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Presidential Lecture

PRL01 Saving the Synapse: Developmental Critical Periods and Alzheimer's Disease

PRL01-01

SAVING THE SYNAPSE: DEVELOPMENTAL CRITICAL PERIODS AND ALZHEIMER'S DISEASE

Carla Shatz
Stanford University, School of Medicine, Stanford, USA

Connections in adult brain are highly precise, but do not start out that way. Precision emerges during development as synaptic connections remodel in a process requiring activity (action potentials and synaptic transmission). Activity also regulates neuronal gene expression. In an unbiased screen, Major Histocompatibility Class I (MHCI) genes were unexpectedly discovered expressed in neurons and regulated by activity and visual experience. To assess requirements for MHCI in CNS, mutant mice lacking stable surface expression of all MHCI, or of 2 specific MHCI genes H2-Db and H2-Kb, were examined. Synapse pruning in developing visual system failed, and ocular dominance (OD) plasticity in visual cortex was greater than in WT. Furthermore, stimulation mimicking natural activity patterns of retinal waves failed to induce LTD at KbDb-/- retinogeniculate synapses, pointing to LTD as an underlying mechanism for synapse pruning. Neuronal expression of just H2-Db in KbDb-/- mice rescued synapse pruning, segregation and LTD in the LGN, despite a compromised immune system (Lee et al, 2014), underscoring H2-Db's unique and separate role in neurons. In a search for receptors that could interact with neuronal MHCI, PirB, an innate immune receptor, was found highly expressed in neurons in mouse CNS. In visual cortex of mutant mice lacking PirB, OD plasticity is increased (Syken et al., 2006; Kim et al, 2013; Bochner et al, 2014), and spine pruning on pyramidal neurons is deficient (Djurisic et al, 2013). The commonality of phenotypes present in these mice suggests a model (Shatz, 2009) in which PirB may bind and transduce signals from MHCI ligands in neurons. This family of molecules, thought previously to function only in immunity, may also act at neuronal synapses to limit how much synapse strength changes in response to new experience. These molecules may be crucial for controlling circuit excitability and stability in developing as well as adult brain. Changes in their function could contribute to developmental disorders such as Autism and Schizophrenia, and Alzheimer's disease where synapse pruning is excessive. Supported by NIH Grants EY02858, MH071666 and the Mathers Charitable Foundation

PRL02 Regulation of Neural-Immune Interactions Following CNS Injury and Infection

PRL02-01

REGULATION OF NEURAL-IMMUNE INTERACTIONS FOLLOWING CNS INJURY AND INFECTION

Dorian McGavern
National Institutes of Health, National Institute of Neurological Disorders & Stroke, Bethesda, USA

The central nervous system (CNS) contains a sophisticated neural network that must be constantly surveyed in order to detect and mitigate a diverse array of challenges. The innate and adaptive immune systems actively participate in this surveillance, which is critical for the maintenance of CNS homeostasis and can facilitate the resolution of infections, degeneration, and tissue damage. Infections and sterile injuries represent two common challenges imposed on the CNS that require a prompt immune response. While the inducers of these two challenges differ in origin, the resultant responses orchestrated by the CNS share some overlapping features and cellular participants. This lecture will focus on how the CNS immunologically discriminates between pathogens and sterile injuries. Using intravital imaging, we have obtained real-time insights into different CNS disorders such as viral meningitis, encephalitis, cerebral malaria, and traumatic brain injury. We have also developed the means to locally manipulate these disease processes using an approach referred to as transcranial drug delivery. These contemporary approaches have enabled us to identify the mechanisms underlying beneficial vs. detrimental neural-immune interactions. The consequence of these interactions depend critically their anatomical location and local guidance cues. The nature of these cues and the immune contribution to the aforementioned disorders will be discussed as well as straightforward therapeutic interventions that can be used to lessen neurological dysfunction.

PRL03 Epigenetic Mechanisms in Memory Function

PRL03-01

EPIGENETIC MECHANISMS IN MEMORY FORMATION

David Sweatt
University of Alabama at Birmingham, Neurobiology, Birmingham, USA

Regulation of chromatin structure and control of direct methylation of DNA are the principal mechanisms of epigenetic regulation. This presentation will address the idea that conservation of epigenetic mechanisms for information storage represents a unifying model in biology, with epigenetic mechanisms being utilized for cellular memory at levels from behavioral memory to development to cellular differentiation. Do epigenetic mechanisms operate in behavioral memory formation, including reward-based learning? We have generated several lines of evidence that support this idea that I will discuss. 1. Contextual fear conditioning and reward conditioning trigger alterations in hippocampal DNA methylation and histone post-translational modifications. 2. Inhibitors of DNA methylation block both hippocampal LTP and associative learning in vivo. 3. Remote contextual fear memory is associated with persisting changes in DNA methylation in the Anterior Cingulate Cortex, and DNMT inhibition can reverse established remote memory. 4. Histone acetylation increases in memory formation, and histone deacetylase (HDAC) inhibitors enhance both memory formation and hippocampal long-term potentiation. 5. Histone subunit exchange controls long-term and remote memory stabilization for threat learning.

PRL04 Deconstructing Smell

PRL04

DECONSTRUCTING SMELL

Linda Buck

Howard Hughes Medical Institute, Molecular Biology, Neuroscience, Seattle, USA

The sense of smell allows mammals to perceive a multitude of environmental chemicals as having a distinct odor. It also mediates the detection of pheromones and predator odors that elicit innate responses. We are interested in how the olfactory system detects different chemicals and how the nervous system translates those chemicals into diverse perceptions and behaviors. Using a combination of molecular, cellular, and genetic approaches, we have identified families of receptors that initially detect odorants and pheromones in peripheral sense organs, asked how those receptors encode the identities of different chemicals, and investigated how the signals they generate are routed and organized in the nervous system to yield distinct perceptions and instinctive responses. Our work also touches on other neural circuits that affect emotions and innate drives that modulate behavior.

Plenary Lectures

PL01 Mechanisms Regulating Mammalian Brain Development

PL01
EPIGENETIC REGULATION OF NEURONAL CONNECTIVITY IN THE MAMMALIAN BRAIN

Azad Bonni
Washington University of St. Louis, Neuroscience, St. Louis, USA

The assembly of neural circuits is critical for the development and function of the brain. Axon and dendrite morphogenesis culminating in synapse formation represent key events that orchestrate the establishment of neuronal connectivity. Our studies suggest that transcriptional and epigenetic regulators play essential roles in neuronal connectivity from axon specification to dendrite morphogenesis to synapse development in the mammalian brain. In recent studies, we have identified a function for the nucleosome and remodeling (NuRD) chromatin remodeling complex in granule neuron/parallel fiber synapse formation in the mouse cerebellum. Remarkably, the NuRD complex promotes the decommissioning of promoters at a subset of developmentally regulated genes and thereby drives synaptic connectivity. At the meeting, I will provide an update on the role of epigenetic mechanisms that control neuronal connectivity in the mammalian brain.

PL01-01
CORTICAL INTERNEURON FATE, A TALE OF TWO PROLIFERATIVE ZONES

Stewart Anderson
Children's Hospital of Philadelphia, School of Medicine, Philadelphia, USA

Fate determination in the mammalian telencephalon, with its diversity of neuronal subtypes and relevance to neuropsychiatric disease, remains a critical area of study in neuroscience. Most studies investigating this topic focus on the diversity of neural progenitors within spatial and temporal domains along the lateral ventricles. Often overlooked is whether the location of neurogenesis within a fate-restricted domain is associated with, or instructive for, distinct neuronal fates.

Here, we use in vivo fate mapping and the manipulation of neurogenic location to demonstrate that apical versus basal neurogenesis influences the fate determination of major subgroups of cortical interneurons derived from the subcortical telencephalon. Somatostatin- expressing interneurons arise mainly from apical divisions along the ventricular surface, whereas parvalbumin-expressing interneurons originate predominantly from basal divisions in the subventricular zone. As manipulations that shift neurogenic location alter interneuron subclass fate, these results add an additional dimension to the spatial-temporal determinants of neuronal fate determination.

PL01-03
CONTROL OF CORTICAL PROGENITOR POTENTIAL BY SHH SIGNALING

Samuel Pleasure, Odessa Yabut
UCSF Institute for Regeneration Medicine, Department of Neurology, San Francisco, USA

Proper lineage progression and diversification of neural stem and progenitor cells during cortical embryonic development ensure the precise generation of projection neuron subtypes in the mammalian neocortex. Here we show that Suppressor of Fused (Sufu) controls cortical projection neuron specification by maintaining the identity of cortical progenitors. Deletion of Sufu from progenitors in the E10.5 mouse neocortex resulted in the impaired production and specification of cortical projection neurons. These defects stemmed from a significant loss of intermediate progenitor cells (IPC) and the misexpression of ventral forebrain progenitor markers in cortical progenitors during corticogenesis. We found that in the absence of Sufu, Gli2 and Gli3 were highly unstable and failed to properly attenuate Shh signaling. The role of Sufu in maintaining progenitor identity is critical at early stages of corticogenesis since deletion of Sufu at E13.5 did not cause similar abnormalities. Our studies revealed that inhibition of Shh signaling at early stages of neurogenesis via Sufu activity is critical for specification and maintenance of cortical progenitors to ensure that cortical projection neurons are properly generated regardless of neuronal birthdate.

PL01-04
CHROMATIN REGULATION OF NEURONAL DIFFERENTIATION

Anne West, Fang Liu, Chris Frank, Greg Crawford
Duke University, Neurobiology, Durham, USA

To identify chromatin mechanisms of neuronal differentiation, we have characterized chromatin accessibility and gene expression in cerebellar granule neurons (CGNs) of the developing mouse. We first used DNase-seq to map accessibility of cis-regulatory elements and RNA-seq to profile transcript abundance across postnatal stages of neuronal differentiation in vivo and in culture. We observed thousands of chromatin accessibility changes as CGNs differentiated, and verified, using ChIP-seq, reporter gene assays and CRISPR-mediated activation, that many of these regions function as neuronal enhancers. Motif discovery in differentially accessible chromatin regions suggested a previously unknown role for the Zic family of transcription factors in CGN maturation. We confirmed the association of Zic with these elements by ChIP-seq and found, using knockdown, that Zic1 and Zic2 are required for coordinating mature neuronal gene expression patterns. Finally our analysis of repressive histone modifications suggests that developmental regulation of gene repression by the induction of histone demethylases

plays an important role in timing the induction of genes required for neuronal differentiation. Together, our data reveal chromatin dynamics at thousands of gene regulatory elements that facilitate the gene expression patterns necessary for neuronal differentiation and function.

PL02 From the Synapse to the Blood Brain Barrier, Glia's Unique Roles in Brain Function and Dysfunction

PL02-01

GLIAL INVOLVEMENT IN THE MOTOR NEURON DISEASE ALS

Brian Kaspar
Nationwide Children's Hospital, Neurology, Columbus, USA

No abstract submitted.

PL02-02

ADHESION MOLECULES OF THE BBB AND NEUROINFLAMMATION

Alexandre Prat
CHUM, Neuroscience, Montreal, Canada

The Blood Brain Barrier (BBB) protects the central nervous system by regulating molecular and cellular exchanges between the brain and the blood. The BBB is made of a network of tightly adherent endothelial cells (ECs) surrounded by astrocytic processes and pericytes which provide factors that contribute to BBB maintenance. Several proteomic based-profiling of human and animal BBB endothelial cells have revealed the presence of unique regulatory proteins involved in BBB physiology and trans-endothelial leukocyte migration, including proteins involved in cellular adhesion, cell structure, BBB development, immunity and defense, transport and trafficking and signal transduction. Recent work, using animal models of MS, as well as human in vitro, in situ and ex vivo analyses revealed that these new BBB candidate proteins, including the adhesion molecules ALCAM, MCAM and DICAM are involved in the regulation of immune cell trafficking across vascular structures of the CNS. This presentation will provide a short overview of the progresses that were made over the last 5 years to identify novel pathways that are involved in the selective recruitment of specific immune cells to the CNS and in the process of CNS immune quiescence. These molecules are currently seen as the basis for the development of future therapies in neuroinflammatory disorders, including multiple sclerosis.

PL02-03

BIOLOGICAL ADAPTIONS OF MALIGNANT GLIA

Harald Sontheimer
Virginia Tech, School of Neuroscience, Blacksburg, USA

Malignant transformation of glial cells or glial committed stem cells gives rise to gliomas, the largest group of primary malignant brain tumors. They developed a number of unique biological traits that facilitate their growth and invasion. Notably, gliomas associate with blood vessels, which they use as guide for migration. In so doing they displace astrocytic endfeet, impair glio-vascular coupling and breach the blood brain barrier, potentially facilitating nutrient access. Rather than spreading hematogenously as is typical of systemic cancers, glioma cells migrate in an amoeboid fashion employing K^+ and Cl^- channels to dynamically regulate their shape and cell volume to maximize their chance of fitting into the narrow extracellular brain spaces. Since there is little room for growth within the skull, gliomas vacate space to support their growth by actively killing surrounding neurons, essentially presenting as a neurodegenerative disease. They do so through assiduous release of glutamate inflicting excitotoxic neuronal death. Glioma-released glutamate is responsible for peritumoral seizures, which in many patients give rise to tumor-associated epilepsy. The transporter responsible for glutamate release is a cysteine-glutamate exchanger that normally serves the production of the cellular antioxidant glutathione. In spite of mutations in tumor suppressor and oncogenes shared with other cancers, the many biological adaptations that support glioma growth and invasion are brain specific and employ pathways that may be specifically targeted therapeutically.

PL02-04

GLIA TARGETS FOR COGNITIVE ENHANCEMENT AND THE TREATMENT OF ALZHEIMER'S DISEASE

Philip Haydon
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In this lecture studies will be presented which demonstrate the therapeutic potential of target distinct glial receptors that are expressed by astrocytes and by microglia in order to enhance brain function in health and disease. Initial results will be presented which show that the activation of nicotinic acetylcholine receptors, either pharmacologically or via optogenetic activation of cholinergic afferents, leads to enhanced occupancy of the co-agonist site of the NMDA receptor as a result of increased release of D-serine from astrocytes. These results are of particular interest for their therapeutic potential in overcoming the negative symptoms of schizophrenia by increasing NMDA receptor function.
Subsequently results of both pre-clinical and clinical studies will be presented concerning P2Y6 receptors, which are expressed by microglia, about how this receptor is a target for novel disease modifying strategy for the treatment of Alzheimer's disease. Modulation of the P2Y6 receptor by an orally bioavailable, safe and well tolerated small molecule leads to enhanced clearance of central amyloid as well as to anti-inflammatory effects. This two-pronged strategy offers a new opportunity for disease modification in neurodegenerative disorders.

PL03 Down the Memory Lane

PL03-01
MECHANISM OF RAPID ANTIDEPRESSANT ACTION

Lisa Monteggia
UT Southwestern Medical Center, Neuroscience, Dallas, USA

Recent clinical studies have demonstrated that a single subpsychotomimetic dose of ketamine, an ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist, produces a rapid antidepressant response in patients with major depressive disorder, with effects lasting up to 2 weeks. Despite enthusiasm about this unexpected efficacy of ketamine, its widespread use as a fast-acting antidepressant in routine clinical settings is curtailed by its abuse potential as well as possible psychotomimetic effects. However, the ability of ketamine to produce a rapid and long-lasting antidepressant response in patients provides a unique opportunity for investigation of mechanisms that mediate these clinically relevant behavioral effects. Recent work from the our laboratory has linked ketamine's mechanism of action to homeostatic synaptic plasticity processes activated after suppression of NMDA-mediated glutamatergic neurotransmission. Data demonstrating that ketamine mediated blockade of NMDA receptors at rest deactivates eukaryotic elongation factor 2 (eEF2) kinase, resulting in reduced eEF2 phosphorylation and desuppression of rapid dendritic protein translation, including BDNF (brain-derived neurotrophic factor), which then contributes to synaptic plasticity mechanisms that mediate long-term effects of the drug will be discussed. The identification of this intracellular signaling pathway to rapid antidepressant action may serve as a viable therapeutic target for fast-acting antidepressant development.

PL03-02
DYNAMIC DNA METHYLATION REGULATES NEURONAL FLEXIBILITY

Hongjun Song
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Epigenetic modifications of chromatin, including the genomic DNA and histone proteins, play critical roles in orchestrating transcriptomes of all cell types. We found that neuronal stimulation induces region-specific active DNA demethylation in a Gadd45b- and TET1-dependent fashion in the adult mouse dentate granule neurons in vivo (Ma et al. *Science* 2009; Guo et al. *Cell* 2011). Our genome-wide analysis further revealed that 1.4% of all CpGs measured exhibit rapid activity-induced demethylation or de novo methylation (Guo et al. *Nat. Neurosci.* 2011). These activity-modified CpGs exhibit a broad genomic distribution with significant enrichment in low-CpG density regions, and are associated with brain-specific genes related to neuronal plasticity. Our single-base methylome analysis discovered significant levels of nonCpG methylation in these neurons and we identified DNMT3A and MeCP2 as a writer and a reader for nonCpG DNA modification (Guo et al. *Nat. Neurosci.* 2013). More recently, our studies suggest that active DNA demethylation and DNA repair serve as a synaptic activity sensor to epigenetically regulate fundamental properties and meta-plasticity of neurons and animal behavior (Yu et al. *Nat. Neurosci.* 2015). Together, our studies implicate novel modifications of the neuronal DNA methylome as a previously under-appreciated mechanism for activity-dependent epigenetic regulation in the adult nervous system under both physiological and pathological conditions.

PL03-03
NOVEL NUCLEOBASE MODIFICATIONS ASSOCIATED WITH MEMORY FORMATION

Timothy Bredy
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RNA modification has emerged as a novel layer of epigenetic control over gene expression that is perfectly suited to serve as a key post-transcriptional regulator in the fine-tuning of gene expression related to adaptation. One of the most prevalent and widely conserved RNA modifications, N6-methyladenosine (m6A), has been shown to be both dynamic and reversible, and is present in neurons. However, whether this epitranscriptomic mechanism is fundamental for the regulation of gene expression underlying learning and memory, has not been explored in any detail. Using m6A capture followed by RNA sequencing, we have discovered an experience-dependent redistribution of m6A in the prefrontal cortex of mice following fear conditioning. This epigenetic mark accumulates in specific regions of the transcriptome and early evidence suggests that it may dictate the fate of messenger RNAs and/or mRNA stability. Importantly, we have also found that the RNA demethylase, FTO, contributes to the formation and maintenance of fear-related memory Thus, our data demonstrate the dynamic nature of the epitranscriptome and suggest that, like DNA methylation, m6A is involved in the regulation of genes directly related to learning and memory.

PL03-04
TARGETS OF HISTONE ACETYLATION IMPORTANT FOR MEMORY STORAGE

Ted Abel
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Transcriptional activation is thought to be a key process in long-lasting forms of memory and synaptic plasticity. This activation is directed by transcription factors and their coactivators, which regulate gene expression via chromatin remodeling, histone modification and interactions with the basal transcription machinery. One type of histone modification associated with transcriptional activation is acetylation, which is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) that add or remove acetyl groups from histones, respectively. The transcriptional coactivator CREB-binding protein (CBP), a potent HAT, is involved in specific forms of long-term memory and synaptic plasticity. Mutant mice in which CBP activity in neurons is reduced

either by the transgenic expression of an inhibitory form of *cbp* lacking the HAT domain or by knocking in a mutation of the CREB transcription factor-binding KIX domain of *cbp* exhibit deficits in spatial and contextual memory and in long-lasting forms of hippocampal synaptic plasticity. A complementary method to study the role of histone acetylation in synaptic plasticity and memory is to examine the effects of HDAC inhibitors, which increase the level of histone acetylation that correlates with transcriptional activation. We found that increasing histone acetylation using the HDAC inhibitor TSA enhances long-term contextual memory and facilitates synaptic plasticity via the transcription factor CREB. Using genetic approaches, we have found that conditional deletion of the transcriptional corepressor Sin3a results in enhanced contextual memory, consistent with the idea that HDACs are recruited to specific genes by Sin3a-containing complexes. We have identified a family of nuclear receptors that appears to be among the gene targets of HDAC inhibition critical for this cognitive enhancing activity. Histone acetylation may provide an epigenetic mechanism for establishing gene-specific modifications that result in the coordinate expression of genes required for long-term memory storage and HDAC inhibitors may provide a novel therapeutic approach to treat the cognitive deficits that accompany many psychiatric disorders.

PL04 Cellular and Molecular Mechanisms of Neurodegeneration

PL04-01

IDENTIFICATION, VALIDATION AND TARGETING OF HUMAN MOLECULAR NETWORKS CONTRIBUTING TO NEURODEGENERATION AND COGNITIVE DECLINE

Philip De Jager
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Using a cohort of older subjects with longitudinal antemortem phenotyping, prospective brain collection and quantitative neuropathological examination, we have initiated a program to identify the regions of the genome that are involved in common neuropathologies. We have examined one region of the brain involved in mood and cognitive circuits – the dorsolateral prefrontal cortex – in each individual using DNA methylation profilling (Illumina 450K, n=708) and chromatin immunoprecipitation for the histone H3K9 acetylation mark (ChIPseq, n=680), and RNA sequencing (n=638). We use these data to define modules of co-expressed genes which are assembled into a directional molecular network that outlines the relationship among modules as wel as between modules an traits such as Alzheimer's disease and cognitive decline. Driver genes are identified for modules associated with neurodegeneration, and we we are currently validating these driver genes by systematic knockdown and overexpressing them in one or more in vitro system: iPSC-derived neurons, human astrocytes,and microglia-like cells. We demonstrate that elements of the associated networks derived from the human cportex are present in these in vitro models. Screening 40 putative driver genes has identified several that influence amyloid secretion. This functional validation along with transcriptomic profiling will identify a set of candidate genes that can be targeted for drug discovery.

PL04-02

INSIGHT INTO HUMAN NEURODEGENERATIVE DISEASE AND AGEING FROM DROSOPHILA

Nancy Bonini
University of Pennsylvania, Biology , Philadelphia, USA

Our laboratory uses the model organism *Drosophila* in order to define mechanisms and pathways associated with human neurodegenerative disease. We have been pursuing mechanisms of toxicity associated with RNA and RNA binding proteins associated with ALS and FTD. Our work has defined a number of pathways that impact disease progression; in particular, our studies indicate that stress pathways are integral to disease mechanisms, and the dysfunction or lack of sufficient protection from stress pathways may contribute to degeneration. In addition, we have found pathways associated with ageing that impact long term brain integrity and disease susceptibility. The miRNA miR-34 is a potent suppressor of polyglutamine toxicity in *Drosophila*. In flies, miR-34 is enriched in the brain, and increases with age. Our studies have shown that loss of miR-34 causes a premature brain aging in flies, highlighted by hallmarks including physical decline of the animal and a gene signature of accelerate aging in the brain. We have been investigating targets of miR-34 to define those that link long-term brain integrity with disease susceptibility. These studies indicate that miR-34 modulates epigenetic pathways; changes in these pathways appear to impact the function of the stress response with age. As the stress response also becomes dampened in mammalian cells with age, these findings suggest that epigenetic impacts on the ability of the stress response to function with age may be an important process that impacts susceptibility to brain disease.

PL04-03

REGULATION OF CNS INFLAMMATION BY ASTROCYTES

Francisco Quintana
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Astrocytes regulate local CNS inflammation in MS, thus it is important to characterize the mechanisms regulating astrocyte function as well as potential targets for the therapeutic modulation of astrocyte activity. We found that glycosphingolipid lactosylceramide (LacCer) is up-regulated in the CNS during chronic experimental autoimmune encephalomyelitis. Moreover, LacCer synthesized by β-1,4-galactosyltransferase 6 (B4GALT6) in astrocytes acts in an autocrine manner to trigger transcriptional programs that promote the recruitment and activation of CNS-infiltrating monocytes and microglia, and neurodegeneration. We also detected increased *B4GALT6* expression and LacCer levels in CNS MS lesions. Finally, the inhibition of glycosphingolipid metabolism suppressed local CNS innate immunity and neurodegeneration in EAE, and interfered with the activation of human astrocytes *in vitro*. Taken together, these results highlight the role of metabolism in controlling CNS inflammation, and identify potential targets for therapeutic intervention in MS.

PL04-04

DYNAMICS OF AUTOPHAGY IN NEURONS

Erika Holzbaur
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Neuronal homeostasis is dependent on efficient degradative pathways such as autophagy. We are examining autophagosome dynamics in primary neurons using live cell microscopy. Constitutive biogenesis of autophagosomes occurs preferentially at the distal end of the axon, via an ordered pathway of protein recruitment following stereotypical kinetics. Egulfment of cargos including aggregated proteins and mitochondrial fragments is observed during autophagosome formation. Following formation, autophagosomes are actively transported along the axon to the cell soma; maturation of the autophagosome and degradation of cargo occur en route. This constitutive form of autophagy can be compared to the dynamics of mitophagy, in which the selective recruitment of receptors such as optineurin to damaged mitochodria

in response to ubiquitination by parkin leads to efficient sequestration of the organelle from the cytosol. This sequestion is followed by degradation of the engulfed cargo by the autophagosome. We find that ALS-linked mutations in both optineurin and the upstream kinase TBK1 inhibit the sequestration of damaged mitochondria, suggesting that defects in mitophagy may contribute to the pathogenesis of motor neuron disease.

