced expression of FGF2 (immunostaining). FGF2 was also strongly upregulated in the cytoplasm, apical membrane and nuclei of virtually all epithelial cells, and downregulated in the extracellular matrix. Interestingly, in the neurohypophysis of the dehydrated animals there was also augmented expression of FGF2 in the Herring bodies, pituicytes and perivascular basement membranes. The upregulation of FGF2 in the choroidal epithelium is similar to the increase in AVP, in response to water deprivation. The similar adjustments of the blood-CSF barrier in regard to the expression and actions of these peptides suggest functional linkage between FGF2 and AVP. Evidence is steadily building for a role by FGF2 in fluid homeostasis.

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C06-05

Vitamin C transporters in ependymal cells
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Ascorbic acid (AA) is best known for its role as an essential nutrient in humans and other species. AA cannot be synthesized within the brain; therefore, high levels in the brain are achieved by specific uptake mechanisms which concentrate AA from the bloodstream into cerebrospinal fluid and from cerebrospinal fluid into intracellular compartment. Recently, two different isoforms of sodium-vitamin C cotransporters (SVCT1 and SVCT2) have been cloned. Both SVCT proteins mediate high affinity Na+-dependent L-ascorbic acid transport and are necessary for the uptake of vitamin C in many tissues. Herein we analyzed the normal localization of SVCT2 in fetal and adult brain by immunohistochemistry and in situ hybridization demonstrating that SVCT2 is selectively expressed in the ventricular and subventricular area of the rat fetal brain. The choroid plexus showed an early expression of SVCT2 in the cytoplasmic area of the cells. A similar expression of SVCT2 was observed in human fetal brain. In adult brain, SVCT2 is mainly expressed in neurons, hypothalamic ependymal cells and choroid plexus cells. Ultrastructural immunohistochemistry demonstrated that SVCT2 is polarized to the apical membrane of ependymal cells. Functional analysis determined the kinetic parameters associated with the uptake of vitamin C in hypothalamic cultured mouse ependymal cells (tanyocytes), neurons and choroid plexus cells isolated from a human papilloma. The results demonstrate that specific ependymal cells express a high-affinity vitamin C transporter. Vitamin C uptake mechanisms present in these cells may perform a neuroprotective role concentrating vitamin C in specific areas of the brain.

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Colloquium C07: The cell biology of myelin repair

Chair: R. Franklin and J. Mason

C07-01
New themes in CNS remyelination
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Unravelling the mechanisms involved in the regenerative process of remyelination is not only of intrinsic biological interest but is also fundamental to understanding the natural history of major demyelinating diseases such as multiple sclerosis and providing the basis from which new therapies may emerge. This presentation will review recent development in the cell and molecular biology of CNS remyelination. Although the biology of remyelination has much in common with the extensively studied developmental process of myelination, recent evidence indicates that important differences occur. For example, the regenerative process appears to exhibit a much greater degree of functional redundancy than the developmental process, implying that in regeneration a network of factors may be more important than the expression of individual factors. The analysis of these networks may be better served by genomic and proteomic analysis than conventional analytical techniques of gene over expression or knock-out.

C07-02
Oligodendrocyte regeneration and remyelination after chronic demyelination
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In multiple sclerosis lesions, remyelination typically fails with repeated or chronic demyelinating episodes. In response to demyelination, growth factors regulate the generation of remyelinating oligodendrocytes from endogenous oligodendrocyte progenitors (OP) in the adult CNS. We used acute cuprizone (0.2% for 6 weeks) demyelination of the corpus callosum in mice with genetic deletions as a model to determine the roles of endogenous growth factors during remyelination. Using platelet-derived growth factor alpha receptor (PDGFαR) heterozygotes we found that PDGFαR signaling is critical for robust OP amplification in response to acute demyelination. Analysis of fibroblast growth factor 2 (FGF2) knockout mice indicated that FGF2 does not cooperate with PDGF to regulate this OP amplification. The predominant role of endogenous FGF2 in vivo is inhibition of OP differentiation into mature oligodendrocytes. Absence of FGF2 enhances oligodendroglial repopulation of acute lesions. Furthermore, in FGF2+/−PDGFαR+/−mice, FGF2 deletion rescues the compromised oligodendrocyte regeneration of PDGFαR heterozygotes. We further tested this FGF2 deletion effect during recovery from chronic demyelination. Wild type mice fed 0.2% cuprizone for 12 weeks have fewer OP cells, compromised oligodendrocyte regeneration, and impaired remyelination. After chronic cuprizone treatment, the extent of remyelination is significantly improved during recovery in FGF2−/−mice. This remyelination in FGF2−/−mice correlates with enhanced oligodendroglial repopulation of the corpus callosum. These findings indicate that blocking signals that inhibit OP differentiation can enhance remyelination of chronically demyelinated axons.

Acknowledgements: NIH grant NS39293, NMSS grant RG3515.

C07-03
IGF-1 protects oligodendrocytes and prevents the formation of chronically demyelinated lesions
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Metabolic insult results in apoptosis and depletion of mature oligodendrocytes during demyelination. Although there is rapid regeneration of the oligodendrocyte population following an acute lesion, most of these newly regenerated cells undergo apoptosis if mice remain on a cuprizone diet. To determine whether insulin-like growth factor-1 (IGF-1) can inhibit the death and deletion of oligodendrocytes during the progression of a demyelinating lesion to a chronic state, we exposed transgenic mice that continuously express IGF-1 (IGF-1 tg) to cuprizone intoxication. Demyelination was observed within the corpus callosum in both wildtype and IGF-1 tg mice 3 weeks after exposure to cuprizone. Wildtype mice showed significant apoptotic death of mature oligodendrocytes within the lesion that resulted in near complete depletion and demyelination by week 5. In contrast, the demyelinated corpus callosum of the IGF-1 tg mice was remyelinated by week 5. Furthermore, if mice were maintained on the cuprizone diet for 12 weeks, the oligodendrocyte population became progressively depleted within the wildtype mice and the corpus callosum became chronically demyelinated. However, there was very pathology or little loss of oligodendrocytes within the corpus callosum of the IGF-1 tg mice. These results suggest that IGF-1 can prevent the depletion of mature oligodendrocytes during a demyelinating insult and thus facilitate a rapid recovery from remyelination, while also preventing the formation of chronically demyelinated lesions.

C07-04
Histone deacetylase activity in oligodendrocyte development and repair
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Remyelination requires the formation of new myelin by resident cells located around the lesion site and is a multi-step process requiring changes in gene expression. We have shown that during the developmental progression of oligodendrocyte progenitors to differentiated oligodendrocytes, histones are deacetylated. Histones are basic components of chromatin and when deacetylated, they can repress transcription. Histone deacetylation in oligodendrocytes progenitors correlated with decreased expression of inhibitors of process outgrowth and of the transcriptional program of differentiation. Blocking histone deacetylase activity with pharmacological inhibitors prevented developmental myelination, both in vivo and in vitro. We have now asked whether similar events occur during repair after demyelinating lesions. Acknowledgements: Supported by NIH-NINDS RO1NS42925 and by NMSS RG 3421-A4.

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Fate-regulating pathways in adult human oligodendrocyte progenitors

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An abundant population of nominally glial progenitor cells resides in the adult human white matter. To identify signal pathways that regulate their homeostasis and fate decisions, we analyzed the transcriptional profile of A2B5⁺ white matter progenitor cells (WMPCs) sorted from human surgical resections. WMPCs expressed high levels of PDGFαR, GD3 synthase and NG2, prototypic oligodendrocyte progenitor genes, yet they also expressed high levels of MASH1 and HES1, suggesting a more primitive phenotype. The members of several parallel signaling pathways were differentially expressed by WMPCs. These included the receptor tyrosine phosphatase (RTP)-β/γ, its ligand pleiotrophin, and its modulators tenascin-R, NrCAM, and the chondroitin sulfate proteoglycans (CPSG2–5); several β-catenin target genes which in turn may be regulated by RTP-β/γ mediated dephosphorylation of β-catenin; and PDGFαR, which may induce pleiotrophin and thereby regulate RTP-β/γ. The functional importance of the RTP-β/γ pathway was tested in vitro by exposing WMPCs to the oxovanadate PTP inhibitor bpV(phen). RTP-β/γ blockade resulted in dose dependent induction of oligodendrocyte differentiation and consequent loss of the progenitor phenotype: increasing the O4/A2B5 ratio in these cultures from <10% to >70%. These observations suggest the necessity of the RTP-β/γ pathway in progenitor self-maintenance. The integrated output of the RTP-β/γ, β-catenin, pleiotrophin, and PDGFαR pathways may thus regulate the maintenance of adult progenitors in an undifferentiated state. As such, these pathways provide targets by which to perturb cell fate choices by progenitor cells of the adult human brain.