Symposia

S01 Myelination: A Biological Process Driven by the Cytoskeleton

S01-01

SUBMEMBRANOUS CYTOSKELETON STABILIZES SPECIALIZED MEMBRANE DOMAINS AT NODES OF RANVIER

Keiichiro Susuki
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Glial cells, oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system, form myelin sheaths surrounding the axons. The myelinating glial cells promote high densities of voltage-gated sodium channels at nodes of Ranvier, short gaps between two adjacent myelin segments. At paranodes flanking nodes, glial cells interact with axons and form junctions that restrict the mobility of the nodal sodium channel complex. These specialized domains are required for rapid and efficient propagation of action potentials along myelinated axons. Recent studies revealed that the cytoskeletal complexes play key roles in organization of nodes of Ranvier. Spectrins are a family of submembranous proteins that function in a multitude of regulatory capacities. They link membrane proteins to actin via ankyrins or 4.1 proteins and stabilize membrane domains. For example, axonal ankyrin-spectrin complexes stabilize sodium channels at nodes. Axonal protein 4.1B-spectrin complexes contribute to paranode and juxtaparanode organization. On the glial side, ankyrinG is enriched at paranodes in oligodendrocytes and facilitates node formation during early development. We recently found that αII and βII spectrin are expressed in both myelinating oligodendrocytes and Schwann cells. βII spectrin in Schwann cells is highly enriched at paranodes. In the mutant mice lacking βII spectrin in myelinating glial cells, we found disruption of nodal and paranodal molecular organization that was progressive with age, suggesting that the glial spectrin complexes contribute to the maintenance of these domains. These findings further underscore the importance of glial cytoskeleton in the organization of myelinated nerve fibers.

S01-02

HOW DOES THE ACTIN CYTOSKELETON DRIVE MYELIN WRAPPING?

J. Bradley Zuchero
Stanford University, Neurobiology, Stanford, USA

Myelination is essential in vertebrates for the rapid propagation of action potentials, and loss of myelin in MS has severe pathological consequences. To myelinate axons, oligodendrocytes undergo a series of defined morphology changes to migrate, extend processes, ensheath axons, wrap multiple times around them, and finally compact to exclude their cytoplasm to form the insulating myelin sheath. The cellular and molecular basis of each step is still largely unknown, but likely requires complex cellular signaling events and a dynamic cytoskeleton. Key proteins that regulate the actin cytoskeleton to drive cell motility in other systems are highly upregulated when OPCs differentiate and begin to myelinate, suggesting that actin dynamics are important for myelination. We characterized the role of the actin cytoskeleton in myelination by studying oligodendrocytes in isolation, in myelinating cocultures, and *in vivo*. We find two distinct stages of actin dynamics during myelination: a first in which actin assembly contributes to the extension of oligodendrocyte processes, and a second characterized by the striking disassembly of the actin cytoskeleton. The first phase is driven by the actin nucleating Arp2/3 complex, which builds lamellipodial networks of actin to push cell membranes forward. Inducing actin disassembly after this initial phase drives myelin membrane growth and myelin wrapping in vivo, and wrapping does not require Arp2/3. Intriguingly, the latter phase of actin filament loss coincides with the simultaneous upregulation of actin disassembly proteins and Myelin Basic Protein (MBP), and in *Shiverer* mice that lack MBP, actin disassembly is blocked. We propose a model in which MBP triggers actin disassembly to drive myelin wrapping and compaction.

S01-03

A GLUTAMATE TRANSPORTER-CAMKIIBETA SIGNALING AXIS AS A REGULATOR OF OLIGODENDROCYTE MORPHOGENESIS AND CNS MYELINATION

Babette Fuss[1], Zila Martinez-Lozada[1, 2], Christopher Waggener[1]
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Oligodendrocytes (OLGs), the myelinating cells of the central nervous system (CNS), undergo extensive changes in morphology when they mature first from bipolar OLG progenitors into premyelinating OLGs extending a complex and expanded process network and then into mature OLGs generating a fully functional myelin sheath. Such OLG morphogenesis is to a large extent driven by changes in the actin cytoskeleton, which occur during development spatially as well as temporally well-coordinated. Currently, however, little is known about the extracellular factors and downstream signaling pathways that are involved in orchestrating these morphological aspects of CNS myelination.
We introduce here a novel signaling cascade that involves the activation of sodium-dependent glutamate transporters and regulation of the actin-binding/bundling domain of

calcium/calmodulin-dependent protein *kinase* IIβ (CaMKIIβ) as a potential modulator of OLG morphogenesis and CNS myelination. More specifically, our findings so far point toward a mechanism by which glutamate transporter-mediated promotion of OLG morphogenesis is mediated by a transient phosphorylation event within CaMKIIβ's actin-binding/bundling domain leading to temporary actin unbundling. Thus, our data suggest that transient and possibly cyclic changes in actin cytoskeletal organization allow morphological remodeling without causing complete actin cytoskeletal collapse. *In vivo*, loss of CaMKIIβ in *Camk2b*$^{-/-}$ mice, and thus loss of specifically the actin binding/bundling function of CaMKIIβ, was found associated with the formation of thinner myelin. These findings are consistent with the idea that F-actin turnover is the driving force in myelin wrapping, which may, at least in part, be mediated by a cyclic acting sodium-dependent glutamate transporter-CaMKIIβ-actin cytoskeleton axis within differentiating OLG.

S01-04
FYN SIGNALING AND THE CYTOSKELETON IN OLIGODENDROGLIA

Donna Osterhout
Upstate Medical University, Cell & Developmental Biology, Syracuse, USA

Myelination in the developing central nervous system (CNS) is a complex process, the result of contact between the oligodendrocyte and neurons. Oligodendroglial progenitor cells (OPCs) are produced in specific regions of the ventricular zone, and migrate throughout the CNS during development. Once these OPCs arrive at the appropriate location, they differentiate into a mature oligodendrocyte, extending numerous processes which will make contact with axons. When an oligodendroglial process contacts an axon, it expands and begins to form the myelin membrane which will ultimately ensheathe the axon. Since mature oligodendrocytes extend many processes, one oligodendrocyte can myelinate many axonal segments.

The morphological transformation of an oligodendroglial progenitor cell, from a simple bipolar cell to a cell with multiple elaborate processes, is a key requirement for myelin formation. However, the signaling pathways that regulate these structural changes are not well understood. The activation of Fyn tyrosine kinase is an early step in the differentiation of OPCs. Fyn activation occurs in OPCs even before any changes in celullar morphology are observed. Fyn regulates the morphological differentiation of these cells by initiating process outgrowth and myelin sheet formation. In Fyn deficient mice, myelin formation is markedly reduced, demonstrating the importance of this kinase in myelination.

Fyn interacts with many downstream effectors, including molecular signaling pathways that interact with the cytoskeleton and initiate process outgrowth and myelination. These published findings, along with new studies, will be discussed in terms of defining the role Fyn kinase plays in regulating myelination.

S02 Axon-Glia Interactions in Development and Disease

S02
GENETIC DISSECTION OF SCHWANN CELLS, OLIGODENDROCYTES, AND MICROGLIA IN ZEBRAFISH

William Talbot
Stanford University, Department of Developmental Biology, Stanford, USA

Glial cells have diverse functions in the development and function of the nervous system, ranging from myelination to control of synaptic activity. Despite the importance of glial cells, much remains unknown about the genetic pathways that regulate their function.

To discover new genes with essential functions in glial cells, including Schwann cells, oligodendrocytes, and microglia, we have conducted several different genetic screens in zebrafish. A screen for abnormalities in myelin basic protein expression identified mutations in a zinc finger protein that regulates oligodendrocyte migration and myelination. A parallel screen for microglial mutants identified a G protein essential for lysosome biogenesis and function; the mutants can engulf apoptotic neurons but are unable to digest them properly. Current analysis on these genes and others will be presented.

S02-03
GLUTAMATERGIC SIGNALING IN MYELINATION AND DEMYELINATION IN RODENT MODELS OF DEVELOPMENT AND DISEASE

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Axon - glia interactions have important implications in development and disease. Proteins involved in glutamatergic signaling are developmentally regulated on oligodendrocytes during maturation suggesting an important physiological role. Our data show that oligodendrocyte progenitor cells (OPCs) respond to activity-dependent glutamate release from axons to turn on transcriptional programs necessary for myelination. This implies that glutamatergic input from axons may be a developmental signal instructing OPCs to form myelin. The goal of these studies is to elucidate sensitive periods when glutamatergic axon-glial signaling is necessary to promote myelination. Activity of glutamatergic processes on oligodendrocytes can also lead to excitotoxic mechanisms in disease states. Several lines of evidence suggest that glutamate dysregulation in the CNS is an important consequence of immune cell infiltration in neuroinflammatory demyelinating diseases like multiple sclerosis. Using conditional knockout mice, glutamate receptors were selectively deleted from oligodendrocytes to examine their response to excitotoxic mechanisms in experimental autoimmune encephalomyelitis (EAE). Mice deficient in glutamate receptors selectively on oligodendrocytes had improved motor function in the chronic phase of the disease compared to littermate controls. This improvement in clinical assessments correlated with less demyelination. Taken together, these data corroborate an important role for glutamatergic signaling on oligodendrocytes in both physiological and pathophysiogical processes.

S02-04
MECHANISMS OF NEUROGLIAL DAMAGE IN MOUSE MODELS OF MULTIPLE SCLEROSIS

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Here, I want to discuss how advances in in vivo microscopy can improve our understanding of the cellular, subcellular and molecular mechanisms that mediate inflammatory damage to the axon-myelin unit in the nervous system. Immune-mediated damage to axons and their myelin sheaths plays a crucial role in inflammatory diseases of the central nervous system (CNS) like multiple sclerosis (MS), as we know by now that the number of neuronal connections that are damaged by immune cells critically determines the clinical disability of MS patients. However we still understand very little about the processes that initiate damage to axons and their surrounding glial cells. In vivo visualization of fluorescently labeled macrophages/microglia and axons recently allowed us to observe the spatially restricted degeneration of axons in mouse models of MS. This “focal axonal degeneration” appears to be a novel type of axonal degeneration that is characterized by intermediated stages that can persist for several days and progress either to the degeneration or full recovery of the affected axons. Refined subcellular and molecular *in vivo* imaging approaches now further allow us to investigate the molecular mediators that drive axonal degeneration and to better understand the relation between structural and functional axon damage in neuroinflammatory lesions. Furthermore to better understand the interrelation of axon and myelin damage we have started to use similar imaging strategies in combination with ultrastructral analysis to reveal the early stages of oligodendrocyte damage in neuroinflammatory lesions. Using these examples, I hope to illustrate how recent advances in light microscopy can help us to reveal and mechanistically dissect the interactions of activated immune cells and CNS target cells as they happen in the living CNS.

S03 Complement in CNS Health and Disease

S03-01

REPAIR FROM THE PERSPECTIVE OF AN INFLAMMATORY NICHE AND THE COMPLEMENT CASCADE

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The interaction between neural stem cells (NSC) and the inflammatory microenvironment may influence the capacity of NSC for repair. We have shown that the inflammatory microenvironment generated by polymorphonuclear neutrophils and macrophages affects NSC fate and migration in vitro and in vivo, and that this is mediated in part via innate immune cell secretion of Complement molecules. Here we have tested the effects of direct exposure to purified Complement components at Neutrophil and Macrophage secreted concentrations on NSC, and investigated the molecular mechanisms involved. NSC were exposed to purified Complement components and cell survival, proliferation and differentiation analyzed. Proximity ligation assay (PLA) and receptor KO strategies were used to identify interactions between complement molecules and candidate transmembrane receptors. Combined treatment of NSC with Complement component combinations at PMN or MAC concentrations decreased proliferation and shifted differentiation away from an oligodendroglial lineage towards an astroglial lineage. Interestingly, this combinatorial effect is different from that detected after exposure to individual ligands, suggesting a complex interactive effect. To test whether the receptors identified in PLA analysis mediated some or all of these effects mouse NSC lines were generated from wildtype and knockout littermate embryos. Elucidating NSC-complement protein interactions, receptor targets, and intracellular pathways mediating these effects will provide a better understanding of the impact of the inflammatory microenvironment on NSC populations. Understanding these mechanisms may be a key approach to enabling effective stem cell therapies in central nervous system trauma, disease and aging.

S03-02

HOW COMPLEMENT ELIMINATES SPECIFIC SYNAPSES IN HEALTH AND DISEASE

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Microglia, the brain's resident immune cells and phagocytes, are emerging as critical regulators of developing synaptic circuits in the healthy brain. Recent studies from our lab and others indicate that microglia engulf synapses in the developing brain; however, how microglia know which specific synapses to target for removal remains a major open question. We found microglia preferentially engulf less active synapses in the visual thalamus (Schafer et al., 2012) and identified innate immune "eat me" signals C1q and C3, and microglial C3 receptor, CR3, as molecules that drive microglial phagocytosis of retinogeniculate inputs. Moreover, emerging data reveal that expression and localization of complement proteins are regulated by neuronal activity, suggesting that complement proteins mark less active synapses for elimination during development.
We hypothesize that protective signals are also required in the CNS to prevent inappropriate microglial engulfment of necessary connections during synaptic refinement. In support of this hypothesis, we have identified a classic 'don't eat me' signal that is enriched in the dLGN during peak pruning and required to prevent excess microglial engulfment of synaptic inputs. Its receptor is expressed by microglia in the dLGN during peak pruning and down regulated by microglia in adulthood. Mice lacking the protective signal and its receptor exhibit increased microglial engulfment of retinogeniculate inputs during peak pruning as well as overpruning of eye-specific territories and reduced synapse numbers in adulthood. These data are the first to demonstrate that "breaks" on microglial phagocytosis are required to protect necessary connections from inappropriate removal. Microglia-mediated synaptic refinement thus appears to depend on a careful balance of positive and negative regulators of phagocytosis, and understanding the consequences of disrupting this balance may provide insight into disorders characterized by immune dysregulation and synaptic circuit abnormalities, such as autism and schizophrenia.

S03-03

COMPLEMENT AS A MODULATOR OF NEURODEGENERATION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease of the elderly, with an incidence doubling every 5 years after 65 years of age, ultimately affecting 50% of people 85 and older. While AD is characterized by the presence of plaques composed of beta-sheet fibrillar β-amyloid (fAβ) and neurofibrillary tangles (NFT) composed of hyperphosphorylated tau, evidence continues to suggest a role for neuroinflammation in the progression, if not initiation, of cognitive loss seen in this disease. Previously, we showed that the C5aR1/CD88 antagonist, PMX205, significantly reduced inflammation and Aβ deposition in the Tg2576 model of AD and additionally reduced NFTs in the 3xTg model of AD. Data from C5aR1 knock out mice support our original pharmacologic finding, and AD mouse models overexpressing C5a in the brain show accelerated cognitive loss. Using MAP-2 staining as a marker of neuronal injury, we show that C5a can enhance the damage to neurons treated with fAβ alone. Blocking C5aR1 with the receptor antagonist PMX53 blocked the loss of MAP2 in these primary neurons treated with fAβ and C5a, providing further support for therapeutic targeting of C5a and C5aR1 for AD and

perhaps other neurodegenerative disorders. However, other data suggest that in early stages of injury or disease, the complement component C1q can rescue deleterious effects of toxicity to promote neuronal survival. Therefore, complement component activities provide insight on novel targets for therapeutic intervention in neurodegenerative disorders.

S03-04

A COMPLEMENT-MICROGLIAL AXIS IS REQUIRED FOR SYNAPSE ELIMINATION DURING VIRUS-INDUCED MEMORY IMPAIRMENT

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Neurocognitive sequelae are observed in >50% of patients who survive neuroinvasive infections with encephalitic arboviruses, such as the mosquito-borne West Nile virus (WNV). Early diagnosis and high survival rates from WNV neuroinvasive disease (WNND) (>90%) have thus led cumulatively to approximately ten thousand patients living with neurocognitive impairments, with 1-3000 cases accruing yearly, yet underlying mechanisms responsible for these deficits have not been investigated. Here, we established a novel murine model of recovery from WNND in which intracranial inoculation of the attenuated mutant WNV-NS5-E218A leads to similar CNS viral loads and inflammation as peripheral inoculation of its parent strain, WNV-NY99, with rates of survival and cognitive dysfunction that mirror human WNND. WNV-NS5-E218A-recovered mice exhibit impaired spatial learning without significant alterations in cortical and hippocampal volume or total neuron numbers, but exhibit persistently activated microglia. Whole transcriptome analysis of hippocampi from WNV-NS5-E218A-recovered mice with poor spatial learning revealed increased expression of genes known to drive microglial effects on synaptic pruning, including the classical complement pathway and phagocytosis. Indeed, the classical complement cascade initiation factor, C1qA, was found to be produced primarily by microglia and localized to infected neurons and synapses during WNND. Electron and confocal microscopy revealed a loss of hippocampal mossy fiber synapses while synaptophysin-positive puncta and phagosomes containing synaptic vesicles were observed within microglia. This loss of mossy fiber synapses was also observed in human WNND post-mortem samples. Importantly, mice with fewer microglia (IL-34 -/-) or mice deficient in complement (C3 -/-) were protected from WNV-induced synapse loss. Our study provides a novel murine model of WNV-induced spatial memory impairment, provides evidence that viral infection of adult neurons may induce complement-mediated elimination of synapses, and identifies a potential mechanism underlying neurocognitive impairments experienced by patients recovering from WNND.

S04 Astrocyte Heterogeneity: From Molecular Subtypes to Function and Pathology

S04
DECODING MOLECULAR AND FUNCTIONAL DIVERSITY OF ASTROCYTES IN THE NORMAL AND PATHOLOGICAL BRAIN

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Astrocytes are the most abundant cell type in the CNS and play vital roles in all facets of brain physiology, ranging from neurotransmission and synaptogenesis, to metabolic support and blood brain barrier formation. This immense functional diversity suggests the existence of heterogeneous astrocyte populations throughout the brain. While hundreds of classes and sub-classes of neurons have been identified, astrocytes remain grouped into two broad categories: fibrous and protoplasmic. In spite of the broad reach of astrocytes across diverse brain functions and all forms of neurological disorder, how this vast functional and cellular diversity is encoded in the brain remains completely undefined. Here we will discuss this longstanding and fundamental question on the nature of astrocyte heterogeneity in the normal adult brain. In the second part of this talk we will describe novel insights into how these principles and newly developed tactics can be harnessed towards gaining new insights into a host of neurological diseases.

S04-02
ASTROCYTE REGIONAL HETEROGENEITY IN SYNAPSE FORMATION AND REMODELING

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Astrocytes are implicated in a growing number of neurodevelopmental diseases and are increasingly recognized as active participants in neural circuit formation. However, a limited appreciation of astrocyte heterogeneity has hampered efforts to understand the role of astrocytes during brain development. We have shown that astrocyte location within the central nervous system is a major determinant of astrocyte heterogeneity. In published work, we demonstrated that the guidance cue Sema3a is uniquely expressed by ventral but not dorsal spinal cord astrocytes. We then demonstrated that this gene is required for proper motor neuron positioning, survival, and synapse formation, with implications for mouse behavior. To identify novel forebrain specific astrocyte-encoded genes and functions, we have extended this approach to perform RNA sequencing of astrocytes in five forebrain regions at postnatal day 9 (thalamus/hypothalamus, hippocampus, subventricular zone, striatum, and dorsal cortex). These data demonstrate significant astrocyte heterogeneity throughout the developing forebrain. In addition we have identified a highly region specific gene signature in developing thalamic astrocytes, including candidate genes with potential roles in synapse development and pruning. In summary, a focus on astrocyte molecular heterogeneity has the potential to elucidate novel functionally relevant astrocyte genes that may be 'averaged out' in more global expression profiling approaches.

S04-03
GENETIC CONTROL OF MORPHOGENESIS AND FUNCTION OF ASTROCYTES IN DROSOPHILA

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Astrocytes play important roles during nervous system development as well as in the proper function of the mature brain: They promote synapse formation, they are required for ion homeostasis, metabolic support of neurons and neurotransmitter clearance, however the underlying molecular mechanisms are only poorly understood.

We recently described a glial subtype in *Drosophila* that shows striking similarities to mammalian astrocytes. *Drosophila* astrocytes show a highly ramified morphology in the neuropil where they tile the volume to closely associate with synapses and they are critically important for the clearance of glutamate as well as GABA making *Drosophila* an attractive model system for understanding astrocyte biology.

To identify genes that are enriched in *Drosophila* astrocytes we conducted expressional profiling using Transcribing Ribosome Affinity Purification (TRAP). About 800 genes were at least twofold enriched in astrocytes over neurons and comparison with previously published data from mammalian astrocytes further supports the similarity of *Drosophila* and mammalian astrocytes.

To identify astrocyte enriched genes that are required for normal morphogenesis as well as function in the mature nervous system we used an RNAi knock down approach as well as analysis of CRISPR induced mutants. We identified a number of candidate genes affecting astrocyte infiltration, astrocyte proliferation as well as modification of sleep and seizure behavior including transcription factors, receptors and transporters. Here we will be presenting our progress in these screening efforts, the further characterization of the candidates and discuss the implications for our understanding of astrocyte biology.

S04-04
MOLECULARLY DEFINED ASTROCYTE SUBPOPULATIONS IN ADULT RODENT AND HUMAN CNS AND RESPONSE TO NEURODEGENERATIVE DISEASE INJURY

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Astrocytes are the most abundant cell types in the central nervous system. Historically, astrocytes have been considered a homogenous population of cells. However, accumulating evidence has shown

that astrocytes may be a heterogeneous cell type. To date, there is a very limited collection of tools available to study different astrocyte populations. We generated a transgenic mouse model containing a genetically encoded fluorescent reporter that labels a specific astrocyte subpopulation in the adult spinal cord and layer specific population in the neocortex. We employed this model to study astrocyte subpopulations and show that the distribution of these astrocytes is enriched in Layers II and V of the neocortex and in the ventral horn of the spinal cord grey matter. We also utilized multiphoton *in vivo* imaging and were able to track individual astrocytes over a time period of weeks in the adult mouse neocortex thus establishing that this fluorescent reporter is static. Furthermore, in order to evaluate astrocyte distribution across the entire CNS, we performed the recently established method, CLARITY. Using a double transgenic mouse line that has unique, astroglial specific genetically encoded fluorescence reporters to visualize all gray matter astrocytes and the layer specific astrocyte subpopulation, we quantified the overall distribution throughout the CNS. We present a further optimized system that includes sequential sectioning of fixed tissue, 3D remodeling of cell structures, tissue reconstruction, and in vivo astrocyte specific quantification. To understand the role of this astrocyte subpopulation in neurodegenerative disease context, we crossed our transgenic astrocyte reporter mice with G93A SOD1 ALS mouse model. We show that this astrocyte subpopulation and markers enriched in this subpopulation are remarkably affected in both the spinal cord and neocortex of the ALS mouse. Finally, we can extend these findings in rodent astroglia to unique human iPS derived astroglia and to human cortex. Taken together, these results shed light on the heterogeneous population of astrocytes in health and the susceptibility of certain astrocyte subpopulations to disease.

S05 Paradoxical Role of Interleukin-1beta in Central Nervous System Pathology

S05-01

INFLAMMASOME ACTIVATION AND IL-1β EFFECT NEURODEGENERATION IN JUVENILE BATTEN DISEASE

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Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), or Juvenile Batten Disease, is a fatal lysosomal storage disease caused by an autosomal recessive mutation in CLN3. Symptom onset occurs between 5-10 years of age, beginning with blindness and intractable seizures, followed by progressive cognitive and motor deterioration, and premature death (late teens-early 20s). Activated microglia and astrocytes are observed in the brains of JNCL patients and CLN3 mutant mouse models, which predict regions that will undergo neurodegeneration. These findings suggest that glial activation may influence neuron survival, which is supported by our results identifying a novel link between CLN3 mutation and aberrant caspase-1/inflammasome activation. The inflammasome is a multi-subunit cytoplasmic complex that catalyzes the proteolytic processing of target substrates, including pro-IL-1β, via its effector enzyme caspase-1 and can also trigger a specialized form of inflammatory cell death, termed pyroptosis. In terms of JNCL, $CLN3^{\Delta ex7/8}$ microglia exhibit exaggerated caspase-1 activation that leads to heightened IL-1β release and neurotoxicity. Exaggerated caspase-1 expression is also observed in the $CLN3^{\Delta ex7/8}$ brain that parallels regions where eventual neuron loss occurs in JNCL, namely the thalamus, various cortical structures, and the hippocampus. At a mechanistic level, we have found that $CLN3^{\Delta ex7/8}$ astrocytes and microglia display impaired mitochondrial function, which may be one trigger to elicit cytoplasmic caspase-1/inflammasome activation by the release of danger-associated molecular patterns (DAMPs), such as mtDNA. We have recently generated $CLN3^{\Delta ex7/8}$/caspase-1 mutant mice that will allow us to directly assess the functional importance of caspase-1 in disease progression and pathology. Targeting inappropriate caspase-1 activity may represent a novel approach to delay/prevent neuron loss in JNCL, which is critical based on the current lack of therapeutics for affected children. Supported by the NIH National Institute of Neurological Disorders and Stroke (R21NS084392) and the UNMC Dean's Pediatric Research Fund.

S05-02

BIMODAL ACTIONS OF IL-1β IN STROKE-RELATED MODELS

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Interleukin-1 (IL-1) is a cytokine released by many cell types that acts in autocrine and/or paracrine fashion, thereby stimulating a variety of signaling pathways. Evidence supports the involvement of IL-1b in the pathogenesis of both acute and chronic neurological disorders/disease although the precise cellular and molecular targets responsible for injury have not been fully elucidated. Previously, we have demonstrated that animals with a deletion for the IL-1β signaling receptor, IL-1R1, are less susceptible to cerebral ischemic damage. Further, we demonstrated that astrocyte-mediated alterations in system x_c^- (cystine/glutamate antiporter) activity contributes to the development and progression of inflammatory (IL-1β-enhanced) hypoxic neuronal injury ─an *in vitro* model of the ischemic penumbra. Interestingly, this same transporter has a well-characterized role in the synthesis and maintenance of the antioxidant molecule glutathione (GSH) raising the intriguing possibility that under certain circumstances, IL-1β could upregulate processes that protect against oxidative stress. Indeed, we find that IL-1β can enhance astrocyte GSH production and release. Further, following IL-1β treatment, astrocyte susceptibility to oxidant-induced injury is significantly attenuated. Hence, under the appropriate conditions, IL-1β may be an important stimulus for increasing total antioxidant capacity in brain. More broadly, our evidence suggests that the increase in IL-1β expression that occurs after insult/injury may be part of a protective response that ultimately goes awry. Supported by 2R01NS051445 – 07.