Forced expression of OLG1 transcription factors promotes neural stem cell-derived oligodendrocytes for myelin repair

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The adult CNS has an inherent capacity to regenerate oligodendrocytes after demyelination, raising the possibility that therapeutic strategies designed to promote endogenous remyelination may have beneficial impacts for the treatment of demyelinating diseases, such as multiple sclerosis. Here, we address whether forced expression of Olig genes represents a possible strategy to enhance remyelination. The bHLH transcription factors Olig1 and Olig2 are known to be essential for oligodendrocyte differentiation during development. Olig2 is necessary for oligodendrocyte specification, while Olig1 is required for myelination and remyelination of the CNS (Xin et al., 2005; Arnett et al., 2004).

In this study, we analyze the expression of Olig genes in neural stem cell (NSC) and their progeny isolated from embryonic mouse and primate CNS. We demonstrate that Olig1 and Olig2 are expressed in NSC and at all stages of the oligodendrocyte lineage. To test whether forced expression of Olig genes in NSC can promote their differentiation in oligodendrocytes, NSC over-expressing Olig1 or Olig2 were obtained by stable transfection. Our data show a two-fold increase of O4⁺ oligodendrocytes in neurospheres over-expressing Olig2. To assess if forced expression of Olig1 or Olig2 in NSC can promote their differentiation for myelin repair, we next generated transgenic mice with inducible expression of Olig1 or Olig2 under the control of the nestin CNS-specific enhancer. Our results indicate that forced expression of Olig genes increases the production of oligodendrocytes and induces early myelination during development. Therefore, these transgenic mice provide a useful model to assess the effects of Olig genes during remyelination.
Workshop W03: Promoting research integrity: do we need better scientists or better science?

Chair: R. DeVries

W03-01
Promoting research integrity: do we need better scientists or better science?

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This workshop will review and advance the debate over what must be done to reduce research misconduct among scientists. Some argue that we need to build better scientists – instructing newcomers on the norms of science, sharpening their consciences and their consciousness of proper and improper behavior. Others believe that it is the structure of science – with high degree of competitiveness and limited rewards – that spawns improper behavior. Professor De Vries will moderate the interactive workshop, which will center on three themes: (1) Brief review of the current research on research integrity/research misconduct. What constitutes research misconduct? What characteristics of researchers AND research settings are associated with integrity/misconduct? (2) ‘We need better training’ – research integrity is best promoted by making better scientists. Review of what works and does not work in training for the responsible conduct of research. (3) ‘We need to alter the structure of science’ – a look at the factors that increase the pressure under which scientists work.
Role of glycogen in supporting energy metabolism of CNS axons
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We tested the hypothesis that astrocytic glycogen supports CNS axon function during glucose deprivation and intense activity. Functional activity of the rat or mouse optic nerve, representative white matter areas, was assessed electrophysiologically as the area under the supermaximal compound action potential (CAP). Area of rodent optic nerve persisted for about 30 min during aglycemia, after which the CAP rapidly failed. Glycogen content measured biochemically fell during glucose withdrawal with a time course compatible with rapid use in the absence of glucose. Increased glycogen content induced by high-glucose pretreatment increased latency to CAP failure and improved CAP recovery from 60 min of aglycemia. Conversely, decrease of glycogen content induced by norepinephrine or low-glucose pretreatment, decreased latency to CAP failure and decreased CAP recovery. Lactate transport blockers, applied during aglycemia, decreased latency to CAP failure and decreased CAP recovery. Glycogen also decreased during high frequency axon discharge and CAP area declined if lactate transfer was blocked. Lactate transporters are expressed on axons and astrocytes. These results indicate that during aglycemia and heightened metabolic demand, astrocytic glycogen was broken down to lactate which was transferred to axons as fuel.

Manipulation of brain glycogen levels in vivo: effects on neuron function and survival during severe hypoglycemia
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Brain glycogen is localized primarily to astrocytes. The manipulation of astrocyte glycogen content is an important tool for furthering our understanding of the normal role of glycogen in brain. Studies using cell culture and optic nerve preparations have shown that increased astrocyte glycogen reserves can prolong the function and survival of neurons during glucose deprivation. This has been difficult to demonstrate in vivo, however, because of the difficulty in manipulating brain glycogen content without also changing brain glucose content or other relevant aspects of brain metabolism. A novel approach to this problem is the use of ‘I-site’ phosphorylase inhibitors. These agents inhibit glycogen phosphorylase in the presence of glucose, and thereby increase basal glycogen levels. Importantly, this inhibitory action is lost at low glucose concentrations, permitting utilization of the increased glycogen store. Astrocyte cultures treated with one of these agents, CP316819, showed a 250% increase in glycogen content. Under substrate-deprivation conditions these cultures consumed this glycogen store and showed increased cell survival. Rats treated in vivo with CP316819 showed an 80% increase in brain glycogen. When subjected to insulin-induced hypoglycemia, these rats maintained EEG activity much longer than control rats, despite equivalent blood glucose concentrations. The treated rats also showed substantially less neuron death than the untreated rats after recovery from hypoglycemia. These studies support prior in vitro studies indicating a major role for astrocyte glycogen in maintaining neuron function and survival.

Role of glycogen in neurotransmission
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Despite the small amount of glycogen in brain, recent findings have indicated that glycogen in brain is essential for proper neuronal function also under normal conditions. It has been suggested that glycogen serves as an energy buffer, capable of providing rapid and short-term energy rather than an energy reserve, for long-term energy requirements. However, the underlying mechanisms are not known. Astroglial cells are of paramount importance for the homeostasis of neurotransmitter glutamate; i.e. glutamate uptake, glutamate degradation and supply of neurotransmitter precursors to the neurons, processes which are all essential for the maintenance of neuronal signaling. It is an interesting question to assign whether these energy requiring mechanisms and reactions need the rapid energy derived from glycogenolysis and/or oxidative metabolism of pyruvate derived from glycogen. Alternatively, glycogen derived lactate may be transported to neurons in order to sustain energy requiring processes involved in neuronal signaling. The role of glycogen turnover in glutamate neurotransmission and homeostasis was investigated in the presence of glucose (2.5 mM) in a co-culture system of cerebellar astrocytes and glutamatergic neurons. The cultures were placed in a superfusion system and release of pre-loaded [3H]D-aspartate was induced by NMDA to monitor neuronal glutamate release and subsequent uptake in the absence or presence of a glycogen phosphorylase inhibitor. The results indicate an effect on the net-release of [3H]D-aspartate by inhibiting glycogen degradation even in the presence of 2.5 mM glucose. Further experiments are needed to elucidate the mechanisms behind this phenomenon.
W04-04  
**Glycogenolysis may reveal a large, ‘hidden cost’ of astrocytic work**  
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Glycogen is consumed during functional activation and energy crises. Its role as an ‘energy provider’ when blood glucose is freely available is uncertain, but it is likely to be an active fuel, not a static reservoir. We recently found very high (11–12 μmol/g) but quite variable (cv = 35%) glycogen levels in cerebral cortex of carefully-handled rats. These values are 3–4-fold greater than usual levels, and the glycogen is biologically available, since 95% is degraded during postmortem ischemia. Glycogen extraction requires great care to minimize loss during the thawing-denaturation step. Generalized sensory stimulation for 5 min reduced glycogen content by 23% ($P = 0.07$, 1-tail t test), and after 15 min recovery it fell further (32%, $P < 0.05$); these findings were obtained in separate studies carried out about a year apart, with similar percent (22, 23%) and absolute (2.7, 3.1 μmol/g) reductions in glycogen level. Also, 16% of $^{13}$C in pre-labeled glycogen was released by a 30s stimulus ($P = 0.06$). Parallel metabolic balance analysis showed that $O_2$ uptake from blood to brain did not match that of glucose, and half of the ‘excess’ glucose uptake was accounted for increased lactate level. Inclusion of glycogen degraded during the stimulus interval in the calculated $O_2$/carbohydrate metabolic ratio increased the magnitude of oxidative mismatch and quantity of ‘missing’ lactate. Together, these findings suggest glycogen was not oxidized, so glycogen-derived lactate is not fuel for other brain cells; it was probably rapidly released from the tissue. Glycogenolysis is not easily measured in vivo and it raised the calculated glucose utilization rate by 50%, suggesting a high, ‘hidden cost’ to support astrocytic activity that might take place in the fine perisynaptic processes that lack mitochondria and surround synapses.