S05-03

SUSTAINED IL-1β OVER EXPRESSION REDUCES AMYLOID PLAQUES VIA ALTERNATIVE MICROGLIAL ACTIVATION

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We previously found that sustained expression of interleukin (IL)-1β in mouse brain led to reduction in plaque pathology associated with increased evidence of glial activation, a finding that has been reproduced by others using proinflammatory stimuli. The present study explored the role of microglia and their activation status on amyloid-β (Aβ) plaque clearance in a proinflammatory setting. APPswe/PS-1dE9 mice were intrahippocampally injected with AAV2-hIL-1β or control virus and assayed 4 weeks later for plaque pathology, microglial phenotypes, and association of Aβ with specific microglia. In separate experiments some mice were also infused with a neutralizing antibody to IL-4rα or control antibody to explore the role of IL-4 receptor activation in microglial phenotype changes. IL-1β overexpression led to increased numbers of argninase-1+ microglia that were preferentially associated with Aβ. Interestingly, IL-4 was elevated, predominantly in infiltrating T cells, following sustained IL-1β expression, and IL-4 injection alone led to increased numbers of argninase-1+ cells and plaque reduction. When IL-4 signaling was blocked with a specific antibody in the context of IL-1β overexpression, we found reduced numbers of arginase-1+ microglia. Moreover, this reduction coincided with less plaque clearance in the Alzheimer's model mice. In conclusion we found that sustained IL-1β expression leads to an IL-4 dependent alternative activation of microglia that appear to be responsible for Aβ

plaque clearance. *This work was funded by NIH grant RO1 AG030149.*

S05-04
ENDOGENOUS INTERLEUKIN-1β SUPPRESSES ACUTE EPILEPTIC SEIZURES AND STATUS EPILEPTICUS

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Reoccurring unprovoked seizure episodes are the hallmark of epilepsy, a common neurological affliction with an estimated lifetime risk of 1 in 26 individuals. Epileptic seizures are thought to result from episodic shifts in the balance between inhibition and excitation that facilitate bursts of abnormal electrical discharges. While numerous antiepileptic drug therapies are currently available, adverse side effects and resistance often hinder their efficacy raising the need for novel drug development. A better understanding of the mechanisms that underlie the disruption of homeostasis in the epileptic brain will facilitate this endeavor. A number of molecular mechanisms have been proposed to explain the pathophysiology of epilepsy, including perturbation of endogenous neuromodulatory pathways that positively or negatively influence neuronal activity. One such putative pathway is the Interleukin-1*beta* (IL-1β) signaling pathway. IL-1β is a pleotropic cytokine that is produced and secreted during immune or inflammatory reactions in response to infection and trauma. Under normal conditions, however, low levels of IL-1β have been reported in certain tissues, including the central nervous system (CNS). This suggests that IL-1β may also serve physiological purposes that are independent of its role in pathophysiology. Evidence supports this notion in the CNS. Our results from acute seizure models in mice show that targeted inactivation of the IL-1β signaling pathway enhances sensitivity to convulsive stimuli. This effect was observed in two different acute seizure models using two different approaches to inactivate the IL-1β signaling pathway. Thus, these results provide compelling evidence to support the contention that this pathway assists in maintaining an elevated seizure threshold in the normal brain and that its perturbation might contribute to the phenotype of epilepsy. Nonetheless, the nature of the role of IL-1β in epilepsy remains controversial. Although it has been suggested that the IL-1β signaling pathway might provide targets for novel antiepileptic drug development, a better understanding of the neuromodulatory role of IL-1β in normal and epileptic brain is necessary before this will be possible.

S06 Emerging Role of Glia in Neuronal Connectivity and Neurodegeneration

S06-01

JUVENILE STRIATAL WHITE MATTER IS RESISTANT TO ISCHEMIA-INDUCED DAMAGE

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A primary focus of research on ischemic stroke is neuronal damage, but white matter injury is also a major element of the pathology. Age-related changes in white matter vulnerability to ischemia have been studied, which suggest that the perinatal and aged periods are times of increased white matter vulnerability. However, white matter damage following stroke in the juvenile brain has not been evaluated. The late pediatric/juvenile period is an important developmental stage, as it is the time of maximal myelination. In our middle cerebral artery occlusion (MCAO) mouse model of juvenile ischemia, postnatal day (P) 20-25 or adult (8-12 weeks old) male mice were subjected to 45 minutes of reversible MCAO. Animals were analyzed at 24 hr, 3, 7 and 30 days of recovery. Neuronal death after ischemia was comparable between juvenile and adult striatum. By contrast, while adult oligodendrocytes were quite sensitive, actively myelinating striatal oligodendrocytes in the juvenile brain were highly resistant to ischemia. As a result, myelin sheaths and axons remained remarkably intact in the injured striatum of juvenile mice, while significant demyelination/axon loss was observed in adult striatum. Additionally, very different glial/endothelial cell responses were seen in juvenile and adult mice, including differences in astrogliosis, fibrosis, NG2-cell reactivity, and vascular integrity. Importantly, the tissue integrity after juvenile ischemia was very robust, and significant proliferation of neuronal progenitor cells occurred to repopulate the striatal tissue. Together, these results demonstrate that the juvenile mouse brain is quite resistant to ischemia, and that the glial responses uniquely preserve the tissue, allowing for significant recovery. Understanding the unique gene expression in juvenile oligodendrocytes that may provide protection is a major focus of the current studies. Supported by the Bugher Foundation.

S06-02

NEW TOOLS TO EXPLORE ASTROCYTE SIGNALING IN NEURAL CIRCUITS

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Astrocytes tile the entire CNS and their existence has been known for as long as that of neurons. Astrocytes are vital for neural circuit function, but have traditionally been viewed as simple homogenous cells that serve the same essential supportive roles everywhere. A critical hurdle to systematically exploring astrocyte functions in specific neural circuits has been the lack of methods to selectively monitor their signaling and the lack of methods to selectively impair astrocyte signaling within intact neural circuits. In the current presentation, we will report the development of methods to selectively monitor bulk intracellular and near-membrane calcium signals in genetically specified astrocytes. We will also report new methods to selectively and genetically suppress astrocyte calcium signals and the development, as well as validation, of wearable miniature microscopes to monitor astrocyte cytosolic calcium signals in freely behaving mice. These tools will be described in detail and deployed to assess astrocyte contributions to neural circuit function in adult mice.

S06-03

AN NFκB/COMPLEMENT-MEDIATED NEURON-GLIA SIGNALING PATHWAY IN NEURONAL HEALTH AND ALZHEIMER'S DISEASE

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Abnormal NFκB activation has been implicated in Alzheimer's disease (AD). However, the signaling pathways governing the regulation and function of NFκB in the brain are poorly understood. Here we demonstrate that NFκB activity is critically controlled by its inhibitor protein IκBα in astrocytes, but not in neurons. We identify complement protein C3 as an astroglial target of NFκB whose levels are increased in response to heightened NFκB activity. Elevated astroglial C3, in a neuronal complement receptor C3aR dependent manner, triggers aberrant intraneuronal calcium levels and disrupts surface AMPA receptor-mediated excitatory synaptic function and dendritic morphology. These effects can be rescued by C3aR blockade. Importantly, we show that astroglial NFκB and C3 can be induced by Ab and are upregulated in AD brains. Thus, deregulation of neuron-glia interaction through IκBα/NFκB/C3/C3aR/calcium signaling may contribute to synaptic dysfunction occurring in AD and our study provides support that C3aR antagonists may be therapeutically beneficial.

S06-04

DYSMYELINATION IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a devastating neurodegenerative disorder that manifests in midlife with motor, cognitive, and psychiatric deficits inexorably progressing to death within 15-20 years of onset. The abnormal expansion of CAG repeats in the

huntingtin gene is the cause of the disease. Currently, no cure is available. The mechanism of mutant huntingtin induced toxicity remains fully understood. The subtle onset of behavioral and psychiatric symptoms long before motor impairment suggests that dysfunction of brain connections controlling emotional and behavioral response may characterize the early phases of the disease, giving a possible explanation to early symptoms and potential early intervention to defeat this devastating condition. MRI imaging indicates that impaired white matter integrity appears to be an early event and related to neuronal loss and disease progression in HD patients. The selective degeneration of myelinated projection neurons with sparing of interneurons in the basal ganglia of HD patients further suggests that a defect of myelination may be important to HD pathogenesis. We demonstrated that white matter abnormalities were also present in HD mouse models, and decreased expression of myelin basic protein and deficient myelination were detected in HD mouse brain. Moreover, diffusion tensor imaging indicated disrupted white matter integrity and electron microscopy revealed thinner myelin sheaths in HD mice. Oligodendrocyte precursor cells also exhibited abnormal proliferation in HD mouse brain. In addition, others reported that overexpression of mutant huntingtin in oligodendrocytes led to age-dependent neurological symptoms in a mouse model. Taken together, current data suggest that dysmyelination is involved in HD pathogenesis, and therapeutic development for HD may also consider non-neuronal cells.

S07 Enhancing Remyelination During Multiple Sclerosis

S07
CHEMICAL CONTROL OF OLIGODENDROCYTE FATE AND FUNCTION

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Oligodendrocyte progenitor cells (OPCs) are a resident stem cell population in the central nervous system that serve as the predominant source of myelinating oligodendrocytes. Oligodendrocyte loss or dysfunction can lead to significant motor and cognitive disability in patients due to myelination deficits. We have developed technologies that enable the rapid and robust generation of OPCs from pluripotent stem cells and via direct cell reprogramming technologies. These in vitro generated OPCs serve as a powerful platform to understand oligodendrocyte development and to discover therapeutic compounds for enhancing myelination. I will discuss our recent efforts to use high-throughput phenotypic screening of pluripotent stem cell-derived OPCs to uncover new aspects of oligodendrocyte biology.

S07-01
GLIAL INTERACTIONS MODULATING DEMYELINATION AND REMYELINATION

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The regulation of central nervous system (CNS) demyelination and remyelination requires complex interactions between multiple cell types. Dissecting the nature and significance of those interactions have been complicated at the molecular level because of multiple overlapping signaling pathways. To begin to understand the role of individual cell populations in promoting myelination, demyelination and myelin repair we have developed the series of transgenic animals in which we are able to selectively deplete specific populations of neural cells through stimulation of the apoptotic pathway. During spinal cord development, depletion of mature oligodendrocytes inhibits myelination. Myelination recovers, however, after oligodendrocytes are allowed to redevelop, the myelin that forms appears normal in thickness and composition although the number of oligodendrocytes in previous affected regions of the CNS are elevated. Subsequent challenge with an alternative demyelinating lesion in the adult animal demonstrates that recovery in previous lesion regions is compromised. This compromised repair reflects alterations in the proliferative capacity of the local OPCs. Astrocytes are the other major family of neural cells implicated in myelination and remyelination. Local deletion of astrocytes during development inhibits myelination probably as a result of a reduction in OPC growth factors, mainly PDGF. Reestablishment of astrocyte numbers results in developmental myelination suggesting that astrocytes are critically important for timely CNS myelination. In the setting of a demyelinating lesion, however, deletion of astrocytes soon after lesion generation results in enhanced protection of oligodendrocytes and myelination suggesting that astrocytes contribute to demyelinating pathology. The molecular mechanisms by which astrocytes contribute to demyelination appear to reflect the functions of NF-kB signaling pathways.

S07-02
DEVELOPMENTAL GLIOGENESIS: NEW PERSPECTIVES AND PARADIGMS FOR REPAIRING THE CNS

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Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS; however, contributions of proximal regulators of the Wnt receptor complex to these processes remain undefined. To identify components of the Wnt pathway that regulate these processes, we applied a multifaceted discovery platform and found that Daam2-PIP5K comprise a novel pathway regulating Wnt signaling and myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 promotes Wnt signaling and receptor complex formation through PIP5K-PIP2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it suppresses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a pharmacological strategy to inhibit Daam2-PIP5K function, application of which stimulates remyelination after WMI. Put together, our studies integrate information from multiple systems to identify a novel regulatory pathway for Wnt signaling and potential therapeutic target for WMI.

S07-03
FUNCTION OF LRP1 DURING REMYELINATION

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Multiple sclerosis (MS) is a debilitating neurodegenerative disease in which myelin of the central nervous system (CNS) is destroyed by an autoimmune response. Chronic demyelination is the major reason why MS patients accumulate disability during their lifetime, as exposed neurons are more prone to neurodegeneration. Unfortunately, currently approved therapies are only aimed at dampening the immune response and do not address the critical need for stimulating myelin repair during/following an MS attack. Understanding the mechanisms of remyelination is critical to prevent neuronal loss, and is paramount to improving the quality of life of MS patients. The adult CNS contains a large population of oligodendrocyte precursor cells (OPC) that have the potential to differentiate into mature oligodendrocytes and remyelinate denuded axons. Although OPC are efficiently recruited into MS lesions, the

process of axon remyelination is impaired. OPC differentiation into mature oligodendrocytes is inhibited by myelin debris, which can linger in the area of MS plaques, where demyelination took place. Unfortunately, the mechanisms by which myelin debris inhibits OPC differentiation are poorly characterized.

Low density lipoprotein-related protein 1 (LRP1) is a multi-functional cell surface receptor involved in phagocytosis and cell signaling. We have recently discovered that, in neurons, LRP1 expression contributes to myelin-mediated inhibition of axonal regeneration. Furthermore, our work demonstrates that siRNA-mediated inhibition of LRP1 expression in primary OPC allows them to overcome myelin inhibition and differentiate *in vitro*. To test and extend our discovery in an *in vivo* model, we have generated mice with the deletion of LRP1 specifically in OPC ($LRP1^{fl/fl}$-Olig1-Cre mice). $LRP1^{fl/fl}$-Olig1-Cre mice are viable and fertile, and we have confirmed LRP1 deletion in OPC. Furthermore, using the cuprizone model of demyelination and remyelination, we have observed accelerated remyelination in $LRP1^{fl/fl}$-Olig1-Cre mice, when compared to control. This project is testing the hypothesis that LRP1 contributes to myelin-mediated inhibition of OPC differentiation. Our findings could open new avenues for the development of therapeutics for MS patients, ultimately aimed at promoting remyelination of denuded axons.

S08 Mechanisms of Mutant C9orf72 Pathogenesis in ALS and FTD

S08-01

SYNAPTIC DYSFUNCTION IN C9ORF72 ALS AND FTD

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The newly discovered gene mutation in *C9orf72* represents the most common genetic abnormality in frontotemporal dementia (FTD; 10-30%) and Amyotrophic Lateral Sclerosis (ALS; 20-50%). This mutation is characterized by an expanded GGGGCC (G4C2) hexanucleotide repeat in the non-coding region of the *C9orf72* gene on chromosome 9p21. Previous studies in our laboratory, using patient derived adult pluripotent stem cells differentiated into neurons (iPSNs) support the presence of RNA toxicity as a mechanism of disease pathogenesis for C9orf72 carriers. We hypothesize that this RNA toxicity leads to aberrant RNA processing of synaptic proteins, which in turn results in synaptic dysfunction and consequently in cognitive impairment as well as increased susceptibility to cellular stressors, including excitotoxicity. To study deficits at the synapse, we examined transduced patient derived (ALS and FTD) adult pluripotent stem cells (iPSCs) differentiated into mixed neurons with a lentivirus overexpressing eGFP and discovered aberrant dendritic branching and spine density. Furthermore, immunohistochemistry for major synaptic proteins (including synapsin and VGluT1) revealed altered expression patterns showing a loss of puncta appearance in C9orf72 iPSNs compared to control, non-diseased iPSNs. To examine the role of RNA toxicity due to sequestration of RNA binding proteins in these cellular phenotypes, we studied the previously identified G4C2 interacting protein ADARB2 and its family member ADARB1, which is responsible for editing of glutamate receptor GluA2. C9 patient tissue and C9 iPS neurons showed significant loss of GluA2 editing. Interestingly, ADARB1, shows significant nuclear/cytoplasmic mislocalization, similar to what has been reported for TDP43 in C9 iPS neurons and patient tissue. We hypothesize that GluA2 editing deficiency, which leads to increased calcium permeability of AMPA receptors, participates in the previously described susceptibility of C9 iPS neurons to excitotoxicity and might also explain the morphological and functional synaptic deficits observed.

S08-02

DIPEPTIDE REPEAT PROTEINS IN C9ORF72 FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS

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A hexanucleotide G_4C_2 repeat expansion in the *C9ORF72* gene is the most common known cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Elucidating how this repeat expansion causes "c9FTD/ALS" has become an important goal of the field. Likely pathogenic mechanisms include toxicity induced by repeat-containing RNAs. For instance, these transcripts undergo repeat associated non-ATG (RAN) translation resulting in the production of six "c9RAN proteins" of repeating dipeptides that form neuronal inclusions throughout the central nervous system of c9FTD/ALS patients. To investigate whether these proteins influence clinical and neuropathological characteristics of *C9ORF72* repeat expansion carriers, we evaluated associations between features of disease and levels of two abundantly expressed c9RAN proteins. To do so, we took a departure from traditional immunohistochemical approaches, and instead employed immunoassays to quantitatively measure poly(GP) and poly(GA) levels in cerebellum, frontal cortex, motor cortex, and/or hippocampus from 55 *C9ORF72* mutation carriers [12 patients with ALS, 24 with frontotemporal lobar degeneration (FTLD) and 19 with FTLD and motor neuron disease (FTLD-MND)]. Poly(GP) levels were highest in the cerebellum, and cerebellar poly(GP) associated with neuropathological diagnosis. Specifically, cerebellar poly(GP) levels were significantly higher in patients with FTLD and FTLD-MND compared to patients with ALS. Furthermore, in a cohort of 15 c9ALS patients for whom neuropsychological data were available, we found that cerebellar poly(GP) associated with cognitive score. Poly(GA) levels in the cerebellum similarly trended lower in the ALS subgroup compared to FTLD or FTLD-MND subgroups, but no association between cerebellar poly(GA) and cognitive score was detected. Both cerebellar poly(GP) and poly(GA) associated with *C9ORF72* variant 3 mRNA expression, but not variant 1, repeat size, disease onset, or survival after onset. Overall, these data indicate that cerebellar abnormalities, as evidenced by poly(GP) accumulation, associate with neuropathological and clinical phenotypes, in particular cognitive impairment, of *C9ORF72* mutation carriers.

S08-03

NUCLEOCYTOPLASMIC TRAFFICKING DEFICITS UNDERLIE C9ORF72 ALS/FTD NEURAL INJURY

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Postmortem CNS tissues from nearly all Amyotrophic Lateral Sclerosis (ALS) patients exhibit cytoplasmic accumulation of proteins regardless of any ALS-causing mutations. These proteins are predominantly nuclear in healthy cells and tissue where they shuttle between the nucleus and cytoplasm but form cytoplasmic inclusion and are depleted from the nucleus in ALS neurons and glia. One hypothesis underlying ALS aggregate pathology is that these proteins are transported to the cytoplasm but cannot return to the nucleus thus promoting inclusion formation. Nuclear pores function to limit molecules ≥ 40 kDa from traversing the nuclear membrane and are exceptionally long-lived in post-mitotic cells. Notably, loss of nuclear pore integrity due to aging or stress can disrupt the localization of cytoplasmic and nuclear proteins in otherwise healthy cells. A G_4C_2 repeat expansion in the first intron of the *C9ORF72*

gene is the most common known genetic cause of both familial and sporadic ALS and the mutation generates G_4C_2 RNA and dipeptide repeat protein (DPR) molecules that confer pathogenesis. We and others have recently showed that these toxic molecules can disrupt the nuclear pore function and nucleocytoplasmic trafficking. Specifically, we show that genetic modulation of nucleocytoplamic trafficking to enhance nuclear import of proteins containing a nuclear localization sequence (NLS) or inhibiting nuclear export protects against neurodegeneration and rescues nucleocytoplasmic trafficking deficits in a C9ORF72 *Drosophila* model. Moreover, we have found that iPSC motoneurons derived from C9ORF72 ALS patients exhibit deficits in cytonuclear protein import. Given these recent studies and the unifying TDP-43 pathology in ALS, we hypothesize that dysfunctional nucleocytoplasmic trafficking plays a global role in ALS/FTD pathobiology.

S08-04

UNDERSTANDING ALS-FTD SPECTRUM DISORDERS: INSIGHTS FROM DROSOPHILA AND IPSC MODELS

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Frontotemporal dementia (FTD) is the second most common presenile dementia and shares clinical, pathological and genetic overlaps with the motor neuron disease amyotrophic lateral sclerosis (ALS). In recent years, many disease-causing mutations in FTD/ALS have been identified such as CHMP2B, progranulin, TDP-43, C9ORF72 and others. However, the underlying pathogenic mechanisms remain poorly understood and there is no effective treatment available. To address this important question, we have been using multidisciplinary approaches including patient-specific iPSCs and *Drosophila* genetics. For instance, we generated iPSC models of FTD with progranulin and *C9ORF72* mutations (Almeida et al., *Cell Reports* 2012; Almeida et al., *Acta Neuropathol.* 2013) as well as different *Drosophila* models and performed several large-scale genetic screens. For instance, in a novel *Drosophila* model of FTD/ALS with *C9ORF72* repeat expansion, we found that low levels of dipeptide repeat (DPR) proteins, but not nuclear sense RNA foci, are a major source of toxicity in vivo (Tran et al., *Neuron* 2015). Among different DPR proteins, we found that $(GR)_{80}$ is mostly localized to the cytoplasm with some inclusions on chromatins and impairs the Notch signaling pathway and that $(GA)_{80}$ decreases $(GR)_{80}$ toxicity through inclusion formation (Yang et al., *Acta Neuropathol*. 2015). In another large-scale unbiased genetic screen done in collaboration with Dr. J. Paul Taylor lab, we found that expression of expanded G_4C_2 repeats in flies compromised nucleocytoplasmic transport, a defect also observed in iPSC-derived human cortical neurons (Freibaum*, Lu* et al., *Nature* 2015). In this presentation, I will discuss the implications of these recent findings and unpublished results for our understanding of ALS/FTD pathogenesis.

S08-05

NEUROTOXICITY MEDIATED BY C9ORF72-LINKED ARGININE-RICH DIPEPTIDE REPEAT PROTEINS

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The noncoding GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The expansion sense and antisense RNA transcripts can be translated through repeat associated non-ATG (RAN) translation to form five dipeptide repeat proteins (DRPs). We have shown that the arginine-rich dipeptides, poly-Proline-Arginine (PR) and poly-Glycine-Arginine (GR), are highly toxic. We have demonstrated this toxicity in primary cortical and motor neuron cultures, transgenic fly models, and in human iPS-derived neurons. Here, we are going to discuss the toxic mechanisms of these arginine-rich dipeptides. We found that toxicity is associated with their aggregation in the nucleus, with strong co-localization with nucleolin and fibrillarin, both nucleoli proteins. To further determine the human relevance of the observed dipeptide toxicity, we performed immunofluorescence (IF) imaging techniques on post mortem human tissues. Human tissues were collected from mutant C9ORF72 positive ALS patients, mutant C9ORF72 negative ALS patients, and non ALS patients. Analyses of the human tissue showed robust perinuclear and nuclear ariginine-rich dipeptides positive cells specifically in mutant C9ORF72 ALS positive patients. Interestingly, PR staining primarily showed aggregation in the nucleus that co-localized in nucleoli, similar to toxicity factors seen in the primary cell cultures. GR staining showed both nuclear aggregate staining and unique rin-like staining pattern around the nucleus which colocalized with lamin B, a nuclear envelope marker. This suggests that GR could possibly be trapped in the nuclear envelop while being transported into the nucleus. In vitro studies have documented increased nucleolar size following expression of arginine-rich dipeptides. In other cells types, this phenotype can be indicative of impaired pre-ribosomal RNA processing and induction of the nucleolar stress response. We will report on and discuss some of these latest findings.

Colloquia

C01 Keeping Time: Roles of Circadian Genes in the Formation & Repair of Neural Circuits

C01-01

CLOCK PLAYS A KEY ROLE IN THE TIMELY DEVELOPMENT AND REPAIR OF NEUROMUSCULAR SYNAPSES

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The formation and repair of neuromuscular junction follows prescribed steps starting with the initial contact between pre- and postsynaptic regions. Here, we asked if motor neurons utilitize the Clock macrinery during axonal growth and as their nerve endings differentiate into presynaptic endings. Analysis of conditional mice lacking Clock specifically in motor neurons revealed defects in the timely formation and maturation of neuromusucular junctions. We also discovered delayed reformation of neuromuscular junctions following seering of motor axons lacking Clock. These defects were exacerbated by deletion of BMAL1 specifically in motor neurons also lacking Clock. Together, our findings strongly suggest critical functions for the Clock machinery in the formation and repair of neuromuscular synapses.

C01-02

LOSS OF CLOCK RESULTS IN DYSFUNCTION OF BRAIN CIRCUITS UNDERLYING FOCAL EPILEPSY

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Medically refractory focal epilepsy, is often treated by resection of the region of epileptogenic tissue, indicating the importance of the "seizure focus". Nevertheless, understanding of the molecular underpinnings of the epileptogenic brain tissue is rudimentary. To study pathogenesis of focal seizures, we studied performed transcriptome analysis of human surgical samples following electrocorticography. Analysis of gene expression, identified significant downregulation of the transcription factor, Circadian Locomotor Output Cycles Kaput (Clock), expression in human epileptogenic foci compared with non-epileptic brain. Neurons with decreased levels of Clock have simplified dendritic morphology, including a decrease in mature spine morphologies and spine density. We further demonstrate that Clock-deficient excitatory neurons exhibit an imbalance in excitation and inhibition due to a defect in spontaneous IPSCs as compared with EPSCs. This electrophysiological abnormality is associated with a selective reduction of inhibitory synaptic proteins. Finally, we show that these cellular and biophysical changes underlie alterations in circuit function, as mice with selective deletion of Clock in excitatory neurons exhibit reduced seizure threshold.Thus, circadian gene expression may be important for pathogenesis of epilepsy, regulating the formation and stability of neuronal circuits. This finding may provide a molecular link between the sleep-wake cycle and seizure thresholds, and may provide a molecular and cellular mechanism underlying the progressive dysfunction of circuitry in human epilepsy.

C01-03

CELL TYPE-SPECIFIC FUNCTIONS OF THE CIRCADIAN CLOCK IN NEUROIFLAMMATION AND NEURODEGENERATION.

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The circadian system serves to coordinate internal physiology with external environmental cues. Circadian clock genes, which are expressed in neurons, astrocytes, and microglia, mediate cell-autonomous oscillations in gene transcription and cellular function. Circadian dysfunction is associated with aging and occurs in several neurodegenerative diseases, including Alzheimer Disease (AD), though the mechanisms by which these processes influence disease pathogenesis are still under investigation. We have found that brain-specific deletion of the master clock gene Bmal1, which abrogates circadian clock function, leads to severe age-related astrogliosis, synaptic damage, and oxidative stress in the mouse brain. This effect is independent of disruptions in sleep:wake cycle or behavioral rhythms. The core clock machinery appears to control cell type-specific transcriptional programs in neurons, astrocytes, and microglia which influence neuroinflammation and neurodegeneration. As an example, deletion of Bmal1 within astrocytes elicits cell-autonomous astrocyte activation in primary cultures by perturbing expression of key inflammatory regulators, including the inflammatory suppressor *Chi3L1* (YKL-40). Genetic and non-genetic perturbation of the circadian clock also accelerates the accumulation of amyloid-beta pathology in a mouse model of AD, suggesting a possible contributory role for circadian clock disruption in AD pathogenesis. I will discuss emerging evidence implicating circadian clock genes in the regulation of neuroinflammation, redox homeostasis, and protein aggregation in the brain, and the potential role of circadian dysfunction in neurodegenerative diseases.

C01-04

SECRETED GLIAL PROTEINS THAT REGULATE ADULT DROSOPHILA BEHAVIOR

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Previous studies from our lab have demonstrated a function for glial cells, in particular glial astrocytes, in the neural regulation of rhythmic behavior. Those studies, for example, identified a glial-specific factor that regulates locomotor activity rhythms (Suh and Jackson, 2007). Our more recent studies have demonstrated that glia-to-neuron communication is important for rhythmic behavior (Ng et al., 2011) and shown that glial vesicular secretion mechanisms function in this type of intercellular signaling (Ng et al., 2015). In related studies, we have employed cell type-specific translational profiling methods to define the genome-wide expression profile of adult fly astrocytes. In addition to verifying expression of vesicular secretion components in fly astrocytes, that analysis revealed the astrocyte expression of genes encoding more than 100 small, secreted proteins. We have employed RNA interference (RNAi)-based genetic screens to identify secreted glial proteins that function in the regulation of adult behavior. Several interesting candidates from the screen, including one that regulates fly sleep, will be discussed.

Suh, J., Jackson, F.R. (2007) Drosophila Ebony activity is required within glia for the circadian regulation of locomotor activity. *Neuron* 55: 435-447.

Ng, F.S., Tangredi, M.M. and Jackson, F.R. (2011) Glial cells physiologically modulate clock neurons and circadian behavior in a calcium-dependent manner. *Current Biology* 21: 625-634.

Ng, F.S. and Jackson, F.R. (2015) The ROP vesicle release factor is required in adult Drosophila glia for normal circadian behavior. *Front. Cell. Neurosci.* (http://dx.doi.org/10.3389/fncel.2015.00256).

C02 Astrocytes as Excitation-Energy Coupling Hubs

C02-01
NORADRENALINE AND AEROBIC GLYCOLYSIS IN ASTROCYTES

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Astrocytes express α- and β-adrenergic receptors, and noradrenaline can influence astrocytic glucose and glycogen metabolism via different signaling pathways, including Ca^{2+} and cAMP. Aerobic glycolysis, the disproportionate utilization of glucose or total carbohydrate compared with oxygen, occurs during brain activation and exercise even though oxygen level and delivery are normal. Increased flux of glucose into three metabolic pathways contributes to aerobic glycolysis during sensory stimulation of awake rats. Pentose shunt flux increases from 7 to 25% of glucose oxidized, and greater decarboxylation of glucose generates CO_2 without oxygen consumption. Glucose phosphorylation rises by ~50-70% and brain lactate levels rise 2-3-fold. However, oxygen consumption rose by ~30% and incorporation of label from [6-^{14}C]glucose into glutamate increased by ~45%, demonstrating that glycolysis exceeds oxidation. These two pathway fluxes may occur in neurons and astrocytes. In contrast, glycogen is mainly in astrocytes, and it is consumed during activation. Release of ^{14}C from prelabeled glycogen exceeds the fall in glycogen level, and the incorporation of [6-^{14}C]glucose into glycogen during stimulation occurs at the resting rate. During the early phase of recovery, label incorporation into glycogen doubles even as its level falls further. Thus, increased glycogen turnover coincides with net consumption. The importance of glycogenolysis to astrocytic energetics is underscored by large, regionally-selective compensatory increases in glucose utilization when glycogenolysis is blocked during sensory stimulation. Shunting of glucose through glycogen prior to entering the glycolytic pathway has a lower ATP yield than direct glycolytic metabolism of glucose (1 vs. 2ATP), and twice as much glucose must be consumed to generate the same amount of ATP. Thus, increased glycogen turnover, glucose shunting, and prolonged resynthesis contribute to aerobic glycolysis. Norepinephrine is known to stimulate glycogenolysis, and pre-treatment of subjects with propranolol, a non-specific β-blocker, prevents aerobic glycolysis during exercise and brain activation. Together, these findings support the conclusion that increased flux into three pathways contributes to aerobic glycolysis, and that astrocytic glycogen turnover and utilization may be a major underlying factor.

C02-02
REGULATION OF GLYCOGEN BREAKDOWN IN ASTROCYTES BY DISTINCT GLYCOGEN PHOSPHORYLASE ISOFORMS

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The brain relies on glucose from the blood, however, astrocytes contain a small, albeit metabolically active amount of glycogen. Glycogen is a highly dynamic storage molecule being degraded and rebuild continuously. Glycogen metabolism seems important for a number of processes including neurotransmitter glutamate uptake and memory formation even under normoglycemic conditions. The rate-limiting step of glycogen degradation is catalyzed by glycogen phosphorylase, and in astrocytes, two isozymes of glycogen phosphorylase exists, GPMM and GPBB. The distinct roles played by these two isozymes in astrocytes are still unknown. To address this, we have employed cultured mouse astrocytes in which we distinctly knocked down the two isozymes employing a siRNA approach. The data suggest that GPBB is acutely activated by an increase in the cytosolic AMP level, whereas GPMM is fully activated by reversible phosphorylation. In another study, we have investigated importance of glycogen to fuel store-operated Ca^{2+} entry, a process involved in re-filling the endoplasmic reticulum (ER) Ca^{2+} pool following signaling events. We found that activation of store-operated Ca^{2+} entry induces glycogen breakdown in a cAMP-dependent manner. This suggests the involvement of an adenylate cyclase isoform activated by Ca^{2+}. Which of the two isozymes of GP is responsible for this observation remains to be determined. Finally, pharmacological inhibition of glycogen degradation depleted the ER Ca^{2+} pool, indicating that glycogen breakdown supports ATP synthesis important for accumulation of Ca^{2+} into the ER.

C02-03
LACTATE RECEPTOR IN THE BRAIN

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Abstract Text should be entered here. This is only an example to show you how the abstract should be formated. You may preview this example to see how it looks like. But after that delete these lines and enter your own text!
The lactate-specific G-protein coupled receptor, GPR81 (HCA1), is present in the brain, including regions of the cerebral cortex and hippocampus. GPR81 is activated by physiological concentrations of lactate and the specific GPR81 agonist 3,5-dihydroxybenzoate to reduce cAMP. Cerebral GPR81 is present on the synaptic membranes of synapses and at the blood-brain barrier. GPR81 immunoreactivity is also located on subplasmalemmal vesicular organelles suggesting trafficking of the protein to and from the plasma membrane. The results indicate roles of lactate in brain signalling, including a neuronal glucose and glycogen saving response to the

supply of lactate. We suggest that lactate, through activation of GPR81 receptors, can act as a signalling molecule that links cerebral energy metabolism and neuronal activity in response to energy substrate availability.

C02-04

ADRENERGIC CONTROL OF ASTROCYTE FUNCTION

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The primary source of noradrenaline in the central nervous system (CNS) is locus coeruleus (LC) in the brain stem. When the LC neurons are activated during arousal and startle response, wide areas of the brain are excited synchronously, including the neocortex. In this part of the CNS, which in humans represents 80% of the brain mass, non-neuronal cells outnumber neurons. Non-neuronal cells include astrocytes, arguably the most heterogeneous glial cells in the CNS, which provide homeostatic support to the neural networks and failure to do so results in many, if not all, of neurologic diseases. Perhaps one of the key functions of astrocytes in health and disease is that they detect space-time coherent signaling by LC neurons, get excited themselves and respond back by releasing gliosignaling molecules, which bind to receptors on adjacent neighbouring glial and endothelial cells as well as neurons. Importantly, astrocytes are the predominant site where glycogen energy stores are present and when astrocytes are excited glycogenolysis is stimulated to activate glycolysis ending with lactate production. As details about this excitation-energy coupling are not clear at cellular level, this lecture will focus into mechanisms controling vesicle-based dynamics and the release of gliosignaling molecules. Moreover, measurements of noradrenergic receptor-mediated second messenger changes and metabolites at cellular level will be discussed.

C03 Neural Development and Regeneration

C03-01

FLOOR PLATE-DERIVED NEUROPILIN 2 FUNCTIONS AS A SECRETED SEMAPHORIN SINK TO FACILITATE COMMISSURAL AXON MIDLINE CROSSING

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Commissural axon guidance depends upon a myriad of cues expressed by intermediate targets. Previously, it has been shown that secreted semaphorins signal through neuropilin-2/plexin-A1 receptor complexes on post-crossing commissural axons to mediate floor plate repulsion in mouse spinal cord. However, we show here that neuropilin-2/plexin-A1 are also co-expressed on commissural axons prior to midline crossing and can mediate pre-crossing semaphorin-induced repulsion *in vitro*. How premature semaphorin-induced repulsion of pre-crossing axons is suppressed *in vivo* is not known. We discover a novel, previously unappreciated, source of floor plated-derived, but not axon-derived, neuropilin-2 is required for pre-crossing axon pathfinding. Floor plate specific deletion of neuropilin-2 significantly reduces the presence of pre-crossing axons in the ventral spinal cord, which can be rescued by inhibiting plexin-A1 signaling *in vivo*. Our results show floor plate-derived neuropilin-2 is developmentally regulated, functioning as a molecular sink to sequester semaphorins, preventing premature repulsion of pre-crossing axons prior to subsequent down-regulation, and allowing for semaphorin-mediated repulsion of post-crossing axons. In sum, this work demonstrated a novel non-cell autonomous mechanism for guidance receptors, and the midline floor plate structure is not only a source for attractive and repulsive molecules but also it has the ability to regulate the activity of these guidance cues during development.

C03-02

MIDLINE RADIAL GLIAL-LIKE CELLS PROMOTE IPSILATERAL LONGITUDINAL GROWTH OF SPINAL CORD DORSAL COLUMN AXONS DURING DEVELOPMENT

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The spinal cord comprises the ascending and descending connections between the brain and periphery, and disruption of these connections can lead to debilitating sensory and motor deficits. However, the mechanisms underlying the formation of the longitudinal axon tracts are poorly understood. The direct dorsal column (DDC) pathway, a longitudinal tract that is composed of ascending axons of primary somatosensory neurons and mediate touch and body position sensation, is one of such tract. We found that a population of radial glial-like cells in the midline of the dorsal column plays a crucial role for the development of this major spinal cord longitudinal tract. Mice with a spontaneous mutation resulting in the disruption of these midline cells (GMCs) exhibit severe growth deficits in DDC fibers. Additionally, although the dorsal column normally contains solely ipsilateral projections, these mutant mice appear to have ectopic crossings. Thus, our data suggest that these GMCs present an important developmental mechanism that promotes long distance ipsilateral growth of longitudinal axon tracts. Our study may provide valuable insight into potential therapeutic regenerative strategies following spinal cord injury.

C03-03

SEEING IS BELIEVING: PERIPHERAL NERVE REGENERATION IN ZEBRAFISH

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Unlike axons of the central nervous system, axons of the peripheral nervous system have retained the capacity to remake functional neuromuscular synapses even after complete nerve transection. Yet despite their unique ability to re-make functional connections, we know remarkably little about how peripheral axons, such as motor axons re-connect with their appropriate msucle targets. This is in part because the dynamic behavior of injured or diseased axons as they respond to insults, interact with neighboring Schwann cells, and begin to pioneer a path to the original targets, has not been examined in real time, in intact vertebrate animals. We have established a laser based nerve transection model in zebrafish, enabling us to visualize the cellular behaviors of transected axons and neighboring Schwann cells simultaneously, in real time, in an intact vertebrate animal (1, 2). I will discuss ongoing projects to understand the interaction between injured axons and neighboring Schwann cell, and present a role for a key regulator of neuromuscular synapse development during peripheral nerve regeneration.

1) Rosenberg et al *J.Neuroscience* 2012; 2) Rosenberg et al *J.Neuroscience* 2014.

C03-04

AKT COORDINATES THE SIGNALING OF MTORC1 AND MTORC2 TO PROMOTE CNS AXON REGENERATION

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Injuries of mature central nervous system (CNS) axons result in loss of vital functions due to the failure of CNS axons to regenerate. Deletion of phosphatase and tensin homolog (PTEN), the negative regulator of phosphatidylinositol 3-kinase (PI3K), induces CNS axon regeneration through activation of mammalian target of rapamycin (mTOR) signaling. We have conducted an extensive molecular dissection of the cross-regulating mechanisms in optic

nerve regeneration that involve the downstream effectors of PI3K, AKT and the two mTOR complexes (mTORC1 and mTORC2). We found that the predominant AKT isoform in brain and retina, AKT3, induces much more robust axon regeneration than AKT1 and that activation of mTORC1 and inhibition of glycogen synthase kinase-3β (GSK3β) are critical for AKT3-induced axon regeneration. Surprisingly, phosphorylation of T305 and S472 of AKT3 play opposite roles in axon regeneration and mTORC2 inhibits axon regeneration through phosphorylation of AKT3-S472. Thus our study revealed a neuron-intrinsic balancing mechanism involving AKT as the nodal point of PI3K and mTORC1/2 that coordinates both positive and negative cues to regulate adult CNS axon regeneration.

C04 Dissecting the Effects of Chronic Stressors on Glial Activation and Neurological Disorder in the CNS

C04-01

MULTIPLE DRIVERS OF PATHOGENESIS IN CD4 T CELL-DRIVEN EPILEPSY

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Epilepsy affects 1% of the population and carries significant unmet need. Immune signaling, including proinflammatory cytokine modulation of astrocytic function, is implicated in epilepsy etiology. Although T-cells have been found in brain tissue of epilepsy patients and experimental animals after seizure, little is known about the role of adaptive immunity in epilepsy etiology. To determine whether an antigen-driven response against an astrocyte target is sufficient to initiate epilepsy, we generated transgenic mouse line (GFAP-HA) where astrocytes expressed a non-functional antigen (HA). GFAP-HA mice were bred to clone 6.5 (HA-TCR) mice in which >95% of CD4+ T cells recognize HA antigen. Unmanipulated GFAP-HA/HA-TCR mice appeared normal. However, after pertussis toxin adjuvant stimulation, animals developed progressive seizures. T cells were observed throughout brain, including brain regions that normally have lower GFAP expression in healthy animals. The T-cells infiltrating brain were activated, CD62L-low and VLA-4+, and this infiltration was antigen-dependent. This suggests that brain-infiltrating T-cells were presented HA antigen by antigen presenting cells (APCs) that also provided costimulatory signal for activation. After robust T-cell infiltration into brain, we see high expression of T-cell cytokines IFNγ and IL-17 –the latter of which can directly influence neurons. Levels of the cytokine IFNγ positively correlated with GFAP transcript and not astrocyte water channel AQP4; whereas IL-17 correlated with AQP4 transcript. This suggests that different T-cell cytokines may be differentially influencing this astrocytopathy. Long-term depth electrode recording showed altered neuronal activity that precedes robust T-cell infiltration and detectable levels of T-cell cytokines within brain. At this early timepoint, there is a large population of brain-infiltrating professional APCs, including B cells. This suggests that early changes in neuronal activity, which precede visually overt seizures, may be mediated by brain-infiltrating professional APCs. Here we show that CD4 T-cells can be pathogenic in epilepsy. Together, this data leads us to hypothesize that distinct cellular and molecular actors direct distinct aspects of pathology in this mouse model of autoimmune epilepsy.

C04-02

ROLE OF EXOSOMES IN PROMOTING CNS MYELINATION/REMYELINATION

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Multiple sclerosis is an inflammatory disorder involving myelin damage and oligodendrocyte loss. Treatment options are limited and consist of conventional immunosuppressors, immunomodulators, or agents to prevent lymphocyte infiltration to the CNS (Tullman *et al.,* 2013). These therapies can induce harmful immune sequelae and do little to promote repair. Instead, we suggest use of exosomes, naturally occurring nanovesicles (40-100nm) that play important roles in physiologic cell function, disease states, and modulation of the immune system. While their exact function in these diverse activities is not yet fully understood, they exert influence through delivery of mRNA, miRNA and protein (Bobrie *et al.,* 2011). In addition, exosomes are a non-toxic delivery platform that can easily cross the blood brain barrier, making them promising candidates for development as neurotherapeutics.

We discovered that exosomes derived from the serum of rats exposed to environmental enrichment (EE; volitionally increased physical, intellectual and social activity) increase oligodendrocyte precursor cells and their differentiation into mature myelin-producing cells – both under normal conditions and after acute demyelination (Pusic and Kraig 2014). To mimic EE and create a scalable source of therapeutic exosomes, we stimulated primary dendritic cell cultures (DCs) with interferon-gamma (IFNγ). IFNγ is physiologically increased during EE, and phasic low-levels significantly increase myelination when applied to slice cultures or administered nasally (Pusic and Kraig 2015).

When applied to slice cultures, exosomes released by IFNγ-stimulated DCs (IFNγ-DC-Exos) increased baseline myelination, reduced oxidative stress, and improved remyelination following acute lysolecithin-induced demyelination. Furthermore, nasally administered IFNγ-DC-Exos increased CNS myelination *in vivo*. Screening of IFNγ-DC-Exo miRNA content identified high levels of miR-219, which is crucial for oligodendrocyte maturation and the formation and maintenance of compact myelin (Dugas *et al*., 2010; Zhao *et al.,* 2010). Finally, tracking studies revealed preferential uptake by oligodendrocytes, suggesting that IFNγ-DC-Exos directly impact oligodendrocytes to increase myelination. Thus, our results show great potential for use of IFNγ-DC-Exos as an adjunct therapy to promote remyelination in multiple sclerosis (Pusic *et al.,* 2014).

C04-03

CNS EFFECTS OF OPIATE EXPOSURE IN A MOUSE MODEL OF NEUROAIDS: SELECTIVE VULNERABILITY OF D1 AND D2-EXPRESSING MEDIUM SPINY NEURONS

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Opiate abuse and HIV are co-morbid conditions that significantly burden society. HIV-1-associated neurocognitive disorders persist despite antiretroviral therapy and can be exacerbated by opiate abuse. HIV-1 patients show selective vulnerability in the basal ganglia that may involve actions of the HIV-1 protein, transactivator of transcription (Tat), which has been noted to increase the neurotoxicity and dendritic pathology of medium spiny striatal neurons (MSNs). Importantly, neurotoxic effects of HIV-1 are heterogeneous among neuron populations and the factors that confer neuronal protection or vulnerability are not well understood and have not been systematically examined. To explore whether subpopulations of MSNs might be selectively vulnerable, the effects of Tat and/or morphine exposure on striatal dopamine 1 and 2 receptor-expressing MSNs (D1- and D2-MSNs) were examined in transgenic Tat mice bred to Drd1a-tdTomato or Drd2-EGFP reporter mice. Alterations in affective/motor behavior were correlated with changes in electrophysiological properties and in the morphology of D1- and D2-MSNs. Mice exposed to Tat or morphine demonstrated greater anxiety-like behavior in a light-dark transition task, compared to controls. Morphine increased spontaneous (open field), but not elicited (rotarod) locomotion. Preliminary data suggest that Tat and morphine affect the excitability of D2-, but not D1-, MSNs, though similar treatment-induced reductions in spine density were apparent between D1- and D2–MSNs. Notably, in Tat-expressing mice, reduced action potentials in D2, but not D1, MSNs were correlated with fewer light/dark-transitions in the anxiety test (no such relationship was observed in Tat-naive controls). We speculate that Tat ± morphine triggers glial inflammation contributing to the neurophysiological and neurobehavioral deficits observed *in vivo* and that D2-MSNs are especially vulnerable to HIV-1 and opiate abuse comorbidity.

C04-04

TOXOPLASMA GONDII INFECTIONS ALTER GABAERGIC SYNAPSES AND SIGNALING IN THE CENTRAL NERVOUS SYSTEM

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During infections with the protozoan parasite, *Toxoplasma gondii*, gamma-aminobutyric acid (GABA) is utilized as a carbon source for parasite metabolism and to also facilitate parasite dissemination by stimulating dendritic cell motility. The best recognized function for GABA, however, is its role in the nervous system as an inhibitory neurotransmitter that regulates the flow and timing of excitatory neurotransmission. We used immunohistochemistry to compare synaptic structures between brains of mock and type II *Toxoplasma*-infected animals. Infection led to a global deficit of the GABA biosynthetic enzyme, GAD67, which is normally clustered at presynaptic release sites of inhibitory GABAergic synapses. These changes appear to be due to altered localization rather than protein expression since GAD67 protein levels were unaltered by infection. Humans suffering from cerebral toxoplasmosis develop seizures, although mechanisms of how *Toxoplasma* dysregulates synaptic transmission are unknown. To address this question, we first tested whether *Toxoplasma*-infected mice develop seizures. We found that mice develop spontaneous and long-lasting seizures 30 days post infection. Our data regarding GAD67 mislocalization in type II *Toxoplasma* infected animals suggested that they would be more susceptible to drugs that inhibited GABAergic signaling. GABAergic protein mislocalization and the response to seizure inducing drugs were observed in mice infected with type II but not type III strain parasites indicating a role for polymorphic parasite factor(s) in regulating GABAergic synapses. Taken together, these data support a model in which seizures and other neurological complications seen in type II *Toxoplasma*-infected individuals are, in part, due to parasite-induced changes in GABAergic signaling.

C05 Functions of Astrogliosis

C05
GLIAL ENGULFMENT FUNCTION IN THE DEVELOPING AND MATURE BRAIN

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During normal development or after nervous system injury or disease, neuronal processes (axons, dendrites, and synapses) often degenerate and neuronal cell bodies undergo apoptotic cell death. Glial cells are the primary cell type responsible for recognizing and clearing this neuronal debris in a timely fashion. We are interested in understanding how neurons autonomously destroy selected cellular compartments during pruning, how they signal to glia to activate these cells, and the molecular basis of glial recognition and phagocytosis of neuronal waste materials. This presentation will discuss recent finding regarding molecular mechanisms of neuron-glia signaling during developmental pruning or after axotomy, and the surprising diversity of molecular programs engaged by glia to engulf unique types of neuronal detritus.

C05-01
REACTIVE ASTROCYTES AND SYNAPTIC PLASTICITY

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The role of astrocyte reactivity in response to remote axonal injuries is not fully understood. After such injuries, a complex process of reorganization of synaptic connections is observed on the dendrites of surviving neurons. This process, named adaptive synaptic plasticity, is crucial for the re-establishment of previously lost connections, for neuronal survival and ultimately for functional recovery. Since astrocytes are key regulators of synaptogenesis during development, we investigated their role in adaptive synaptic plasticity following neuronal injury. Using transgenic approaches, we evaluated the effects of astrocyte reactivity on neuronal integrity and synapse recovery following extracranial facial nerve transection in mice. We showed that after axotomy, the activation of signal transducer and activator of transcription-3 (STAT3) signaling mediates reactivity of perineuronal astrocytes, inducing astrocyte process formation, and promoting neuronal survival. Using a combination of in vitro and in vivo models, we also revealed a novel mechanism in adaptive synaptic plasticity in the adult CNS. We showed that in reactive astrocytes STAT3 directly controls the re-expression and release of the synaptogenic molecule thrombospondin-1 (TSP-1), which promotes the restoration of excitatory input onto axotomized motor neurons. These data may provide insights for developing novel synapto- or neuro-protective strategies.

C05-02
DISSECTING REGION-SPECIFIC FORMS OF TRAUMA-REACTIVE ASTROGLIOSIS IN VIVO BY CELL-TYPE SPECIFIC ANALYSIS OF TRANSLATING MRNA

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In addition to upholding normal brain function, astrocytes respond to diverse forms of CNS injury with dynamic changes in gene expression, metabolism, morphology, proliferative capacity and function collectively referred to as reactive astrogliosis. Many lines of evidence indicate that astrocytes tune their responses to varying degrees of cell death, axonal injury, vascular disruption and inflammation, resulting in distinct regional and likely function-specific reactivity profiles that may be informed by differences in molecular expression. To begin characterizing how distinct local post-injury environments differentially alter the molecules expressed by reactive astrocytes *in vivo*, we are using the transgenically targeted (*J Neurosci 28:7231; 2008*) RiboTag procedure (*PNAS 106:13939; 2009*) and RNA sequencing to obtain gene translation profiles of reactive astrocytes from the mouse spinal cord after traumatic injury (SCI). Bioinformatics analyses of astrocyte ribosome-associated mRNA (ramRNA) is being used to identify gene expression fingerprints that distinguish scar-forming from non-scar forming hypertrophic reactive astrocytes. Many changes in astrocyte-specific gene expression differ from changes observed for non-astrocyte RNA derived from whole spinal cord tissue after SCI. This study provides a qualitative and quantitative measurement of gene expression by reactive astrocytes across different traumatic injury microenvironments *in vivo*. Further investigation into the significance of these heterogeneous manifestations of reactive astrogliosis will aid our evolving understanding of mechanisms influencing neuroregeneration and repair. Funded by Adelson Medical Research Foundation and NINDS.