W04-05  
**In vivo studies of glycogen: turnover vs. net changes**  
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Glycogen is present in the brain in substantial quantities, that have historically probably been underestimated due to difficulties in biochemical measurement. In vivo, brain glycogen can be measured using non-invasive NMR spectroscopy in conjunction with administration of the stable $^{13}$C isotope (using 1–$^{13}$C glucose). Using this method in rats and humans, brain glycogen was reported to have an active, yet slow turnover when brain glucose concentration was not rate-limiting for metabolism. Due to the use of label and potential confounding effects from label scrambling, conservative estimation of glycogen changes suggested that brain glycogen accounts for the majority of the glucose supply deficit in hypoglycemia, is influenced by plasma glucose/insulin levels and supercompensates following a single episode of hypoglycemia. In chronic hypoglycemia, brain glycogen levels were unchanged but brain glucose levels were elevated, suggesting control of brain glycogen levels by brain glucose concentrations. Despite the potential confound of label turnover the changes in total brain glycogen concentration deduced from the NMR measurements were confirmed using biochemical assay following microwave fixation in 1.4s with minimal agonal artifact as verified by the low tissue lactate concentration. It is concluded that brain glycogen can cover the partial glucose supply deficit for extended periods of time, which emphasizes the important role of brain glycogen and its neuroprotective role in vivo.

W04-06  
**Inhibition of glycogenolysis at specific time points abolishes learning in day-old chick**  
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Transient reductions in glycogen content in the forebrain of day-old chicks occur at specific time points following one-trial aversive learning where chicks learn to distinguish between two differently colored glass beads, one of which had been tainted with an aversively tasting compound (O’Dowd et al. Dev. Brain Res 1994; 78: 137–141; Herz et al. Brain Res 2003; 994 : 226–233). The sequence of metabolic events during subsequent memory consolidation is known in detail, as are the windows of susceptibility for many types of pharmacological intervention (Gibbs and Summers. Prog Neurobiol 2002; 67: 345–391). However it is not known whether glycogenolysis is essential for learning. Glycogen-derived pyruvate may function as an energy substrate or as precursor for de novo formation of glutamate by astrocytic pyruvate carboxylation. We have used the specific phosphorylase inhibitor, 1,4-dideoxy-1,4-arabinitol (DAB), to prevent breakdown of glycogen to determine if there is any effect on memory. Intracerebral injection of arabinitol caused dose-dependent inhibition of learning, and there were three time periods during the memory sequence when memory processes are susceptible to inhibition by DAB: 5 min before training and 25–35 and 55 min post-training. Two of these times are similar to the reported decreases in glycogen content. There was also a marked similarity in the timing of the participation of glutamate and glutamine during memory consolidation and two of the times when glycogen is involved. Our data demonstrate that glycogen breakdown is essential for normal learning in this paradigm and that an important role of glycogenolysis might be to provide pyruvate as the astrocytic precursor for transmitter glutamate.
O02-01 Specification of optic nerve oligodendrocyte precursors by retinal ganglion cell axons

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Commitment to a particular cell fate in the developing CNS frequently depends on localized cell–cell interactions. In the avian visual system, a population of optic nerve oligodendrocytes are derived from founder cells located at the floor of the 3rd ventricle. What controls the location of these cells has not been defined. Here we show that the induction of these cells is directly dependent on signaling from the axons of the retinal ganglion cells (RGC). In vitro retinal cues are capable of inducing oligodendrocytes from responsive tissues. In vivo, the appearance of oligodendrocyte precursor cells (OPCs) correlates spatially and temporally and is dependent on RGC axonal projections. Eye removal prior to optic nerve formation dramatically reduced the number of OPCs in the floor of the third ventricle. Direct signaling from RGC axons to responsive tissue was demonstrated in compartmental chamber culture. Retinal axon induction of OPCs was dependent on both sonic hedgehog and neuregulin and inhibition of either signal reduced OPC induction in vivo and in vitro. These data suggest that in the visual system, the initial induction of oligodendrocyte lineage cells is directly dependent on retinal axons, the ultimate target for ensheathment by the mature cells.