C05-03
NOVEL IN VITRO SYSTEMS TO STUDY REACTIVE ASTROCYTES

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Astrocytes undergo profound changes in morphology and gene expression in response to brain injury and disease. But whether reactive astrocytes are harmful or helpful has been unclear. We recently found that genes induced in reactive astrocytes depends on the nature of the inducing injury. After ischemia, reactive astrocytes

upregulate neurotrophic factors suggesting they are beneficial, whereas after systemic injection of lipopolysaccharide (LPS) they strongly upregulate multiple complement cascade components needed to drive synapse destruction suggesting they are detrimental. These findings suggest that, like macrophages which exist on a spectrum from bad (M1) to good (M2) states, reactive astrocytes also exist in bad (A1) and good (A2) states.

Here we show that LPS-induced M1 microglia are sufficient to induce A1 reactive astrocytes. M1 microglia do this by releasing IL1α, TNFα and C1q, which together are sufficient to induce A1 (bad) reactivity in purified astrocytes, and are all required for M1 microglia to induce the A1 state. Using IL1α, TNFα and C1q together, allowed us to create the first defined serum-free cultures of pure A1 reactive astrocytes enabling us to investigate their function. By directly comparing functions of normal astrocytes with A1 astrocytes *in vitro*, we found that A1 astrocytes are unable to promote neuronal survival, axon outgrowth, synapse formation or synapse function, and have lost the ability to phagocytose synaptosomes and myelin debris. In addition to loss of their normal functions, A1 reactive astrocytes gained a powerfully neurotoxic function, releasing a protein that specifically induces apoptosis of neurons and oligodendrocytes.

In conclusion, Il1α, TNFα and C1q treatment of purified astrocytes *in vitro* provides a serum-free recapitulation of *in vivo* neuroinflammatory reactive astrocytes - allowing for investigation of a range of interesting molecular and physiological aspects of CNS trauma/disease. Understanding multidimensional roles of reactive astrocytes in diseased/injured CNS will undoubtedly contribute to development of treatment strategies that will enable more controlled modulation of reactive astrogliosis - enabling promotion of brain repair and reduced CNS cell loss and neurological impairment.

C06 Dynamics of DNA Damage Responses, Brain Aging and Neurodegenerative Diseases

C06-01

NEW EVIDENCE FOR RNA/DNA BINDING PROTEINS'-MEDIATED GENOME REPAIR DEFECTS IN MOTOR NEURON DISEASE

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Accumulation of genome damage including oxidized bases, single- and double-strand breaks, in affected brain cells has been linked to neurodegenerative diseases whose underlying cause(s) are not completely understood. Here, we provide evidence for the first time that RNA/DNA binding protein TDP-43, whose nuclear clearance and simultaneous cytoplasmic deposition is a hallmark feature in Amyotrophic Lateral Sclerosis (ALS) and other neurodegenerative diseases, is required for efficient DNA double strand break repair (DSBR) in neurons. We demonstrate that (1) TDP-43 stably interacts with DSBR proteins in human iPSC/stem cell-derived primary motor neurons, which was enhanced after treatment with DSB-inducing agents. (2) TDP-43 is recruited to the DSB sites neuronal cells, and (3) TDP-43's overall as well as nucleus-specific depletion markedly increased accumulation of DSBs. (4) TDP-43 controls the expression of key DNA damage response proteins 53BP1 and MDC1 via specific micro RNAs. (5) TDP-43 knock out causes significant accumulation of DNA damage and DDR activation which mediates neuronal cell death. These results are consistent with the dramatic accumulation of unrepaired DSBs in postmortem brains of ALS-affected human patients and a distinct nuclear clearance of TDP-43 in these affected neurons. In summary these results reveal that **TDP-43 and FUS are critical components of genome damage response in the neurons whose loss of function(s) causing DNA repair deficiency is a key etiological factor in ALS and possibly other neurodegenerative diseases.** Thus defects in genome damage repair in neuronal genome represents a common basis for ALS and other neurodegenerative diseases. Furthermore, our current studies are focussed on exploring genome repair and DDR-tergeted therapeutic strategies for motor neuron disease (Research supported by UNIH/NINDS, ALS Association and Muscular Dystrophy Association).

C06-02

DNA REPAIR GONE WRONG: MECHANISMS OF TRINUCLEOTIDE EXPANSION IN HUMAN NEURODEGENERATIVE DISEASE.

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Mammalian cells have evolved sophisticated DNA repair systems to correct mispaired or damaged bases and extra helical loops. However, we find that two DNA repair systems, the mismatch recognition system (MMR) and the base excision repair, are turned into mutational machines, rather than safeguards of genomic integrity. We previously reported that the 7,8-dihydro-8-oxo-guanine (8-oxo-G) glycosylase (OGG1) is a causative factor in CAG expansion. Loss of somatic expansion in the *HdhQ150(+/+)/OGG1(-/-)* crosses delays the onset of disease by around 8 months relative to their *HdhQ150(+/+)* littermates, although they both inherit a similar disease-length HD allele. OGG1, however, cooperates with MSH2-MSH3. Both OGG1 and MSH2-MSH3 bind well to hairpins containing 8-oxo-G lesions, suggesting that the two proteins either compete for hairpin site binding or interact there upon damage. MSH2-MSH3 interacts with the BER endonuclease (AP-endonuclease or Polβ) to increase gap-filling synthesis. Targeting of MT with a pharmacological radical scavengers stop expansion, indicating that the oxidation derives from mitochondrial metabolism. Indeed, using new phosphor imaging, we demonstrate that hyperactive MT are present in the regions targeted for death, and predicts which regions die first. Altogether expansion arises from a toxic oxidation cycle, in which removal of oxidize damage by BER creates a single strand break, and expansion occurs during gap filling synthesis. Continued hyperactive MT perpetuates the cycle.

C06-03

STUDIES ON SYNUCLEIN INDUCED DNA CONFORMATION/DAMAGE IN RELEVANCE TO PARKINSON DISEASE

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Alpha-synuclein conformational modulation leading to fibrillation has been centrally implicated in Parkinson's disease. Here we present the alpha-synuclein's DNA binding property and further characterized the effect of DNA binding on the conformation and fibrillation kinetics of alpha-synuclein. It was observed that single-stranded circular DNA induce alpha-helix conformation in alpha-synuclein while plasmid supercoiled DNA has dual effect inducing a partially folded conformation and alpha-helix under different experimental conditions. Interestingly, alpha-synuclein showed a specificity for GC* nucleotide sequence in its binding ability to DNA. The aggregation kinetics data showed that DNA which induced partially folded conformation in alpha-synuclein promoted the fibrillation while DNA which induced alpha-helix delayed the fibrillation, indicating that the partially folded intermediate conformation is critical in the aggregation process. Further, the mechanism of DNA-induced folding/aggregation of alpha-synuclein was studied using effect of osmolytes on alpha-synuclein as a model system. Among the five osmolytes used, Glycerol, trimethylamine-N-oxide, Betaine, and Taurine induced partially folded conformation and in turn enhanced the aggregation of alpha-synuclein. The ability of DNA and osmolytes in inducing conformational transition in alpha-synuclein, indicates that two factors are critical in modulating alpha-synuclein folding: (i)

electrostatic interaction as in the case of DNA, and (ii) hydrophobic interactions as in the case of osmolytes. The property of DNA inducing alpha-helical conformation in alpha-synuclein and inhibiting the fibrillation may be of significance in engineering DNA-chip based therapeutic approaches to PD and other amyloid disorders. Also further we present mechanistic study on DNA damaging ability of synuclein and AGE-α-synuclein and found that very interesting results. The lucid evidence for the presence of Synuclein in the nucleus and DNA binding ability provide newer understanding of DNA instability in Parkinson disease (Rao KS thankful to SENACYT-Panama for financial support through SNI system)

C06-04

NUCLEAR TO MITOCHONDRIAL DNA DAMAGE SIGNALING IN NEURODEGENERATION

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We find that some DNA repair defective diseases with severe neurodegeneration have mitochondrial defects. Our studies involve cell lines, the worm (c.elegans), and mouse models and involve the conditions Xeroderma pigmentosum group A, Cockaynes syndrome and Ataxia telangiectasia. We find a pattern of hyperparylation, deficiency in the $NAD^+$and Sirtuin signaling and mitochondrial stress. We are pursuing mechanistic studies of this signaling and interventions at different steps to improve mitochondrial health and the neurodegeneration.

C06-05

OXIDATIVE GENOME DAMAGE AND ITS RESPONSE IN PROLIFERATING VS. POSTMITOTIC CELLS: IMPLICATIONS IN NEURODEGENERATIVE DISEASES

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Reactive oxygen species (ROS), the predominant genotoxic agents, generated both endogenously as respiration by-products and also after exposure to environmental agents including toxic substances, continuously damage DNA. Multiple DNA lesions include oxidized bases, abasic (AP) sites, and single-strand breaks (SSBs) which are converted into toxic double-strand breaks (DSBs) during replication. Persistent genome damage, in particular, DSBs, which are also generated directly by drugs and radiation, activate damage response pathways to promote cell survival and cell death via multiple mechanisms. Unrepaired lesions could induce mutations which in oncogenes and tumor suppressor genes could lead to cancer. In contrast, neurodegenerative diseases are caused by altered signaling culminating in neuronal death via apoptosis, necrosis and other pathways. Several neurological diseases, selectively caused by deficiency in strand break-processing enzymes, may reflect the reduced repair in postmitotic neuronal cells compared to replicating cells. Oxidative genome damage is repaired by base excision/single-strand break repair initiated by base lesion-excising DNA glycosylases (DGs) and by break termini-cleaning enzymes. Studies from our lab and others over some four decades have contributed to the following key discoveries about the scope and regulation of BER/SSBR in mammalian cells.**(a)** DGs which initiate BER have overlapping substrate range, thus acting as each other's back-up**. (b)** BER involves repair complexes stabilized by direct, pairwise interaction among repair proteins independent of DNA. Covalent modifications, (e.g., ROS-activated acetylation and phosphorylation) stabilize such 'BERosome' complexes**. (c)** Distinct BER complexes act in replicating vs. non-replicating cells. **(d)** BER is active in both nucleus and mitochondria. Persistent strand breaks in the mitochondrial genome triggers apoptosis. **(e)** Several non-canonical RNA-binding proteins are present in repair complexes which enhance repair efficiency. **(f)** Mammalian DGs, unlike their *E.coli* counterparts, invariably have non-conserved, intrinsically disordered terminal segments which, while dispensable for activity, enhance repair efficiency by providing common interaction interface**. (g)** Oxidative damage in promoter/enhancer sequences are induced by histone/5-mecytosine demethylases. Finally, our extensive understanding of oxidative genome damage repair notwithstanding, unraveling its complex regulation still poses a major challenge.

C07 Glia-Neuron Interactions in CNS Injury

C07-01

MICROGLIA-NEURON COMMUNICATION IN EPILEPSY

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Epilepsy represents a neurological disorder that can manifest in uncontrolled seizures in patients. Microglia are exquisitely sensitive to disruptions in the central nervous system. Since epilepsy is characterized by neuronal hyperactivity rooted in excessive glutamate release and ionic imbalance, it is conceivable that microglia respond to and regulate neuronal activities during the pathology. Here, we found an increased number of microglial primary processes in the hippocampus during kainic acid-induced seizure activity. Consistently, global glutamate induced robust microglial process extension (MPE) towards neurons making increased contact with neurons in both brain slices and in the intact brain in vivo. The mechanism of the glutamate-induced MPE involves the activation of neuronal NMDA receptors, calcium influx, subsequent ATP release, and microglial response through P2Y12 receptors. In addition, we further found that extracellular Ca^{2+} reduction or transient glutamate exposure induced microglial processes to converge at distinct sites, a phenomena we termed microglial process convergence (MPC). Our studies revealed that MPC occurs independent of astrocytic functions and are not directed towards astrocytes but target neuronal dendrites. MPC is also mediated by ATP and microglial P2Y12 receptor; fractalkine signaling regulates MPC via IL-1β release as downstream effectors. Importantly, we found that MPC was triggered in experimental seizures and simulated ischemia in mice. In P2Y12 knockout mice with reduced seizure-induced MPE and MPC, kainic acid-induced seizure behaviors were significantly worsened. These studies are the first to investigate the microglial dynamics and discovered MPE and MPC during acute epilepsy. These results provide significant insights into the dynamics, mechanisms and molecular regulation of a novel bi-directional microglial-neuronal communication axis that may be especially relevant during epilepsy and ischemia.

C07-02

MECHANISMS OF SPREADING DEPOLARIZATION-INDUCED CYTOTOXIC EDEMA

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Spreading depolarizations (SDs) are waves of sustained, near-complete neuronal and glial depolarization. In stroke and trauma patients, SDs exacerbate tissue damage in the at-risk cortical territory supporting the view that SD may be an important mechanistic endpoint in clinical studies. A massive disruption of the physiological ion gradients in SD results in intracellular water accumulation, leading to astroglial and neuronal swelling and dendritic beading (focal swelling) with the disruption of dendritic spines. While it has been assumed that neurons swell during SD because osmotically obligated water follows sodium, chloride and calcium influx, the molecular mechanisms by which water crosses neuronal membrane are unclear. Aquaporins are most likely facilitating the balancing of astroglial water content after the ionic movements during SD. In comparison, pyramidal neurons that do not express functional aquaporins are largely water-impermeable under acute osmotic stress. This implies that SD-induced neuronal swelling does not occur as a simple osmotic event following a net gain of excess electrolytes in the neuronal cytoplasm. Several neuronal chloride cotransporters translocate a significant amount of water together with substrate. Accordingly, we have recently shown that these cotransporters activated by the shifts in the ion and proton gradients during SD participate, at least in part, in focal dendritic swelling. Yet, transporter-mediated ion and water fluxes could be paralleled by water entry through additional pathways, such as large-pore pannexin-1 (Panx1) channels. Panx1 are activated by depolarization, high extracellular potassium, strongly elevated intracellular calcium, and by mechanical stretch. All of these conditions are present during SD, indicating that the opening of Panx1 could instantly "flood" neurons with water. Using *in vivo* 2PLSM in combination with pharmacological and genetic approaches, we have shown that pannexin-1 channels are not required for SD-induced focal dendritic swelling, further emphasizing the important contribution of chloride-coupled transport mechanisms to focal dendritic swelling.

This work was supported by National Institutes of Health Grant NS083858.

C07-03

THE GLIA-DERIVED ALARMIN IL-33 ORCHESTRATES THE IMMUNE RESPONSE AND PROMOTES RECOVERY FOLLOWING CNS INJURY

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Inflammation is a prominent feature of central nervous system (CNS) injury that heavily influences neuronal survival, yet the signals that initiate and control it remain poorly understood. We have identified the nuclear alarmin, interleukin (IL)-33, as an important regulator of the innate immune response after CNS injury. IL-33 is expressed widely throughout the healthy brain, and is concentrated in white mater due to predominant expression in oligodendrocytes. IL-33 is released immediately following CNS injury from damaged glia, acting on local astrocytes to induce chemokines critical for monocyte recruitment. Mice lacking IL-33 have impaired recovery following CNS injury, which is associated with reduced monocyte infiltration to the injury site. These results demonstrate a novel molecular mediator contributing to immune cell recruitment to the injured CNS and may lead to new therapeutic insights in CNS injury and neurodegenerative diseases.

C07-04

MICROGLIA, NEUROGENESIS AND STROKE

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Stroke results in areas of neuronal cell death with concomitant inflammation and edema in the surrounding tissues. Ischemia in the thalamus can present as an accumulation of many smaller lacunar infarcts leading to potential cognitive deficits and central post-stroke pain syndromes, for which curative therapies are lacking. The capacity of the brain to repair itself rests upon the robust response of adult neurogenesis, and gliogenesis, to formidable ischemic conditions. Here we evaluate neurogenesis/gliogenesis in the context of adult thalamic ischemia. Firstly, we demonstrate that the chromatin protein HMGB2 can affect the transition between the creation of new neurons and glial cells during development. Secondly, following the induction of stroke in the thalamus, loss of HMGB2 lowers the rate of apoptotic cell death and differentially modulates behavioral outcomes of the ischemic lesion. Our results point to diverse array of brain functions for HMGB2, which makes it an important molecule. We further explore whether microglia facilitate and regulate the neural stem and progenitor cells (NSC/NPC) migration towards the ischemic lesion site.

C07-05

ROLE OF ASTROCYTIC GDNF IN NEURONAL DEATH AND BRAIN DAMAGE AFTER ISCHEMIC STROKE

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Astrocytes play a non-cell autonomous role in neuroprotection after ischemic stroke. Glial cell-derived neurotrophic factor (GDNF) was originally isolated from a rat glioma cell-line supernatant and has been found to be a potent survival neurotrophic factor for dopaminergic, noradrenergic and motor neurons. However, whether and how astrocytic GDNF protects neurons and brain after ischemic stroke has not been investigated. Here we used astrocyte-specific GDNF inducible conditional knockout mice, i.e., Glast-CreERT:GDNF$^{flox/flox}$ (Glast-GDNF cKO) mice to investigate the effect of endogenous astrocytic GDNF on neuronal death and brain damage after photothrombosis (PT)-induced ischemic stroke. Under non-ischemic conditions, we found that Glast-GDNF cKO mice exhibited significant lower number of Brdu+ and Ki67 cells as well as DCX+ cells in the dentate gyrus in adult mice, indicating astrocytic GDNF can promote adult neurogenesis. Under ischemic conditions, we found that PT induced a dramatic increase in GDNF in penumbral region in WT mice in a time dependent manner. Glast-GDNF cKO mice exhibited a significant reduction in infarct volume after 2, 4 and 14 days after PT as compared with WT litter mates. Glast-GDNF cKO mice also exhibit a significant reduction in FJB+ degenerating neurons in the penumbra. We also examined the effect of astrocytic GDNF on cell proliferating in the penumbra using Brdu labeling and immunohistochemistry. We found that Glast-GDNF cKO mice have lower densities of Brdu+ and Ki67+ cell in the penumbra than WT mice. From these results we conclude that astrocytic GDNF plays important roles in reducing neuronal death and brain damage and cell proliferation in the peri-infarct region after PT.

C08 Non-Traditional Approaches to Study Glial Cell Biology

C08-01

GENETIC SCREENS IN ZEBRAFISH UNCOVER NEW REGULATORS OF MYELINATING GLIAL CELL DEVELOPMENT

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Myelin is the multilayered membrane formed by the spiral wrapping of glial cells around axons. In the central nervous system (CNS), oligodendrocytes (OLs) form the myelin sheath, while SCs generate myelin in the peripheral nervous system (PNS). The importance of myelin in normal nervous system function is underscored by myelin disruption in multiple sclerosis and Charcot-Marie-Tooth diesase. Currently, no treatments exist to restore myelin in these diseases, and there is a pressing need to develop therapies that address this issue. Therefore, a basic understanding of the genetic and molecular bases of myelin formation is of direct relevance to human patients with myelin disease.

Myelin is an evolutionary innovation of the vertebrate lineage; thus, zebrafish represents the simplest model system to elucidate the molecular mechanisms that regulate myelination. Zebrafish embryos develop externally and are transparent, allowing real-time visualization of dynamic events that are impossible to observe directly in mammals. Zebrafish are also a powerful tool for forward genetics; accordingly, we have recently completed a large-scale, three-generation forward genetic screen, and we have recovered 28 mutants with defects in glial cell development and myelination. Three of the most striking mutants are *stl90*, *stl64*, and *stl83*, which present with CNS amyelination (*stl90*), CNS and PNS hypermyelination (*stl64*), and CNS and PNS hypomyelination (*stl83*). Our work to delinate the molecular mechanisms of glial cell development controlled by these genes will be discussed.

C08-02

GLIA ESTABLISH STRUCTURAL AND FUNCTIONAL SET POINTS IN THE NERVOUS SYSTEM

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Neurons receive input from the outside world or from other neurons through neuronal receptive endings (NREs). Glia envelop NREs to create specialized microenvironments; however, glial functions at these sites are poorly understood. We have studied glial roles at both sensory NREs and at synapses, and have found that glia play a critical role in setting activity and shape setpoints. At a sensory NRE we uncovered a novel molecular mechanism by which glia control NRE shape and associated animal behavior. The *C. elegans* AMsh glial cell ensheaths NREs of twelve neurons, including the thermosensory neuron AFD. KCC-3, a K/Cl transporter, localizes specifically to a glial microdomain surrounding AFD receptive-ending microvilli, where it regulates K^+ and Cl^- levels. Cl^- ions, in turn, function as novel inhibitors of an NRE-localized receptor-guanylyl-cyclase, GCY-8, which synthesizes cGMP. High cGMP mediates the effects of glial KCC-3 on AFD shape by antagonizing the actin-regulator WSP-1/NWASP. Components of this pathway are broadly expressed throughout vertebrate nervous systems, suggesting that ionic regulation of the NRE microenvironment may be a conserved mechanism by which glia control neuron shape and function. We have also explored the roles of glia at glutamatergic synapses in *C. elegans*, have identified key glial genes regulating glutamate-mediated responses in the animal, and uncovered similarities with astrocyte functions in vertebrates. Our results suggest that glia determine synaptic activity thresholds, that when disrupted lead to profound behavioral changes.

C08-03

TRANSCRIPTIONAL PROFILING OF DROSOPHILA GLIA RESPONDING TO INJURY

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Glial cells are the first immune responders to neuronal stress and damage. Any form of neural trauma triggers swift responses from glia, including glial migration to injury sites, release of neuroprotective and neurotoxic factors and glial clearance of damaged neurons through phagocytic engulfment. This complex set of responses is, in part, orchestrated by injury-induced changes in glial gene expression, but our knowledge of the transcriptional programs that govern glial responses to neuronal injury is still incomplete. Importantly, there is striking molecular and cellular conservation in how glia respond to trauma across species. Thus, we are using *Drosophila* as a tractable genetic model system to generate a transcriptional snapshot of glia responding to axon degeneration in vivo. We developed a novel assay that includes severing peripheral nerves to elicit widespread glial responses throughout the ventral nerve cord (VNC) of the fly and then performed RNAseq analysis to profile injury-induced changes in gene expression. Peripheral nerve axotomy triggered robust upregulation of genes that fall into discrete signaling classes, including Toll-like signaling, phagocytic activity (e.g. Draper/MEGF10 pathway) and extracellular matrix remodeling (e.g. MMPs), and we have validated induction of subsets of these genes by quantitative PCR and/or immunostaining. Finally, we have streamlined our bioinformatics pipeline incorporating DIOPT, OMIM and GWAS databases, as well as Annokey software, to identify high priority genes that are 1) conserved in human and 2) not previously implicated in neurodegenerative/glial immunity pathways. We are now exploring the functional role of high priority genes to gain novel insight into the molecular underpinnings of innate glial response to axon degeneration.

C08-04

ENGINEERING PSEUDO-AXONAL SCAFFOLDS FOR MYELINATION: IMPLICATIONS FOR DEVELOPMENT, DISEASE AND HIGH THROUGHPUT SCREENING

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Functional screening for compounds that promote remyelination represents a major hurdle in the development of rational therapeutics for multiple sclerosis (MS). Screening for remyelination is problematic, as myelination requires the presence of axons. Standard methods do not resolve cell autonomous effects and are not suited for high-throughput formats. Here we describe a **B**inary **I**ndicant for myelination using **M**icropillar **A**rrays (**BIMA**). Engineered with conical dimensions, micropillars permit resolution of extent and length of membrane wrapping from a single two-dimensional image. Confocal imaging acquired from the base to the tip of the pillars allows for detection of concentric wrapping observed as " rings" of myelin. The platform is formatted in 96 and 384-well formats, amenable to semi-automated random acquisition and automated detection and quantification. Upon screening a 250-compound GPCR library, we discovered two clusters of molecules that modulate two specific GPCRs, one that inhibits (G_q) and one that promotes (G_i/G_o) differentiation and myelination of oligodendrocytes both during development and after demyelination. Testing of compounds in a focal demyelinating mouse model and in human iPSC-derived OPCs suggests that our screening platform is ideally suited to identify potential regenerative therapeutics in MS.

C08-05

PEERING THROUGH THE LOOKING GLASS: THE ROLE OF GLIAL-GLIAL INTERACTIONS ACROSS TRANSITION ZONES IN DEVELOPMENT AND DISEASE

Sarah Kucenas
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Motor nerves play the critical role of shunting information out of the CNS to targets in the periphery, including muscle. Their formation requires the coordinated assembly of distinct cellular components, including motor axons, the glial cells that ensheath them and the surrounding muscle. Currently, we have a broad perspective of the developmental events that lead to spinal motor nerve formation. However, we don't know: 1) the exact steps that drive the earliest stage of spinal motor nerve development, establishment of motor exit point transition zones (MEP TZ), 2) how these locations are selectively permeable, restricting myelinating cells from mixing, while allowing the passage of other populations, or 3) the nature of the cellular interactions that occurs across them. Using in vivo, time-lapse imaging in zebrafish, we've begun characterizing the distinct glial populations found on either side of the MEP TZ, their roles in motor nerve development and injury, and the molecular mechanisms that mediate their dynamic interactions. Utilizing an in vivo system to directly investigate the MEP TZ and the glial-glial interactions that establish it, we are poised to provide important insights into how functional nervous systems are assembled, maintained and behave during disease.

C09 Unmyelinated and Unappreciated: Novel Roles of Non-myelinating Peripheral Glia

C09-01

DEVELOPMENT AND FUNCTION OF DROSOPHILA WRAPPING GLIA

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In the vertebrate peripheral nervous system, unmyelinated axons are separated and ensheathed by glial membranes within Remak bundles. Despite the considerable number of Remak-ensheathed axons, the development, maintenance, and function of nonmyelinating glia remain poorly understood. Like Remak Schwann cells, the wrapping glia of *Drosophila* peripheral nerves ensheath multiple individual axons, and recent studies have identified conserved molecular mechanisms that control both wrapping glia and Schwann cell differentiation. Fly wrapping glia thus constitute a powerful model to study neuron-glia interactions *in vivo* and identify novel molecular mechanisms that drive axonal ensheathment during development. We recently completed a wrapping glia-specific *in vivo* RNAi screen of ~2,000 genes to identify genes required for proper ensheathment. This screen has identified several candidates necessary for proper wrapping. Importantly, many candidates have homologs that are strongly enriched in vertebrate glial cells. Experiments to determine how these genes drive ensheathment, as well as to investigate the consequences of incomplete wrapping on neuronal health and function are ongoing. Our studies should provide important insights about axon-glia interactions during development that will inform our understanding of ensheathing glial cells in health and disease.

C09-02

REGULATION OF ENTERIC NEURAL NETWORKS BY ENTERIC GLIA IN HEALTH AND DISEASE

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Reflex behaviors of the intestine such as peristalsis are orchestrated by the enteric nervous system (ENS); a complex neural network embedded in the gut wall. Like the brain, the ENS is composed of neurons that are surrounded by glial cells. Enteric glia are a unique type of non-myelinating peripheral glia that exhibit many astrocyte-like properties. Enteric glia were previously considered passive support cells for neurons. Yet, our recent findings challenge this view by demonstrating that enteric glia detect, and in turn, influence neuron activity. Our data show that in the context of health, glial signalling is both necessary and sufficient to control the enteric motor programs that underlie gut motility. Specifically, we used a transgenic mouse model where enteric glia express designer receptors exclusively activated by designer drugs (Gq-DREADD; *GFAP::hM3Dq*) to show that the activation of enteric glia calls up motor programs in the ENS. Likewise, we used *GFAP::Cre$^{ERT2+/-}$/Cx43$^{f/f}$* mice to ablate intercellular communication by enteric glia and found that gut motility was significantly disrupted. These data show that neuron-glia communication is required for the maintenance of normal intestinal reflexes. However, we also find that the stimulation of glial cells by neuronal danger cues, such as ATP, that are released under pathological conditions contributes to neurodegeneration. Our data show that enteric glia kill neurons during neuroinflammation using mechanisms that involve nitric oxide, the release of ATP through connexin-43 hemichannels and the activation of neuronal P2X7 purine receptors. Glial-driven neuron death contributes to permanent alterations in the neural control of gut motility by disrupting ENS structure and function. Thus, our data show that enteric glial cells play essential roles in the coordination of enteric reflexes and in the disruption of ENS function during disease.

C09-03

ENTERIC GLIA PROTECT THE MUCOSAL BARRIER

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Glial cells represent an extensive but understudied cell lineage in the enteric nervous system. Although previously regarded as passive support cells for enteric neurons, there is now an emerging recognition that enteric glia directly regulate enteric nervous system function and mucosal homeostasis through nitric oxide signal transduction. Fulminant intestinal inflammation is associated with a loss of glia and neurons in the intestine, and barrier regulation plays a pathogenic role in the disease onset. Our work has actively investigated the functional analogy between glial regulation of barrier function in the gut and brain, identifying *S*-nitrosoglutathione (GSNO) as an active anti-inflammatory nitric oxide-derived signal in the intestine. New systems biology tools for quantifying S-nitrosylation signals and molecular targets will be discussed in the context of the regulatory mechanisms of action of GSNO on enteric nervous system function and intestinal homeostasis.

C09-04

UNDERSTANDING AND TARGETING DYSFUNCTIONS OF THE DIGESTIVE NEURO-GLIO- EPITHELIAL UNIT IN DIGESTIVE AND BRAIN DISORDERS

Michel Neunlist, Malvyne Rolli Derkinderen, Pascal Derkinderen
Laurianne Van Landeghem, Jeremy Bregeon, Francois Cossais
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Chronic diseases (CD) are multifactorial and multiorgan and combine genetic risk factors and environmental triggers. Forming a privileged exchange surface with the environment, the gastrointestinal tract is widely recognized as a key organ involved in the development of CD. Intestinal epithelial barrier dysfunctions (IEB)

leading to a “leaky gut” is a hallmark of a large number of CD. Changes in permeability and / or alterations of barrier repair processes form a large part of these lesions. IEB dysfunctions are linked to changes its cellular (immune cell response) and luminal microenvironment (dysbiosis; toxic challenges). Among the constituents of the microenvironment recently identified as playing a central role in the control of the IEB functions is the enteric nervous system (ENS). The components of the ENS (enteric neurons and glial cells) form a real anatomical and functional unit, designed as a neuro- glial epithelial unit (NGEU). Recent studies highlighted the central role of the neurons and glial cells in strengthening the IEB and in enhancing IEB repair processes. In addition, the structural and molecular organization of the NGEU is highly similar to the neuro- glial endothelial unit in the brain regulating blood-brain barrier. This parallelism between gut and brain is further reinforced by recent data gained in Parkinson’s disease suggesting that exploring the UNGE could open a window in the brain during neurodegenerative diseases. In this context, the talk will be organized into three parts. First, it will set the anatomical and functional basis of the NGEU. It will describe the ability of the ENS, and especially glial cells to control, during life, the main functions of the IEB. Second, it will describe the development of tools for the study of NGEU dysfunctions, in patient, and its application to the study of Parkinson's disease. Finally, it will present the development of therapeutic approaches targeting the NGEU.

C09-05

PULMONARY GLIA

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Autonomic nerves regulate important functions in visceral organs, including the lung. The postganglionic portion of these nerves is ensheathed by glial cells known as non-myelinating Schwann cells. In the brain, glia play important functional roles in neurotransmission, neuroinflammation, and maintenance of the blood brain barrier. Similarly, enteric glia are now known to have analogous roles in gastrointestinal neurotransmission, inflammatory response and barrier formation. In contrast to this, very little is known about the function of glia in other visceral organs. Like the gut, the lung forms a barrier between airborne pathogens and the bloodstream, and autonomic lung innervation is known to affect pulmonary inflammation and lung function. Lung glia are described as non-myelinating Schwann cells but their function is not known, and indeed no transgenic tools have previously been validated to study them *in vivo*. This talk will discuss such a transgenic tool, and explain the relationship between non-myelinating Schwann cells and pulmonary nerves in the airways and vasculature, and describe the morphology, marker expression, and potential functions of non-myelinating Schwann cells in the lung.

C10 Old Friends and New Roles: Brain Patterning and Signal Transduction

C10-01

FUNCTIONS OF ERK/MAP KINASE SIGNALING IN DEVELOPING CORTICAL CIRCUITS

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The Extracellular-signal Regulated Kinases 1/2 (ERK/MAPK) signal transduction cascade plays a central role in the intracellular response to important extracellular cues in the nervous system. Mutations in components of the ERK/MAPK pathway have been discovered in a family of human syndromes, known as 'RAS'opathies, NCFC, or RAS/MAPK syndromes. Intellectual disability, learning abnormalities, neurodevelopmental delay, and seizures are commonly observed in these patients. Perturbations in ERK/MAPK activity have also been noted in models of autism disorders, traumatic brain injury, neonatal hypoxia-ischemia, and childhood epilepsy. Yet, the pathogenesis of neurological defects resulting from aberrant ERK/MAPK activity in the developing nervous system remain poorly understood. We have examined whether ERK/MAPK signaling regulates subtype-specific or common neurodevelopmental mechanisms in the developing cortical excitatory and inhibitory neurons. Our data reveal a critical requirement for ERK/MAPK signaling in the morphological development and survival of large $Ctip2^+$ neurons in layer 5. Loss of *Mek1/2* led to deficits in corticospinal tract formation and subsequent corticospinal neuron apoptosis. ERK/MAPK hyperactivation also led to reduced corticospinal axon elongation, but was associated with enhanced arborization. ERK/MAPK signaling was dispensable for axonal outgrowth and survival of layer 2/3 callosal neurons. However, *Mek1/2* deletion led to reduced expression of Arc and enhanced intrinsic excitability in both layers 2/3 and 5, in addition to imbalanced synaptic excitation and inhibition. These data demonstrate selective requirements for ERK/MAPK signaling in layer 5 circuit development and general effects on cortical pyramidal neuron excitability. Moreover, developing cortical inhibitory interneurons exhibit specific deficits in $Parvalbumin^+$ neuron number that are dependent upon the precise trajectory of ERK/MAPK perturbation. Overall, our findings provide insight into the highly subtype-specific functions of ERK/MAPK signaling in the regulation of neuronal number and connectivity in developing cortical circuits.

C10-02

JNK SIGNALING: A MOLECULAR COMPASS FOR MIGRATORY CORTICAL INTERNEURONS

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Aberrant migration of cortical interneurons can alter the formation and function of neural circuitry in the cerebral cortex and lead to severe neurological and psychiatric disorders including epilepsy, autism, and schizophrenia. During fetal development, cortical interneurons travel dorsally in tangentially ordered migratory streams to reach the cerebral cortex and then turn radially to exit migratory streams and invade the nascent cortical plate. Molecular mechanisms governing the timing of interneuron arrival to the cerebral cortex and subsequent departure from migratory streams remain poorly understood, yet are of fundamental importance to cortical development. Our lab recently demonstrated that cortical interneurons rely on the c-Jun N-terminal Kinase (JNK) signaling pathway to enter and navigate the developing cortical rudiment. Current work from our lab indicates that after arriving to the cerebral cortex, cortical interneurons utilize JNK signaling to maintain tangential progression in migratory streams. Indeed, *ex vivo* pharmacological inhibition of JNK activity leads to rapid migratory stream dispersion and precocious accumulation of interneurons in the cortical plate, while complimentary *in vivo* analyses show similar alterations to the migratory orientations of JNK deficient cortical interneurons. Together, these observations indicate that JNK signaling orients the migration of cortical interneurons by controlling the timing of interneuron entry to the cerebral cortex and departure from migratory streams. Thus, JNK—a ubiquitous intracellular signaling pathway—plays the unexpected role of a molecular compass by directing the migration of cortical interneurons into and within the cerebral cortex. Ongoing experiments aim to unravel the molecular pathways acting up and downstream of JNK signaling to control the migration and differentiation of cortical interneurons. We anticipate that our results will provide new insight into the role of JNK signaling in cortical development and neurodevelopmental disorders associated with improper cortical connectivity.

C10-03

NEW INSIGHT INTO NCL-ASSOCIATED PROTEINS: NOVEL REGULATORS OF CARGO TRANSPORT IN NEURONS

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The neuronal ceroid lipofuscinoses (NCLs) are a family of devastating neurodegenerative diseases resulting from mutations in as many as 14 different genes. Researchers have long sought a molecular link between various NCLs. Recent studies suggest a common NCL pathway associated specifically with membrane associated protein forms of the disease (CLN3, CLN6, CLN5, CLN8) may be intracellular transport via disrupted interaction with the cytoskeletal network. In support of this concept, we have identified a novel complex containing the ER-associated CLN6, whose mutation results in a variant late infantile NCL (vLINCL), the collapsin response mediator protein 2 (CRMP2), and the kinesin motor protein, KLC4. Acting through a network of protein interactions, CRMP2 regulates axonal/dendritic specification and extension during neurodevelopment and contributes to maintenance/regeneration in the mature brain. We hypothesize that the

CRMP2/CLN6/KLC4 (CCK) complex utilizes CLN6 as a "molecular tag" on ER-vesicles for segregation of cargo to distal sites in dendrites and axons. Disruption of this signaling complex could contribute to the pathogenesis of vLINCL through altered neuronal process outgrowth and maintenance. The studies we will present focus on 1) determining how the CCK complex regulates ER-vesicle transport development and maintenance of neurons; 2) defining how CCK complex transport is linked to early events in neuronal differentiation; and 3) determining if stabilization of CRMP2-associated complexes, independent of CLN6 rescue, could ameliorate neurological deficits in a pre-clinical NCL mouse model. These studies will expands our understanding of CLN6's contribution to crucial cellular processes and start to unravel the biological significance of the CCK complex in developing and mature neurons, as well as its role and the role of intracellular trafficking in neurological disorders such as the NCLs.

C10-04

ENDOLYSOSOMAL TRAFFICKING AND SIGNALING DURING BRAIN DEVELOPMENT

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It is known that rapid and persistent remodeling occurs on many scales within the developing brain, yet the mechanisms by which individual cells navigate this challenge remain unclear. Alterations in the complement of surface membrane proteins is one mechanism that cells can use to adapt to this changing molecular landscape. This is accomplished through a process of new protein insertion and degradation of those proteins that have become damaged or obsolete. The trafficking itinerary of such vesicle-based cargos varies greatly depending on developmental status, metabolic demand and signaling activity. Here, we have used genetic loss-of-function in the prenatal mouse brain to explore the link between protein trafficking and neural development. Our results indicate multiple brain defects associated with perturbation of the endolysosomal pathway including brain dysmorphologies, increased apoptosis and decreased cell proliferation, altered autophagic processing, and perinatal viability. I will discuss our results characterizing these phenotypes using a combination of biochemical, cell biological and electron microscopic approaches to reveal novel developmental impacts of this well-known pathway. The work presented opens new avenues for understanding how critical membrane-associated proteins necessary from proper brain patterning are regulated between cell surface expression and their ultimate degradation. It will also shed new light on the vesicular trafficking mechanisms orchestrating proper neurodevelopment by revealing heretofore unappreciated aspects of patterning that are dependent on the precise control of membrane protein sorting and turnover.

Selected Oral Presentations

OR01 Oral Session I

OR01-01

OLFACTORY BULB BUT NOT CORTICAL CAPILLARIES ARE SUSCEPTIBLE TO VIRUS-INDUCED VASCULAR LEAK AND PROMOTE VIRAL NEUROINVASION

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Viral neuroinvasion is a critical step in neurovirulent virus disease pathogenesis. Multiple mechanisms of neuroinvasion have been identified, but their relative contribution to central nervous system (CNS) infection remains unclear for many viruses. In this study, we examined neuroinvasion of the mosquito-borne bunyavirus La Crosse (LACV), the leading cause of pediatric viral encephalitis in the USA. We found that the olfactory bulb (OB) and tract were the initial areas of the CNS virus infection. Removal of the OB reduced the incidence of LACV encephalitis demonstrating the importance of this area to neuroinvasion. However, we determined infection of the OB was not due to axonal transport of virus from olfactory sensory neurons as ablation of these cells did not affect viral pathogenesis. Instead, we found OB capillaries were compromised allowing leakage of virus-sized particles into the brain. Analysis of OB capillaries demonstrated specific alteration in cytoskeletal and Rho GTPase protein expression not observed in capillaries from other brain areas such as the cortex where leakage did not occur. Collectively, these findings indicate that LACV neuroinvasion occurs through hematogenous spread in specific brain regions where capillaries are prone to virus-induced activation such as the OB. Capillaries in these areas may be "hot spots" that are more susceptible to neuroinvasion not only for LACV, but other neurovirulent viruses as well.

OR01-02

AN AUTOMATED BEHAVIORAL PARADIGM REVEALS CEREBELLAR DEFICITS IN A MOUSE MODEL OF PREMATURE BIRTH INJURY

Aaron Sathyanesan, Vittorio Gallo
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Premature births constitute a rapidly rising percentage of live births in the US. Annually ~65,000 preterm infants are born 'very low birth weight' or 'VLBW' (<1500 g; 32 weeks gestation), making them 40-100 times more likely to develop disruptive motor disorders such as ataxia compared to regular birth weight infants. Biomedical studies have established perinatal hypoxia as a major cause of grey and white matter abnormalities in the CNS of VLBW infants, with the cerebellum being particularly vulnerable to hypoxic injury. Previous work from our laboratory has shown that GABAergic signaling is significantly reduced in the white matter of the cerebellum in an established mouse model of perinatal hypoxic injury [Zonouzi, M. *et al.* GABAergic regulation of cerebellar NG2 cell development is altered in perinatal white matter injury. *Nature Neuroscience*, 2015]. Quite strikingly, the cerebellar cortex is also affected post-hypoxic injury (Hx). Hx results in a drastic reduction in number of molecular layer interneurons, and dramatically altered Purkinje cell arborization compared to normoxic controls (Nx). However, whether this cellular alteration in the cerebellar cortex due to hypoxic insult results in a difference in motor behavior remains unexplored. Using an automated behavioral apparatus – the Erasmus Ladder – to monitor motor performance and learning, we have studied cerebellar behavior in Hx mice. This horizontally-oriented computerized ladder allows us to measure stepping patterns using pressure-sensitive rungs. Our results show that compared to normoxic controls, P25 Hx mice display profound deficits in both motor performance – measured as number of missteps on the ladder, as well as conditioned motor learning – measured as adaptability to adjust stepping patterns to avoid a computer-controlled obstacle preceded by a warning tone. This deficit in motor performance and learning is present even in naïve P45 Hx mice, albeit to a lesser extent than P25 Hx deficits. Thus, our data indicates a long-term ataxic phenotype characterized by motor malperformance, as well as cerebellar-learning deficits in a mouse model of premature birth injury.

OR01-03

DIFFERENTIAL PERIPHERAL AND CENTRAL NERVOUS SYSTEM REGULATION OF SELF-REACTIVE CD4 T CELLS DURING VIRAL ENCEPHALOMYELITIS

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Viral infections have been implicated in initiating and enhancing the severity of the demyelinating disease Multiple Sclerosis (MS), by activating self reactive (SR) T cells through mechanisms that include epitope spreading, molecular mimicry, cryptic antigen and bystander activation. Neurotropic coronavirus (JHMV strain) induced demyelination is indeed associated with induction of SR T cells. Nevertheless, despite sustained demyelination and central nervous system (CNS) inflammation, SR T cells declined during chronic infection and were not associated with autoimmune symptoms, suggesting regulatory mechanisms limit the SR T cell response.

JHMV infection induces both antigen-specific IL-10 secreting CD4 T cells (Tr1) and Foxp3 regulatory T cells known to control autoimmunity. To assess their relative implication in limiting SR T cells, we infected both IL-27 receptor deficient (IL-27R$^{-/-}$) mice, characterized by a drastic reduction of Tr1 cells and DEREG mice, which express the diphtheria toxin (DT) receptor under control of the Foxp3 promoter to specifically deplete Foxp3 Tregs.

Ablation of Foxp3 Tregs during chronic JHMV infection was associated with a significant increase of SR CD4 T cells within cervical lymph nodes, but no differences in their numbers or activation within the CNS compared to controls. In contrast, SR CD4 T cells were increased within the CNS of IL-27R$^{-/-}$ mice, while the peripheral SR T cell response remained unchanged.

Altogether, these data suggest that JHMV infection establishes differential regulatory mechanisms in the CNS and periphery to limit SR T cells. The JHMV model is thus unique to decipher the differential mechanisms preventing autoimmunity during persistent viral infection.

OR01-04

CHEMOGENETIC ACTIVATION OF SATELLITE GLIAL GQ-GPCR SIGNALING ENHANCES CARDIAC FUNCTION IN VIVO

Alison Xie, Jakovin Lee, Ken McCarthy
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The autonomic nervous system (ANS) exerts profound regulatory influence over cardiovascular physiology. The integrated network among autonomic nuclei in the central nervous system (CNS), as well as ganglionic innervation in the peripheral nervous system (PNS) are essential for homeostasis and for coordinating adaptive changes in physiology and behavior. In addition to neurons, the ANS contains large numbers of Glial Fibrillary Acidic Protein (GFAP) - expressing cells ($GFAP^+$ glial cells), which serve many functions in assisting neuronal network activity in both the CNS and the PNS. The present study explores the active role of $GFAP^+$ glial cells in regulating cardiovascular function *in vivo*. Selective activation of Gq-GPCR signaling in $GFAP^+$ glial cells using DREADD technology led to fast and long-lasting increases in heart rate and blood pressure *in vivo*. Pharmacological studies suggest that the changes in cardiovascular function are due to activation of ganglionic SGCs and consequent increases in neuronal output from sympathetic ganglia. Ongoing *ex-vivo* studies using superior cervical ganglia cultures focus on identifying the mechanism underlying the interaction between sympathetic SGCs and postganglionic neurons.

OR01-05

BRAIN ENDOTHELIAL CXCL10 MEDIATES LPS-INDUCED MICROGLIAL ACTIVATION AND NEUROPROTECTION IN MICE

Zhihong Chen, Weiwei Hu, Zachary Gossman, Grahame Kidd
Timothy Phares, Cornelia Bergmann, Bruce Trapp
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Microglia are the resident innate immune cells of the central nervous system (CNS) and are the primary responders in a defense network that covers the entire brain parenchyma. Intraperitoneal (IP) injection of the Gram-negative bacterial endotoxin lipopolysaccharide (LPS) elicits microglial activation, which provides neuroprotection through synaptic stripping. However, the mechanism by which microglia respond to the peripheral LPS challenge, whether directly or indirectly, is still undefined. In this study, by taking advantage of cell-specific conditional KO, we demonstrated that the integrity of TLR4 signaling pathway in brain endothelial cells is required to transduce microglial activation and neuroprotection. By using microarray analysis, we identified brain endothelial CXCL10 is a critical molecule in mediating microglial response and neuroprotection. Knocking-out CXCL10 abolishes LPS-induced neuroprotection. We further established that CXCL10 is not only essential for LPS-induced microglial activation, but also microglia-mediated synaptic striping, which is one of the underlying mechanisms known to confer neuroprotection against brain trauma. The significance of these findings rests not only in establishing brain endothelial TLR4 as the initial activation site of peripheral LPS stimulation, but also in identifying endothelial CXCL10 as a crucial molecule enabling these sequential events.

OR02 Oral Session II

OR02-01

MICROGLIAL LIPOPROTEIN RECEPTOR LRP1 REGULATES NEUROINFLAMMATION: APPLICATIONS FOR MULTIPLE SCLEROSIS

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The functions of myeloid cells are inextricably linked to disease progression in Multiple Sclerosis (MS). Besides phagocytosis of the copious amounts of cell debris in the MS lesion, myeloid cells also express inflammatory response proteins that exacerbate clinical disease. Myeloid cells are the most abundant immune cell type within MS lesions and increased number of myeloid cells correlates with enhanced disease severity. Current literature suggests that in animal models of MS, modifying the inflammatory activity of myeloid cells–either microglia or recruited macrophages– correlates with better disease outcomes. Our data show that the expression of the receptor LRP1 on myeloid cells is increased in actively demyelinating lesions in MS patients. We find that mice with deficient LRP1 expression in their microglia have higher incidence and severity of EAE. Additional molecular analysis shows that LRP1 attenuates the inflammatory activity of myeloid cells by decreasing inflammatory cytokine expression. Thus, LRP1 is a putative anti-inflammatory protein. Understanding the mechanism by which LRP1 mediates its protective effect in MS will shed light on physiological modulation of neuroinflammation, and contribute to the development of new therapeutic agents for preventing MS progression and severity.

OR02-02

GPR126/ADGRG6 HAS SCHWANN CELL AUTONOMOUS AND NON-AUTONOMOUS FUNCTIONS IN PERIPHERAL NERVE INJURY AND REPAIR

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Adhesion G protein-coupled receptors (aGPCRs) are a unique class of GPCRs defined by a large extracellular region containing various domains that may be involved in cell-cell or cell-matrix adhesion as well as the classical 7-transmembrane (7TM) region that may be involved in cell signaling. Being a rather understudied class of GPCRs, in general, it is unclear if aGPCRs function as adhesion molecules by virtue of the long N-terminus, as traditional GPCRs that signal through heterotrimeric G proteins by virtue of the 7TM, or if the same molecule can perform both functions. We previously showed that the aGPCR Gpr126/Adgrg6 is essential for Schwann cell development and myelination in the zebrafish and mouse PNS. More recently, we showed that Gpr126 performs these functions by directly regulating cAMP concentrations via coupling to heterotrimeric G-proteins in Schwann cells. Interestingly, the expression of Gpr126 is maintained in adult Schwann cells, suggestive of a function in the adult PNS. We have therefore begun to analyze the role of Gpr126 in myelin maintenance and remyelination after injury by studying a Schwann cell-specific tamoxifen-inducible knockout PLPCre-ERT2;Gpr126fl/fl mouse model. Here, we show that deletion of Gpr126 in mature Schwann cells does not affect myelin maintenance, but remyelination is severely delayed after nerve crush injury. Moreover, we observe several non-cell autonomous defects in injured nerved with reduced Gpr126 in Schwann cells. This work demonstrates that Gpr126 is dispensable for myelin maintenance but essential for proper nerve repair.

OR02-03

A PIVOTAL ROLE OF TESTOSTERONE AND THE ANDROGEN RECEPTOR IN MYELIN REGENERATION

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We have previously shown that testosterone therapy promotes the regeneration of central nervous system (CNS) myelin in a model chronic demyelination, where no spontaneous axon remyelination was observed (Rashad Hussain et al. 2013, Brain 136, 132-146). Here, we report that the spontaneous remyelination of axons by oligodendrocytes fails in the absence of testes and requires testosterone signaling via the intracellular androgen receptor (AR). Moreover, in the absence of testosterone or its receptor, oligodendrocyte progenitor cells (OPC) preferentially differentiated into peripheral myelin-synthesizing cells, i.e. Schwann cells. This was shown by using an experimental model in which axons of the right ventrolateral white matter tract of the adult male mouse spinal cord were locally demyelinated by stereotaxic infusion of lysophosphatidylcholine (LPC). Four weeks after LPC, axons were completely remyelinated by oligodendrocytes within the area of demyelination. However, no remyelination by oligodendrocytes was observed in the absence of testosterone after surgical castration, or after selective ablation of the AR within the CNS. Instead, axons became remyelinated by Schwann cells. Our findings thus reveal an unforeseen key role of the male gonad, testosterone and the AR in OPC specification and myelin regeneration. This may come as a surprise, as testosterone is well known as a male sexual hormone with reproductive functions. However, the AR is abundant and widely distributed throughout the brain and spinal cord, strongly suggesting multiple functions of testosterone to neurons and glial cells. Of note, AR has evolved from an ancestral steroid receptor through gene duplication, precisely at the time when myelin appeared in jawed vertebrates. Thus, both the evolutionary history

of AR and its large distribution in the CNS are consistent with a new signaling function in myelin formation.