O02-02 The dynamics and cellular transport of PLP is altered in the rumpshaker mutant

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The X-linked Plp gene encodes the most abundant myelin protein of the CNS, proteolipid protein (PLP), and its smaller isoform DM20. The spontaneous mouse Plp mutation rumpshaker (rsh Ile186Thre) is a dysmyelinating model in which the severity of the phenotype is influenced by the genetic background. This study sought to determine the influence of the rsh mutation on PLP turnover kinetics and the efficiency of PLP incorporation into myelin. The steady state level of PLP translation rate was comparable to WT but its degradation was blocked when the Golgi was disrupted with Brefeldin A, while rsh PLP incorporation was unaffected. These results suggest the reduced steady state level of PLP in the rsh mutant is due to accelerated degradation and a significant proportion of PLP can insert into myelin via a Golgi independent pathway.

O02-03 Gas6/Axl signaling protects oligodendrocytes from TNF-induced apoptosis via the PI3 kinase/Akt survival pathway

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Previously, we demonstrated that growth arrest-specific protein 6 (gas6) prolonged human oligodendrocyte survival in vitro and protected against growth factor withdrawal. In this study we examined whether rhgas6 protects oligodendrocytes against tumor necrosis factor alpha (TNFα) cytotoxicity and if so, identify the receptor involved in the protective effect. Cytotoxicity was quantified by TUNEL staining (% survival = 100% (O4+/TUNEL+)/total O4+)) and was shown to be caspase-dependent. The survival rate was 18% in oligodendrocyte cultures treated with TNFα alone, compared with 65% and 63% in cultures treated with the caspase inhibitors IETD-fmk and zVAD-fmk respectively. Oligodendrocyte cultures treated with TNFα (100 ng/ml) and rhgas6 (5.6 nM) had a survival rate of 64% and reduced active caspase-3 immunoreactivity. The gas6 protective effect was specifically blocked by the Axl decoy receptor Axl-Fc, and by the PI3 kinase/Akt inhibitor LY294002. Oligodendrocyte cultures established from wild-type and Rse−/− mice, but not oligodendrocytes from Axl−/− mice were protected from TNFα-induced cell death when maintained in rhgas6. In addition, a significant increase in phosphoAkt (Ser473) immunoreactivity was detected 15 min. after gas6 administration to TNFα-treated wildtype oligodendrocytes, but not in gas6-untreated, TNFα-treated cultures. We conclude that gas6 signaling through the Axl receptor and downstream to the PI3 kinase/Akt survival pathway protects oligodendrocytes from TNFα-mediated cell death.
The origin of the remyelination defect in multiple sclerosis (MS) is unknown. However, oligodendrocytes (OL) submitted to a single lyso-phosphatidyl choline (LPC) treatment (TT) in vitro, partially recover and synthesize myelin-like membranes; this process is enhanced by bFGF or PDGF (Fressinaud et al., 1996). Nevertheless, the number of myelinated fibers per OL is decreased in chronic MS plaques (Fressinaud and Jean, 2003), suggesting that relapses could alter the capability of resident mature OL to remyelinate. To determine how repeated insults impair OL survival and maturation, pure OL secondary cultures from newborn rat brain, grown in chemically-defined medium (CDM), were submitted to several LPC TT: split LPC TT (0.5 × 10⁻¹⁰⁸M, 4 × 6h), or multiple LPC TT (2.10⁻¹⁰⁸M, 2 × 24h), and were compared to unique TT (2.10⁻¹⁰⁸M LPC, 24h), and to controls (CDM alone). PDGF and NT-3, which improve remyelination in vivo after a unique injury (Allamargot et al., 2001; Jean et al., 2003), also have been tested in these conditions. Multiple LPC TT killed virtually all OL (mature and progenitors), and recovery did not occur. Cell loss was increased by split LPC TT (−92% versus control) compared to unique TT (−66%). Mature OL disappeared after split or multiple LPC TT. PDGF, and NT-3, rescued some injured OL and induced O-2A progenitor proliferation, without delaying their maturation. Thus, repeated attacks increase OL loss compared to unique injury, and there is no significant spontaneous recovery. Since the differentiation of surviving OL progenitors is impaired, and the plasticity of mature OL is restricted after repeated attacks, it appears likely that relapses in MS kill resident OL and alter the maturation of surviving cells, impairing thereby remyelination.