OR02-04

NOVEL ROLES FOR SPON1 IN ESTABLISHING CIRCUITS AND BEHAVIORS ASSOCIATED WITH CIRCADIAN PHOTOENTRAINMENT

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Over 20 classes of retinal ganglion cells exist in the mammalian retina, each with unique functions, morphologies and projection patterns. In previous studies aimed at elucidating how different classes of RGC axons target different retino-recipient nuclei, we identified Reelin – an extracelluar matrix protein – as being important in directing the targeting of M1 intrinsically photosensitive RGCs (ipRGCs) to the ventral lateral geniculate nucleus (vLGN) and the intergeniculate leaflet (IGL). In mice lacking Reelin, axons from M1 ipRGCs were misrouted into inappropriate regions of the mouse thalamus. However, this specific class of ipRGCs, which encodes for non-image forming responses to light that are necessary for circadian photoentrainment, target other regions of the brain where Reelin is not expressed, such as the suprachiasmatic nucleus (SCN). In the current study we sought to understand what unique cues M1 ipRGCs use to target the SCN. Using a bio-informatic approach, we identified Spon1 (also called F-Spondin): an extracellular matrix protein whose expression is dramatically enriched in the SCN compared with adjacent hypopthalamiuc nuclei. Spon1 appeared as a good candidate synaptic targeting cue for ipRGCs since it binds and signals through the same receptors as Reelin. Behavioral analyses of *spon1*$^{-/-}$ mutant mice reveal reduced activity and defects in circadian photoentrainment, suggesting significant defects in the circuitry associated with circadian behavior. Here we have applied anatomical approaches to further assess the formation and maintenance of the retino-hypothalamic tract in novel targeted mouse mutants that lack Spon1.

OR02-05

MYELIN REMODELING AND EXPERIENCE-DEPENDENT OLIGODENDROGENESIS PERSIST IN THE MIDDLE-AGED BRAIN

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Oligodendrocytes generate myelin sheaths that enwrap axons in the CNS. Although most oligodendrocytes are generated in early postnatal life, the development of myelin continues into adulthood, and recent studies indicate that myelin can be modified by experience. Moreover, cortical axons exhibit discontinuous myelination and blocking the formation of new oligodendrocytes impairs motor learning, raising the possibility that myelin is continually adjusted to modify the information processing capabilities of cortical circuits. However, these studies have been performed during early adulthood, when developmental myelination is still ongoing. It is unclear whether the ability to remodel myelin is retained with age. To explore how myelination changes with age, we used *in vivo* two-photon time-lapse imaging in the somatosensory cortex of transgenic mice in which EGFP is expressed in mature oligodendrocytes (*MOBP-EGFP* mice). EGFP is expressed by nearly all myelinating oligodendrocytes in *MOBP-EGFP* mice and permits the visualization of oligodendrocyte somata and myelin sheaths *in vivo*. Our studies indicate that cortical oligodendrogenesis is very prolonged, with more than half of the oligodendrocytes produced after 3 months of age. However, isolated myelin internodes were still observed in the cortex in middle-aged animals (10-14 months), indicating that discontinuous myelination is not a transient developmental phenomenon. To determine whether the ability to remodel myelin is retained with age, we repeatedly imaged oligodendrocytes and myelin internodes for 1.5 months in middle-aged mice. Although most myelin internodes remained stable, approximately 1% were remodeled, either increasing or decreasing in length. To determine whether sensory experience affects oligodendrogenesis in the middle-aged brain, *MOBP-EGFP* mice were housed in standard cages or cages enriched with hanging beads. Sensory enrichment resulted in five-times the number of newly generated oligodendrocytes. Together, these results indicate that addition and reorganization of myelin internodes persists in the middle-aged brain and may help to alter the pattern of myelination with life experience, perhaps contributing to the plasticity of adult cortical circuits.

Pre-Meeting Workshops

PMW01 Probing Outside the Synapse: Astrocyte-NEURON REGULATION of Excitatory Signaling

PMW01-01

CIRCUIT-SPECIFIC SYNAPTIC REGULATION BY ASTROCYTES

Alfonso Araque
University of Minnesota, Neurosccience, Minneapolis, USA

Accumulating evidence indicates that astrocytes exchange signals with neuronal synaptic elements, establishing a functional unit called “tripartite synapse”. The signaling exchange between astrocytes and neurons within the tripartite synapse results in the regulation of synaptic transmission and plasticity.

I will present and discuss recent evidence indicating that the astrocyte synaptic regulation is not restricted to the active tripartite synapse but can be manifested through astrocyte signaling at synapses relatively distant from active synapses, a process termed lateral astrocyte synaptic regulation. This phenomenon resembles the classical heterosynaptic modulation but is mechanistically different because it involves astrocytes and its properties critically depend on the morphological and functional features of astrocytes.

I will also present recent evidence indicating the existence of circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. I will discuss our recent results showing that subpopulations of astrocytes selectively responded to specific medium spiny neurons subtypes in the dorsal striatum, and that these subpopulations of astrocytes release glutamate that selectively regulates neuronal excitability and synaptic transmission in homotypic, but not heterotypic, medium spiny neurons, which indicates that the bidirectional astrocyte-neuron signaling selectively occurs between specific subpopulations of astrocytes, neurons, and synapses.

PMW01-02

NICOTINIC INPUT PROMOTES GLUTAMATERGIC SYNAPSES VIA ASTROCYTES

Darwin Berg
University of California, San Diego, San Diego, United States

Nicotinic cholinergic signaling is well known to modulate glutamatergic transmission throughout the brain. It acts both pre-and postsynaptically, often through α7-containing nicotinic receptors, to alter the amount of glutamate released, the number of postsynaptic receptors available to respond, and the consequences of the response. Recently it has been shown that endogenous nicotinic signaling early in development actually helps promote glutamatergic synapse formation, again acting through α7 receptors. Intriguingly, astrocytes express α7-containing nicotinic receptors. We find that activation of these receptors on astrocytes releases a component that recruits glutamate receptors to sites on neurons, rendering the sites functional as synapses. Ongoing studies show that early exposure to nicotine itself can abnormally increase the number of glutamatergic synapses in long-lasting ways and effectively re-wire the network for excessive responses well into adulthood. The role of astrocytes in this is an interesting question for the future.

PMW01-03

CONTROL YOUR EXCITEMENT: ASTROCYTE REGULATION OF GLUTAMATE SIGNALING IN THE DEVELOPING AND INJURED BRAIN

Chris Dulla
Tufts University School of Medicine, Neuroscience, Boston, USA

Glutamatergic neurotransmission is controlled by a family of molecules known as the excitatory amino acid transporters (EAATs). These transporter have a high affinity for glutamate and utilize the sodium gradient to move glutamate into the intracellular compartment against its concentration gradient. GLT1 and GLAST, two members of the EAAT family, are heavily expressed by astrocytes in the developing and mature brain. These important proteins are developmentally regulated and play a critical role in protecting the brain from seizures and excitoxicity. When EAATs remove released glutamate, they terminate extracellular glutamate transients and shield glutamate receptors from over-activation. Interestingly, EAAT expression and function are disrupted by brain injury. In this presentation, I will discuss the developmental regulation of glutamate transporters in the cerebral cortex as well as how this developmental maturation contributes to glutamate receptor activation and extracellular glutamatergic tone. Next, I will discuss how neonatal cortical insult leads to formation of hyperexcitable cortical circuity, and the role that glutamate transporters play in this circuit-level transformation. Lastly, I will present exciting new data highlighting how neuronal activity associated with both normal and pathological brain activity, rapidly shapes extracellular glutamate dynamics via modulation of glutamate transporter activity. These studies employ innovative imaging, electrophysiological, and genetic approaches to understand how astrocytic control of glutamate signaling contributes to neuronal excitation. The overall goal of this presentation will be to highlight the exciting dynamics of EAAT control of glutamate signaling in the developing and mature cerebral cortex and how disruption of this system following injury system can contribute to the development of epilepsy.

PMW01-04

DYSREGULATION OF ASTROCYTES AND PATHOLOGICAL EXCITATORY SIGNALING

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Gaps in our understanding of glutamate, which is present in the majority of synapses, are a fundamental barrier to treating CNS diseases. While models of excitatory signaling have primarily incorporated synaptic mechanisms, it is apparent that glutamate signaling is regulated by a number of extrasynaptic processes, including Glu release from astrocytes. System xc- (Sxc) is a non-vesicular glutamate release mechanism primarily expressed by astrocytes that has been shown to exert profound control over synaptic transmission and may be altered in CNS diseases. To determine whether Sxc is essential for complex behavioral phenotypes, we created Sxc loss of function transgenic rats (MSxc) by mutating the Slc7A11 gene. While mutant Sxc (MSxc) rats displayed normal survival rates, growth patterns, and basal levels of locomotor activity, deletion of Sxc resulted in a number of maladaptive behaviors including heightened drug seeking and impaired cognitive flexibility. Because glutamate signaling requires coordinated activity of neurons and astrocytes and because Sxc activity is blunted in CNS diseases, we next examined whether neuron-astrocyte interactions influenced Sxc activity. Interestingly, we discovered that astrocytes exposed to neurons or neuronal conditioned media displayed higher levels of Sxc activity. Moreover, we found that the pituitary adenylate cyclase-activating polypeptide (PACAP) may be a neuronal factor capable of regulating astrocytes. In support, blockade of PACAP receptors in rat cortical cultures comprised of astrocytes and neurons significantly decreased Sxc activity to the level observed in purified astrocyte cultures. In contrast, application of PACAP to purified astrocyte cultures increased Sxc activity to the level observed in cultures comprised of neurons and astrocytes. In order to demonstrate the in vivo relevance of this regulation, we found that PACAP microinjected into the nucleus accumbens blocked cocaine reinstatement. Collectively, these data establish Sxc and its regulation by PACAP as a novel form of neuron-astrocyte signaling that may be relevant in the study and treatment of CNS diseases.

PMW02 From Single Cell Transcriptomes to Functional Circuits: New Findings from the Allen Institute for Brain Science Frontline

PMW02

HUMAN AND PRIMATE-SPECIFIC FEATURES OF NEOCORTICAL DEVELOPMENT, STRUCTURE AND FUNCTION

Ed Lein
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Understanding the function of the human neocortex in health and disease necessitates an understanding of its fundamental circuitry, cell types, developmental processes and molecular underpinnings. To tackle the complexity of this problem we began by studying the underlying molecular code, creating a series of large-scale transcriptional atlases of the mouse, non-human primate and human neocortex. This spatiotemporal map allows an identification of the transcriptomic architecture of the cortex as it develops from a germinal zone through its mature laminar and areal architecture, and "canonical" gene networks that describe the majority of the transcriptional variance in the adult cortex. These data reveal many features of cortical organization and highlight the magnitude of differences between human and model organisms. On the order of 25-30% of genes analyzed show differences in cellular and temporal patterning between primates and rodents, with many fewer differences between human and non-human primate. These findings highlight the importance of studying the human brain itself to understand its detailed function in health and disease, and have led us to develop a new suite of technologies to study the molecular and cellular architecture of the postmortem and living human cortex. Specifically, we are developing a suite of scalable, standardized methods for single cell transcriptomics, quantitative cellular morphology and intrinsic physiological properties, as well as viral tools for molecular genetic manipulation of specific cell types in human cortical slices derived from neurosurgical resections. These efforts aim to derive a quantitative taxonomy of cell types and help understand conserved features of cortical architecture that can be studied in model organisms versus unique features of the human neocortex.

PMW02-01

MAPPING CORTICAL AREAS IN GCAMP6 REPORTER MICE WITH WIDEFIELD IMAGING

Jack Waters
Allen Institute for Brain Science, ---, Seattle, USA

We employed fluorescence imaging and GCaMP6 reporter mice to map visual areas in the mouse. Our opdated map includes 5 new retinotopically-organized regions that extend into barrel cortex and retrosplenial cortex, bringing the total number of regions of mouse cortex with distinct retinotopic maps to 15. In light of the revised map, we reassessed the representation of visual space within each visual area, finding that the four higher visual areas immediately surrounding V1 (called LM, P, PM and RL) display complementary representation, such that these areas together form a single map of the visual hemifield. A similar arrangement exists in primates, where V2 is the ring of cortical tissue surrounding V1 that contains a single secondary representation of visual space. Hence our results reveal new features of the organization of mouse visual cortex and suggest that the hierarchy of mouse visual areas may be more similar to that of primates than previously recognized.

PMW02-02

ADULT CORTICAL CELL TAXONOMY BY SINGLE CELL TRANSCRIPTOMICS

Vilas Menon
Allen Institute for Brain Science, Modeling, Analysis, and Theory, Seattle, USA

Nervous systems are composed of numerous cell types, but the extent of cell type diversity is poorly understood. Here, we construct a cellular taxonomy of primary visual cortex in adult mice based on single-cell transcriptomic profiling. Having developed methods to reliably isolate individual neurons labeled by specific transgenic Cre lines, we used single-cell RNA-sequencing methods to obtain a transcriptional readout of ~1600 individual excitatory and inhibitory cells from multiple cortical layers. We identify 49 transcriptomic cell types - 23 GABAergic, 19 glutamatergic, and 7 non-neuronal types - that exhibit varying degrees of interrelatedness. Based on these data-driven clusters, we extract a set of well-known and lesser-studied genes to generate combinatorial codes to distinguish each of these neuron types. We also analyze cell-type specific mRNA processing and characterize genetic access to these transcriptomic types by many transgenic Cre lines. In addition, we show that some of our transcriptomic cell types display specific and differential electrophysiological and axon projection properties, thereby confirming that the single cell transcriptional signatures can be associated with specific cellular properties. Finally, we examine key surface molecule and neurotransmitter genes to propose hypothetical interactions among some of these transcriptomic types.

PMW02-03

MAPPING STRUCTURAL CONNECTIVITY OF THE MOUSE BRAIN

Julie Harris
Allen Institute for Brain Science, Cell and Circuit Genetics, Seattle, USA

A comprehensive brain wiring diagram should include both interareal pathways as well as specific connectivity patterns from unique

cell types within a region. The current Allen Mouse Brain Connectivity Atlas consists of projection mapping from ~300 anatomically-defined regions sampled from all major brain structures in cortical and subcortical areas. We have also completed a large-scale effort to map projections from genetically-identified cell populations throughout the whole brain using Cre driver lines and rAAVs expressing Cre-dependent fluorescent proteins. Over 100 Cre lines from a variety of sources have been systematically characterized through ISH expression profiling of reporter genes in the whole brain. For all tracing experiments, fluorescently labeled axons are imaged by serial two-photon tomography at high throughput, high resolution, and with high sensitivity through the entire brain. These large datasets of image series are registered into a common 3-D reference atlas space to build axonal projection models and derive quantitative values of signal strength across all brain regions, which also facilitates searching and comparison between this Atlas and other resources. Our recent efforts focus on higher precision targeting and labeling of cell type specific connectivity in the mouse visual system. All data are freely available via the Allen Brain Atlas data portal (http://www.brain-map.org).

PMW03 Hands-on Workshop: Navigating the Allen Brain Atlas Resources

PMW03-01

NAVIGATION AND EFFECTIVE USE OF THE ALLEN BRAIN ATLAS RESOURCES

Terri Gilbert
Allen Institute for Brain Science, -, Seattle, USA

The Allen Institute for Brain Science was launched in 2003 to develop a comprehensive gene expression (*In Situ* Hybridization) map of the mouse brain, and rapidly expanded to include the spinal cord, developing brain and the Institute's first forays into the human brain. Since the first atlas of the mouse brain was completed in 2006, the Allen Institute has led the way in spearheading open access-open data brain science, and in 2012 was re-funded for a new generation of open science projects aimed at more deeply understanding the human brain. Developing a comprehensive research plan to understand brain function, the Allen Institute now has bold ambitions to piece together and integrate a multidimensional level of understanding of circuit function - scaling from single cell gene expression in mouse and human brain cells – up to understanding the dynamic structure and function of cortical circuits important for vision. This workshop will demonstrate how to use the various informatics tools that overlay the vast amount of data collected by scientists at the Allen Institute, and provide a hands-on experience in using the tools to forward your own research. This workshop will cover both the legacy applications as well as coaching on diving into the new datasets and a preview of the kinds of data and resources to come in the future.

Poster session SUNDAY/MONDAY (MAR 20-21)

PSM01 Development, Differentiation and Disorders

PSM01-01

NUCLEAR IMPORT OF NEUROFIBROMIN IS DEVELOPMENTALLY REGULATED, CELL CYCLE-DEPENDENT AND REQUIRED FOR PROPER CHROMOSOME CONGRESSION

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Neurofibromin, primarily expressed in neurons and astrocytes in the CNS, is a RasGAP, a property that has yet to explain aneuploidy often observed in patients with neurofibromatosis, which carry often private mutations in the *NF1* gene and have a 5-fold increased incidence of glioblastoma, one of the most malignant primary brain tumors. One of the least studied properties of neurofibromin is its ability to enter the nucleus through a nuclear localization sequence (NLS) in the C-terminal domain; exon43 that bears the NLS may be alternatively transcribed with ΔE43 splice variants expressed in tissues not implicated in NF-1 pathology. Focusing on this property, we examined the expression of Nf1-NLS transcripts most early in development and found that it is expressed as early as the zygote. When we studied temporal and spatial expression patterns of transcripts from the beginning of neuronal cell lineage, that is blastocyst inner mass stem cells onto retinoic acid (RA)-induced neurons, we found increases in DNLS transcripts only after the embryoid body stage. Moreover in the chick or mouse embryos cerebral tissues, expression of ΔE43 became apparent only after the first cohorts of postmitotic neurons were established. Astrocytes in culture or glioblastoma cells expressed throughout only NLS transcripts. Next, we demonstrated that, in astrocytic cellular backgrounds, neurofibromin's expression and nuclear localization are cell cycle-regulated, resulting in its nuclear accumulation by G2. At this point, neurofibromin is phosphorylated on Ser2808, a residue adjacent to NLS by PKC-ε. A combination of techiques showed that neurofibromin localizes on the mitotic spindle throughout mitosis, in glioblastoma cells and primary astrocytes. More importantly, analysis of mitotic phenotypes after siRNA-mediated depletion showed that acute loss of this tumor suppressor protein leads to aberrant chromosome congression at the metaphase plate. Taken together, we provide a mechanistic model according to which, neurofibromin nuclear import is mechanistically linked to an error-free chromosome congression.

PSM01-02

SIRT1 REGULATES OLIGODENDROCYTE PROGENITOR PROLIFERATION AFTER WHITE MATTER INJURY

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Childrens National Medical Center, Neuroscience, Washington,, USA

Regenerative processes in brain pathologies require generation of distinct neural cell populations from endogenous progenitors. We have previously demonstrated a novel role of Sirt1 in oligodendrocyte progenitor cell (OPC) proliferation in the developing subcortical white matter (WM) induced by perinatal hypoxia (Hx). Sirt1 phosphorylation occurs at residue 47 [Sirt1 (ser47)] as a result of post-translational modifications of Cdk2 and Rb. We also found that Hif1α activation and NAD production occur simultaneously with enhanced OPC proliferation in WM. Here, we further established a causal relationship between Hif1α activation, Sirt1 and Cdk2 in Hx-induced WM injury. Using $Cdk2^{-/-}$ (KO) mice, in which exon 4 and 5 were not functional, we found that Hx increased the number of $Hif1\alpha^{+}$ cells, as well as Sirt1 $(ser47)^{+}$ cells in WM. This indicates that Sirt1 is upstream of Cdk2. Furthermore, these data also demonstrate that Hif1α acts on Sirt1 through kinases other than Cdk2. Consistently with this interpretation, Western Blot analysis confirmed higher level of Hif1α and Sirt1 (ser 47) after Hx in both WT and KO mice. Further analysis also established a link between NAD and Sirt1 activation after Hx. Cultured cells from Nx and Hx WM were incubated in control medium (with EGF + FGF), as well as with NAD or NADH for 24 hours. Immunoprecipitation analysis demonstrated lower levels of acetylated Cdk2 after Hx in control untreated cultures, suggesting increased deacetylation of Cdk2. In the presence of NADH, level of deacetylated Cdk2 was similar in Nx and Hx cells; however NAD dramatically upregulated the level of deacetylated Cdk2 after Hx, indicating that the increase of NAD observed after Hx plays a crucial role in Sirt1 activation. Together, these results indicate that Sirt1 is a major Hif1α- and NAD-dependent mediator of Hx-induced OPC proliferation in WM, and that Sirt1 directly activates Cdk2.

Supported by NIH R01NS045702, P01NS062686 and IDDRC P30HD40677

PSM01-03

SENSORY ACTIVITY AND AGGRECAN REGULATE THE DEVELOPMENT OF CORTICOTHALAMIC PROJECTIONS

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Neural circuit formation demands precise timing of innervation by different classes of axons, however the mechanisms underlying such activity remain largely unknown. In the dorsal lateral geniculate nucleus (dLGN), axons from retina and visual cortex innervate thalamic relay neurons in a highly coordinated manner, with those from the cortex arriving well after those from retina. The differential timing of retino- and corticogeniculate innervation is not a coincidence but is orchestrated by retinal inputs and we recently identified aggrecan, a chondroitin sulfate proteoglycan (CSPG), that regulates this process. Is this mechanisms specific to this specific dorsal thalamic nucleus, or is it more general to other regions of the dorsal thalamus? To answer this question we asked two general questions: Does activity in the "driving" sensory inputs regulate the timing of the arrival and arborization of "modulatory" corticothalamic inputs? And, does the timing of corticothalamic innervation in other dorsal thalamic nuclei require aggrecan? We have initially focused our attention on the medial geniculate nucleus (MGN), which receives and processes auditory information. Thus far, we have discovered that removing auditory stimuli (in tmc1-/- mutant mice) leads to premature cortical axon arborization in MGN, suggesting that environmental signals are indeed important for the timing of corticothalamic innervation into many dorsal thalamic nuclei. We next analyzed aggrecan distribution in tmc1-/- mutant mice and corticothalamic innervation in aggrecan-deficient mutants (acancmd/cmd). Our results demonstrate that aggrecan may be critical for preventing some lateral corticothalamic fibers from entering MGN, however, analysis in acancmd/cmd suggests that the role of aggrecan in MGN may differ from that in dLGN.

PSM01-04

REDUCED ACTIVITY OF MONOAMINE OXIDASE A IN THE BRAIN OF CHILDREN WITH AUTISM

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Autism is a neurodevelopmental disorder characterized by abnormal social and behavioral abnormalities. Extensive evidence from our and other groups has suggested oxidative stress and mitochondrial dysfunction in autism. Monoamine oxidase A (MAOA), a mitochondrial-bound enzyme, catalyzes the oxidation of endogenous amine-containing neurotransmitters such as serotonin and norepinephrine. The role of MAOA in autism is of particular interest because this enzyme affects the levels of serotonin, which are known to be abnormal in some individuals with autism. In comparison to other alleles of MAOA, the 3-repeat allele is associated with reduced transcription and therefore, reduced activity of MAOA. A few studies have reported an association of the low-activity, 3-repeat MAOA-uVNTR allele with autism. In this study, we analyzed the MAOA activity in the cerebellum and frontal cortex from autistic subjects and age-matched control subjects. In the cerebellum, the activity of MAOA was significantly lower in autism than in control subjects. When the subjects were divided into two subgroups according to their ages: children (ages 4-12 years) and adults (ages 13-38 years), a significant decrease in the activity of MAOA was observed in only children with autism but not in adult autistic group. In the frontal cortex, the MAOA activity in autistic children group was also reduced by 30% than in controls, but there was no significant difference. These results suggest that the brain MAOA activity is lower in autistic children than in control subjects. Lower MAOA activity will cause increase in the levels of related neurotransmitters, such as serotonin, which is one of the most important neurotransmitters influencing behavior and has been reported to have critical relationship with autism.

PSM01-05

EMBRYONIC IRON DEFICIENCY SHIFTS THE INHIBITORY/EXCITATORY BALANCE OF THE ADULT BRAIN

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Iron deficiency is the most common micronutrient deficiency, affecting more than nine percent of reproductive age women in the United States and over a billion women worldwide. When an iron deficient woman becomes pregnant, her body cannot provide adequate iron to the fetus during critical windows of development, and there is an increased risk of psychiatric disorders, behavioral problems, and learning difficulties later in the child's life. Despite an awareness of this risk, the developmental mechanism linking embryonic iron availability to these diseases remains largely unknown.

We established a mouse model of gestational iron deficiency in which the dam is given a diet with sufficient iron to meet her daily needs but too little to support the increased iron requirement during pregnancy. As is often seen in humans, this model produces mild anemia during the third trimester which is then treated with post-natal iron supplementation. We found that adult mice, which were gestationally iron deficient, had a significantly impaired response to both a GABA antagonist and a glycine agonist. These mice had an increased number of inhibitory GABAergic interneurons in the cerebral cortex, with no apparent decrease in excitatory pyramidal neurons. We also examined the region which gives rise to GABAergic interneurons during embryonic development and found an expansion of both Nkx2.1 and Gli1 signaling: two factors which are important for appropriate specification of GABAergic interneurons. Taken together, our data suggests that embryonic iron deficiency increases inhibitory signaling in the adult brain by expanding the region which produces inhibitory GABAergic interneurons during development, thereby increasing the number of inhibitory interneurons in the cortex and disrupting the balance between inhibition and excitation in the adult brain.

PSM01-06

DISRUPTION OF ENDOLYSOSOMAL TRAFFICKING AFFECTS BRAIN MORPHOGENESIS DURING EMBRYONIC DEVELOPMENT

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The developing brain is characterized by rapid and persistent remodeling. Neural progenitor cells respond to changing molecular landscapes by adapting their complement of cell surface membrane proteins through highly ordered and regulated mechanisms of membrane insertion and degradation. The trafficking itinerary of such vesicle-based cargos varies greatly depending on developmental status, metabolic demand and signaling activity. The small GTPase Rab7A operates within autophagic and endocytic pathways as a master regulator of lysosomal degradation and membrane trafficking. However, the impact of full loss of Rab7A function in the brain has not been explored in vivo. Here, we have used genetic loss-of-function of Rab7A in the prenatal mouse brain to explored the link between protein trafficking and neural development. Our results indicate multiple brain defects associated with the loss of Rab7a in the developing mouse brain and reveal new aspects of brain development that require Rab7a function. This work opens new avenues to identifying and understanding critical membrane-associated proteins necessary for proper brain patterning and their trafficking itineraries during development. It will also shed new light on the vesicular trafficking mechanisms orchestrating proper neurodevelopment by revealing heretofore unappreciated aspects of patterning that are dependent on the precise control of membrane protein sorting and turnover.