O02-05
BMP regulation of adult human oligodendrocyte progenitor fate
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To identify signaling pathways that regulate the self-renewal and lineage determination of adult human oligodendrocyte progenitors (OPCs), we compared the gene expression of sorted OPCs to the white matter from which they derived. This analysis revealed a set of differentially expressed transcripts that highlighted the significance of several parallel and interactive pathways. Prominent among these was bone morphogenetic protein signaling, in that isolated adult OPCs differentially expressed the ligands BMP2 and BMP7, while concurrently expressing two BMP4 antagonists, BAMBI and neuralin/ventroptin. In vitro challenge with BMP4 resulted in a strong dose-dependent increase in GFAP-defined astroglisisis, and a corresponding depletion of A2B5-defined OPCs. Conversely, neither BMP2 nor BMP7 exposure were associated with increased astroglisisis, though high levels of each were associated with a relative depletion of OPCs. Glial lineage commitment was further examined by treating the cells with noggin, a broad spectrum BMP antagonist. Treatment with noggin together with bFGF permitted the prolonged maintenance of undifferentiated OPCs, and suppressed BMP-induced astrocytic differentiation. These results support a model of autocrine maintenance of OPCs by BMP2 and 7-regulated pathways, with a concurrent tonic inhibition of BMP4, the activity of which would otherwise serve as a strong stimulus to astrocytic production by resident OPCs of the adult human white matter.

O02-06
Induction of bone morphogenetic proteins in mouse spinal cord during experimental autoimmune encephalomyelitis
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The extent of myelin repair in multiple sclerosis is generally insufficient to prevent the progression of disability. The persistence in MS plaques of precursor and immature oligodendrocytes suggests that the myelination program in these cells either lacks a positive signal to effect completion, or is restricted by an inhibitory signal. Bone morphogenetic proteins (BMPs) are among the signals implicated as repressors of oligodendrocyte development. We hypothesize that BMPs are upregulated in MS lesions and play a role in demyelination and astroglisisis in MS. We examined the expression of BMPs in an animal model of MS, chronic experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein peptide 35–55 (MOG peptide) in C57BL/6 mice. Real-time TaqMan polymerase chain reaction (PCR) was used to measure the expression of BMP-2, 4, 6, 7 mRNA in lumbar spinal cord (LSC) during chronic EAE. The expression of BMP-4, 6, and 7 in lumbar spinal cords of EAE vs vehicle-treated controls was upregulated more than three fold at day 14, 21, or 42 post-immunization. The relative increase in BMP-4, 6 and 7 mRNA expression correlated with clinical score of the disease. BMP4 mRNA was the most strongly expressed of the BMPs; its abundance was 40 fold higher than that of BMP6 and 7. In contrast, BMP2 mRNA levels were not significantly altered in EAE mice. Further investigation is needed to determine if the up-regulation of BMPs plays a role in demyelination by creating an environment that inhibits the oligodendrocyte precursor cells from differentiating into myelinating oligodendrocytes and increases astroglisisis.

O02-07
Neutralization of IL-16 reduces inflammation, demyelination, axonal damage, and reverses paralysis during relapsing-remitting EAE
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Infiltration of the CNS by CD4+ Th1 cells precedes onset and relapses of experimental autoimmune encephalomyelitis (EAE). We reported that (B6 × SJL) F1 (H-2b/s) mice, with severe relapsing-remitting disease, had extensive infiltration by CD4+ T cells compared to C57BL/6 (B6) (H-2b) mice, which developed mild low-relapsing disease in response to myelin oligodendrocyte peptide 35–55 (MOG35–55). This observation led us to search for mechanisms that specifically regulate trafficking of CD4+ cells in relapsing R2b/s mice. In this report we show that the CD4+ cell chemoattractant cytokine IL-16, has an important role in regulation of relapsing EAE induced by MOG35–55 in the (B6 × SJL) F1, (H-2b/s) mice. We found production of IL-16 within the CNS of mice with EAE. Levels of IL-16 in the CNS correlated well with the extent of CD4+ T cells and B cells infiltration during acute and relapsing disease. Production of IL-16 was observed by infiltrating CD4+ T cells, CD8+ T cells and B cells. Treatment with neutralizing anti-IL-16 antibody successfully reversed paralysis and ameliorated relapsing disease. In treated mice, diminished infiltration by CD4+ T cells, lesser demyelination and more sparing of axons were observed. Taken together, we show an important role of IL-16 in regulation of relapsing EAE. We describe a novel therapeutic approach to specifically impede CD4+ T cell chemoattraction in EAE, based on IL-16 neutralization. Our findings have high relevance for the development of new therapies for relapsing EAE and potentially MS.