PSM01-07

LRRTM1 IS A DEVELOPMENTALLY REGULATED, TARGET-DERIVED CUE THAT DRIVES RETINAL TERMINAL DIFFERENTIATION IN MOUSE VISUAL THALAMUS

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Retinal synapses onto relay cells in visual thalamus differ from retinal terminals in all other retino-recipient nuclei. Not only are retinal terminals significantly large in the dorsal lateral geniculate nucleus (dLGN), but they can be grouped into two distinct classes: simple retinogeniculate (RG) synapses which contain a single retinal terminal or complex RG synapses which contain numerous terminals on the same region of relay cell dendrite. In the present study we aimed to identify target-derived factors enriched in dLGN that could drive the unique differentiation of retinal terminals in this region. RNAseq analysis identified LRRTM1 (Leucine-Rich Repeat Transmembrane Neuronal 1) as a developmentally regulated synaptic organizer enriched in dLGN compared with other retino-recipient nuclei. In mouse, *lrrtm1* mRNA shows a significant enrichment in dLGN during the development of retinogeniculate synapses. To test the role of LRRTM1 in RG synapse formation, we assessed retinal terminals in targeted mutant mice lacking LRRTM1 (*lrrtm1*$^{-/-}$). Both anterograde labeling of retinal terminals by intraocular injection of fluorophore-conjugated Cholera Toxin B (CTB) and immunostaining for Vesicular Glutamate Transporter 2 (VGluT2), which is specifically enriched in retinal terminals in dLGN, showed an increase in the proportion of smaller terminals in *lrrtm1*$^{-/-}$ mutant mice. These results could mean that each retinal terminal was smaller in mutants or that complex RG synapses were absent or impaired in their formation. To answer this question we used serial block face scanning electron microscopy (SBFSEM). Our ultrastructural data in mutant and control dLGN indicate a reduced number of complex retinogeniculate synapses in the absence of LRRTM1. Thus, complex RG synapses require LRRTM1. This study provides the first insight into the role of a transynaptic synaptic adhesion molecule in regulating the assembly of complex, multi-bouton synapses. Further studies are needed to investigate the physiological consequences and behavioral impact of this defect in retinogeniculate synapses in the absence of LRRTM1.

PSM01-08

GANGLIOSIDE GM1 MODULATES EPIGENETIC GENE REGULATION IN NEURONAL CELLS

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The structural diversity and localization of cell surface glycosphingolipids, including gangliosides in glycolipid-enriched microdomains (GEMs, also known as lipid rafts or glycosynapses) places them in an ideal environment from which they can mediate processes such as intercellular recognition, interactions, adhesion, receptor function, and signaling. Recently, nuclear lipid domains on the nuclear envelope have also been suggested to play a similar role. Gangliosides are sialic acid-containing glycosphingolipids that are most abundant in the nerve tissues. The quantity and expression pattern of gangliosides in brain change drastically throughout development and are mainly regulated through stage-specific expression of glycosyltransferase genes. We have previously reported that efficient histone acetylation of the glycosyltransferase genes in mouse brain contributes to the developmental alteration of ganglioside expression (Suzuki et al., J. Neurochem., 2011). Here, we investigated the hypothesis that nuclear GM1 is associated with gene regulation in differentiated neuronal cells. We demonstrated that acetylation of histones H3 and H4 on the N-acetylgalactosaminyltransferase I (GalNAcT, GA2/GM2/GD2/GT2-synthase) gene promoter resulted in recruitment of *trans*-activation factors (Tsai et al., J. Neurochem. 2013). In addition, we showed that epigenetic activation of the GalNAcT gene was detected as accompanied by an apparent induction of neuronal differentiation in neural stem cells responding to an exogenous supplement of ganglioside GM1. We found that nuclear GM1 binds with acetylated histones on the promoters of the GalNAcT as well as on the NeuroD1 genes in differentiated neurons. Our study

demonstrated for the first time that GM1 interacts with active chromatin via acetylated histones at the nuclear periphery, resulting in changes in gene expression.

PSM01-09

UNEXPECTED ROLE OF INTERFERON-γ IN REGULATING NEURONAL CONNECTIVITY AND SOCIAL BEHAVIOR

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Immune dysfunction is commonly associated with autism spectrum disorder (ASD). In mice that model ASD, replacing the compromised peripheral immune system can rescue behavioral deficits, suggesting that peripheral immunity contributes to ASD. However, the mechanism(s) by which peripheral immunity influences neuronal function and social behavior is unknown. Here we show that adaptive immunity, particularly in the meninges, is necessary for normal social behavior. Mice deficient in adaptive immunity exhibit social deficits and hyper-connectivity of fronto-cortical brain regions. Intact adaptive immunity promotes pro-social behavior via signaling mediated by interferon gamma (IFN-γ). Activation of IFN-γ receptors stimulates inhibitory layer I cortical neurons and increased GABAergic currents in neighboring pyramidal neurons in the prefrontal cortex. This stabilizing effect on cortical activity contributes to proper social behavior. This study implicates adaptive immune dysfunction, in particular a disruption in the IFN-γ signaling, in neurological disorders characterized by social dysfunction.

PSM01-10

THE ROLE OF ADAM10 IN CENTRAL NERVOUS SYSTEM MYELINATION

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Oligodendrocytes (OLs) are the myelin forming cells of the central nervous system (CNS) and are considered to be important targets of therapies designed to promote remyelination in demyelinating disorders such as multiple sclerosis (MS). In the later stages of MS, there is a failure in the process of remyelination. Interestingly, this does not come as a result of the absence of oligodendrocyte progenitors (OPs; precursors to myelinating OLs), but rather, by a deficiency in the process of OP differentiation. As such, the identification of molecules capable of inducing OL lineage progression and promoting myelin repair has been a primary goal of regenerative medicine in myelin disorders. A disintegrin and metalloproteinase (ADAM) family members have been extensively studied in animal models of cancer, Alzheimer's disease, and immune disorders. Importantly, these molecules exhibit protease activity against a number of membrane-localized growth factors, cytokines, and receptors highly relevant to oligodendrocyte biology. In the context of myelination, our laboratory has demonstrated that ADAM17 promotes OL survival during white matter development and repair following cuprizone-induced demyelination. In an extension of these studies, we have also identified ADAM10 as a potent regulator of the OL lineage. In an initial qPCR screen of ADAMs family members using the *PDGFR*a-EGFP mouse line, ADAM10 was determined to be enriched in FAC-sorted GFP+ OPs. This finding was validated by immunocytochemistry, as ADAM10 was observed to be localized to the cell membrane of PDGFRa+ OPs. Further analysis *in vitro* revealed that overexpression of ADAM10 reduced *MBP* promoter activity. Finally, histological analysis demonstrated that pharmacological activation of ADAM10 delays white matter development *in vivo*. Together, these findings suggest that ADAM10 functions to inhibit myelination and implicate ADAM10 as a target for therapeutic intervention following white matter injury.

PSM01-11

PRONT-3 REGULATES GRANULE CELL PROLIFERATION DURING CEREBELLAR DEVELOPMENT VIA THE P75 NEUROTROPHIN RECEPTOR

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During embryogenesis proliferation and differentiation of progenitors and the precise transition among these processes is critical for normal development. Granule cell progenitors (GCP) are located in the External Granule Layer (EGL) of the cerebellum where they proliferate extensively. Post-mitotic granule cells leave the EGL and migrate inwards to form the Internal Granular Layer (IGL) where the differentiation of granule neurons is completed. Many studies have examined effects of mitogenic ligands such as Sonic Hedgehog (shh), however, little is known about signaling pathways that promote withdrawal from the cell cycle and favor differentiation.

The p75 neurotrophin receptor ($p75^{NTR}$) is highly expressed in the EGL, although its function there is unknown. The $p75^{NTR}$ can mediate multiple cellular activities by binding to different co-receptors and different intracellular binding proteins for signaling in response to a particular ligand, such as a neurotrophin (NT) or pro-neurotrophin. In the developing cerebellum, as cells start to differentiate and migrate toward the IGL, they down-regulate the $p75^{NTR}$, suggesting that $p75^{NTR}$ may maintain cells in the EGL in a proliferative state.

In the present work, we observed a delay in cell cycle exit in $p75^{NTR}$ KO mice compared with WT animals. This delay was sufficient to increase the size of the cerebellum, a difference that persisted in adult animals, compromising the normal motor/balance functions of the animals. Using a p75fl/fl; Atoh1-Cre animals we eliminated $p75^{NTR}$ specifically from EGL. These mice also showed an enlarged cerebellum and motor deficits, suggesting that the deficit in motor

functions might be, at least in part, due to deregulation of cell cycle in the GCP. Finally, we showed that proNT-3 is produced and secreted in the cerebellum. Using dissociated GCP cultures, we showed that proNT-3 was the only NT that antagonized shh-induced proliferation.

Our results suggest that $p75^{NTR}$ expression must be spatio-temporally regulated during development of the cerebellum for the proper control of cell cycle in granule cell progenitors and for normal development of the CNS.

PSM02 Drugs of Abuse: Alcohol, Cocaine, Methamphetamine

PSM02-01

FUNCTIONAL CONSEQUENCES AND PRODUCTIVE INFECTION OF HUMAN FETAL BRAIN-DERIVED NPCS BY HIV-1; INTERACTION WITH MORPHINE.

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Opiate drug abuse is strongly linked to CNS complications mediated by human immunodeficiency virus type 1 (HIV-1). Opiates are thought to augment HIV-1 neuropathology by interacting with opioid receptor-expressing CNS cells, leading to accelerated disease progression. Accumulating evidence suggests that neural progenitor cells (NPCs) are also targets of the deleterious effects of HIV-1 and opiates. Here, we investigated the functional consequences of HIV-1 and morphine co-exposure on the proliferation, survival, and differentiation of human, fetal, brain-derived primary NPCs (hNPCs). Treatment with supernatant from HIV-infected primary monocytes (HIV_{sup}) for 24 and 48 h, significantly decreased BrdU incorporation in hNPCs, which was exacerbated by morphine co-exposure. Doubling time of hNPCs was also prolonged in response to HIV_{sup} + morphine, further indicating proliferation changes. Significant cell death was not observed at 12-48 h of exposure. In addition, HIV_{sup} + morphine treatment for 12 d increased MAP2 and GFAP-expressing hNPCs, demonstrating an interactive effect on differentiation. To understand the basis of these responses, we explored earlier reports suggesting that hNPCs might be infected by HIV. Using multiple biochemical methods and a serial dilution approach, we confirmed the productive and sustained infection of hNPCs by R5 HIV-1_{BaL}. Interestingly, morphine enhanced viral production, particularly at later stages of infection, demonstrating that morphine exposure might enhance hNPCs infectivity. We tested whether HIV infection mediated the aberrant proliferation of hNPCs. Neither UV-inactivated HIV_{sup} or purified virions altered hNPC proliferation, suggesting that the effect did not require hNPC infection, but rather was due to other factors in the HIV_{sup}. Our findings suggest that hNPCs can be infected by HIV and may also actively contribute to the ongoing infection. Critical functions of hNPCs can also be undermined by the reactive milieu created by infection. Through these mechanisms hNPCs may contribute to CNS complications in adult and pediatric HIV/AIDS patients. DA024661

PSM03 Neuroinflammation

PSM03-01

ENTERIC GLIAL CELLS REACTION TO INFLAMMATION IS LOST IN CROHN'S DISEASE

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Enteric glial cells (EGC) are essential to intestinal epithelial barrier (IEB) homeostasis. In healthy intestines, EGC reduce IEB permeability and promote mucosal healing. In inflammatory bowel disease (IBD) such as Crohn's Disease (CD) and Ulcerative Colitis (UC), both EGC phenotype and IEB functions are altered, but putative involvement of EGC in IBD pathogenesis remains unknown. If the astrocyte reactivity is well studied, the reaction of EGC to chronic inflammation is not well documented, We investigated whether EGC impact on IEB permeability was altered in an inflammatory environment and in EGC from IBD patients. Rat EGC as well as human EGC from control, CD and UC patients were stimulated with the cytomix TI (TNFalpha+IL1beta ; 1 to 100ng/ml) or LPS for 2 or 4 days. Reactive EGC phenotype were characterized and reactive EGC functional impact on IEB permeability was studied (i) *in vitro* using human intestinal epithelial cells (IEC) in a non-contact co-culture model, or (ii) *in vivo* by grafting the treated rat EGC in colon wall of Sprague Dawley rats. Rat and human control EGC induced a significant reduction of IEB paracellular permeability after TI treatment when compared with untreated or LPS treated EGC. LPS or TI treatment had no significant effects on IEC alone. *In vivo* colon wall grafting with control EGC did not modified the permeability whereas colon wall grafting with EGC preconditioned by TI significantly reduced the permeability when compared to control animals. Human EGC from control or UC patients treated with TI induced a decrease in IEB permeability too, but EGC from CD patients did not. This work is not only the first evidence showing that reactive EGC can have beneficial effects upon IEB permeability, but also shows that EGC from CD but not UC patients have lost these reactivity. This could define EGC as active players in CD pathogenesis.

PSM03-02

GLIAL PGE2 PRODUCTION INDUCED BY INFLAMMATION REGULATES GLIAL RESPONSE TO ATP

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The enteric nervous system (ENS), composed of enteric neurons and enteric glial cells (EGC) regulates gut homeostasis. In inflammatory bowel disease (IBD) such as Crohn's Disease (CD) and Ulcerative Colitis (UC), ENS phenotype is modified but the causes and consequences of this remodelling remain unclear. If reactive astrocytes is well studied, the reaction of EGC to chronic inflammation is not well documented. We have analyzed here the production and role of one inflammatory mediator produced by EGC, the prostaglandin E2 (PGE2), in the IBD context.

Rat EGC as well as human EGC from control, CD and UC patients were stimulated with the cytomix TI (TNFalpha+IL1beta; 1 ng/ml) for 1 or 4 days. PGE2 production was measured by mass spectrometry in EGC conditioned media but also in biopsy supernatant from control CD and UC patients, in healthy and inflamed areas. We have measured the impact of TI or PGE2 treatment on EGC phenotype (glial marker expression and proliferation) and response to ATP (calcium intracellular concentration).

PGE2 concentration was significantly increased in UC biopsies supernatant but not in CD patients. No differences were detected between healthy and inflamed areas. PGE2 production by rat and human control EGC was significantly increased after TI treatment, but EGC from CD patients produced significantly less PGE2 than control or UC EGC. Whereas expression of GFAP was not affected, S100b and Sox10 expressions were increased after 4 days of TI treatment. GFAP, S100b and Sox10 expressions were not modified by PGE2. TI as well as PGE2 reduced EGC proliferation. In addition, PGE2 treatment also increased calcium flux (amplitude and duration) induced by ATP stimulation.

Previous works have shown that glial PGE2 sensitize neuronal response to bradykinin. Our work suggests that PGE2 glial production induced by inflammation can have autocrine effects that encompass EGC number control and an increased reactivity. In addition our work suggests that PGE2 glial production could participate in the differences observed between CD and UC pathological features.

PSM03-03

FATTY ACID INDUCED CYTOKINE EXPRESSION IN MICROGLIAL CELLS

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Abstract The systemic level of free fatty acids (FFAs) and FFA uptake in the brain has been found to be significantly higher in patients with metabolic syndrome compared with healthy subjects. Palmitate, one of the most abundant FFAs in plasma, has been found to aggravate inflammation by promoting secretion of pro-inflammatory cytokines in various cells. For example, palmitate treatment has been found to increase the expression of tumor necrosis factor (TNF)-α in C2C12 skeletal muscle cells, RAW264.7 macrophage cells, 3T3-L1 adipocytes, and astrocytes. Furthermore, rats exposed to high-fat diet have been reported to exhibit increased hypothalamic inflammation. However, how FFAs affect the function of microglial cells, the resident immune cells of the brain, has not been well-defined. In this study, BV2 microglial cells were treated with FFAs for different periods of time, and the expression of various cytokines was examined. Forty-eight hour treatment with 200 μM palmitic acid was found to increase the mRNA and protein expression of (TNF)-α and interleukin (IL)-6 in microglial cells, which suggests that palmitate could activate inflammatory signaling in microglial cells.

PSM03-04

INDUCIBLE EXPRESSION OF CXCL1 WITHIN THE CNS AMPLIFIES DEMYELINATION IN PRE-CLINICAL MODELS OF MULTIPLE SCLEROSIS

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The functional role of the ELR^+ chemokine CXCL1 in host defense and disease in two pre-clinical mouse models of the human demyelinating disease multiple sclerosis (MS) was assessed. Mice in which expression of CXCL1 is under the control of a tetracycline-inducible promoter active within GFAP-positive cells were generated and infected intracranially with the neurotropic JHM strain of mouse hepatitis virus (JHMV) or immunized with myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ to induce experimental autoimmune encephalomyelitis (EAE). In both JHMV-induced neurologic disease and EAE, overexpression of CXCL1 by astrocytes resulted in increased clinical disease severity. Immunophenotyping cells infiltrating into the central nervous system (CNS) revealed a selective increase in $Ly6G^+CD11b^+$ neutrophils (but not $Ly6G^-Ly6C^+CD11b^+$ monocytes) present within the CNS. In JHMV infected mice and MOG_{35-55}-immunized mice, the T cell response to antigen with regard to proliferation and cytokine production was not altered, nor was trafficking into the CNS affected. In both models, increased CXCL1 expression within the CNS resulted in increased morbidity that correlated with selectively elevated neutrophil infiltration, diminished numbers of mature oligodendrocytes, and an increase in the severity of demyelination. Neutrophil ablation in CXCL1-transgenic mice reduced the severity of demyelination in mice arguing a role for these cells in white matter damage. Collectively, these findings illustrate that sustained CXCL1 expression amplifies the severity of white matter damage and neutrophils can contribute to this process in two different mouse models of MS.

PSM03-05

DISSECTING THE ROLE OF THE HUMAN FRACTALKINE RECEPTOR DURING EAE: NEW APPROACH UTILIZING A HUMANIZED ANIMAL MODEL

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The transmembrane chemokine fractalkine expressed by neurons and peripheral endothelial cells acts as an adhesion molecule, as well as a soluble chemoattractant upon proteolytic cleavage. In the CNS, fractalkine functions by signaling through its unique receptor, CX3CR1 expressed by microglia. Fractalkine/CX3CR1 signaling regulates microglia neurotoxicity in selected models of neurodegeneration. During experimental autoimmune encephalomyelitis (EAE), CX3CR1 deficiency confers more severe clinical disease which correlates with exacerbated inflammation and neuropathology. Interestingly, among the CX3CR1 human polymorphisms, the $CX3CR1^{I249/M280}$ variant is present in about 20% of the population and exhibits reduced adhesion for fractalkine and thus confers defective signaling. However, the role of CX3CR1, microglia function and its effect on neuronal damage during multiple sclerosis remains unsolved. The aim of this study is to assess the effect of weaker signaling through the human $CX3CR1^{I249/M280}$ receptor on EAE disease, axonal damage and expression of ciliary neurotrophic factor (CNTF). We hypothesize that dysregulated microglial responses in absence of CX3CR1 signaling enhance neuronal/axonal damage via downregulation of CNTF, a key survival factor for neurons and oligodendrocytes. For this, an animal model was generated by insertion of the $CX3CR1^{I249/M280}$ human variant into the mouse CX3CR1 locus. Active EAE was induced in humanized mice via $MOG_{(35-55)}$ peptide immunization. Our results show an exacerbated EAE phenotype in mice expressing the human $CX3CR1^{I249/M280}$ receptor, characterized by accelerated disease onset and higher maximum EAE score in comparison to WT mice. These results correlated with severe CNS inflammation, a microglia activated morphology and increased demyelination in the cerebellum, a similar phenotype observed in mice lacking the mouse *Cx3cr1* gene. Interestingly, flow cytometry data showed slight downregulation of MHC-II and CD68 activation markers in $CX3CR1^{I249/M280}$ expressing mice, suggesting an alteration in microglia function induced by defective CX3CR1 signaling. Our results provide instrumental validation of defective function of the $CX3CR1^{I249/M280}$ human variant and the foundation to broaden the understanding of microglia dysfunction during neuroinflammation.

PSM03-06

A TRANSLATIONAL APPROACH TO TARGET INFLAMMATION FOLLOWING TBI: ROLE OF THE INTRACELLULAR P38 MAPK SIGNAL TRANSDUCTION PATHWAY

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Closed head traumatic brain injury (TBI) triggers a broad innate immune and acute inflammation response that involves resident glia and other immune cells. A failure in resolution of the injury-induced innate immune responses can cause worsen neurologic outcome to the TBI. Therefore, therapeutic interventions are needed in order to reduce the dysregulated inflammation that is causally linked to the neuropathologic sequelae. Previous work has generated a causal link between the p38α mitogen-activated protein kinase (MAPK) mediated intracellular signaling pathway and the injurious proinflammatory cytokine response in neurodegenerative animal models of disease. The recent availability of highly specific in vivo molecular probes for p38α MAPK inhibition allow a more refined in vivo analysis of this intracellular signaling pathway and its link to dysregulated glia function and neuroinflammation in TBI. We have recently explored these processes in TBI through the combined use of these in vivo p38α MAPK dynamic molecular probes

and genetics based in vivo tools, such as targeted knockdown of p38α MAPK in specific inflammatory cell types. We found that genetic suppression of p38α MAPK in myeloid cells resulted in less TBI induced deficits in a running wheel behavioral task and cognitive deficits as measured by the radial arm water maze. Suppression of p38α MAPK activity through selective pharmacological action or through reduction of p38α MAPK protein levels generated reduction of injury induced cytokine levels in the brain. The congruence of outcomes from genetic and pharmacological approaches provides a unique battery of outcomes consistent with p38α MAPK as a potential therapeutic target in TBI.

PSM03-07

OBESITY-INDUCED NEUROINFLAMMATION AFTER STROKE

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People who are obese are 64% more likely to have a stroke than those who are not. Obesity is also associated with chronic peripheral inflammation, and neuroinflammation following stroke can have profound effects on outcomes. However the influence of obesity-induced peripheral inflammation on stroke outcomes has not been well studied. We hypothesized increased peripheral inflammation in mice with diet-induced obesity would detrimentally affect post-stroke neuroinflammation by increasing activation of microglia and astrocytes, and causing larger strokes. To test this, we produced obese mice by administering a 60% kCal high fat diet for one or three months. We verified weight changes and measured the neuroinflammatory response and infarct size following stroke. Animals fed a high fat diet or control diet for either 1 or 3 months (N = 10/group) significantly ($p < 0.0001$) gain weight in a proportional manner. Animals were 36% heavier after 1 month, and 78% heavier after 3 months of high fat diet. We quantified astrogliosis and microgliosis by immunostaining for GFAP and CD68 3 days post-stroke. In the peri-infarct cortex there was a significantly ($p < 0.005$) increased (195%) area covered by GFAP+ immunostaining and an even higher increase in the % area covered by CD68 (290%; $p < 0.0001$), suggesting a stronger astrocytic response as well as more macrophages in the peri-infarct cortex, respectively. We also immunostained for IgG to assess blood brain barrier integrity and found increased staining not only adjacent to the stroke but also in the cortex contralateral to the infarct (184%; $p < 0.05$), suggesting that a high fat diet damages the blood brain barrier in the whole forebrain 3 days post-stroke. Importantly, animals either fed with a 1 or 3-month high fat diet had significantly ($p < 0.05$) increased (130%) infarcts compared to the control diet. Thus, consuming a high fat diet before stroke causes larger strokes and increases neuroinflammation. Future studies will investigate the mechanisms responsible so that we can develop treatments to help people with obesity who suffer from a stroke.

PSM03-08

INTRASPINAL TLR4 ACTIVATION PROMOTES IRON STORAGE BUT DOES NOT PROTECT NEURONS OR OLIGODENDROCYTES FROM IRON-MEDIATED DAMAGE

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CNS trauma induces hemorrhage-induced intraparenchymal iron accumulation that persists in the lesion, leading to oxidative damage and cell loss. A potential strategy to combat iron-mediated damage is stimulating iron sequestration by macrophages through toll-like receptor 4 (TLR4) activation. TLR4 stimulation promotes iron uptake and storage by macrophages, and work from our group showed intraspinal TLR4 activation also promotes oligodendrocyte (OL) progenitor (OPC) proliferation and new OL formation. Therefore, TLR4 is an attractive target for promoting tissue repair after CNS injury. To test the hypothesis that TLR4 activation protects against iron-mediated damage, we microinjected iron, a TLR4 agonist (lipopolysaccharide; LPS), or iron+LPS into the intact spinal cord and determined if iron sequestration was enhanced and tissue damage reduced by concomitant TLR4 activation. LPS treatment concomitant with iron did promote iron storage as detected by significantly increased ferritin expression and ferritin+ microglia in the injection site. However at 1d, OLs were reduced by 70% in all groups revealing that TLR4 activation did not protect OLs from iron-induced loss. Similarly, significant neuron loss occurred in iron and iron+LPS groups by 1d, and in all groups by 7d. Interestingly, OPCs were also lost acutely in LPS and iron+LPS groups, but not within the iron alone injection sites. By 7d post-injection, OPCs had completely replaced OLs lost in the LPS and iron injection sites; however, OLs in the iron+LPS injection sites only recovered to 75% of baseline levels. Since OPC proliferation was similar in all groups, it is likely that OPC differentiation was differentially affected by the iron+LPS injection. Predictably, a reduced number of OPCs in the iron+LPS injection sites had phosphorylated STAT3 signaling, a pathway implicated in OPC differentiation. Collectively, this work reveals that TLR4 activation does stimulate iron sequestration within the CNS; however, to be neuroprotective TLR4 activation may need to occur prior to iron exposure. Thus, the timing of treatments will be examined in future experiments.

PSM03-09

DELETION OF SCAVENGER RECEPTOR LRP1 IN OLIG1+ CELLS PROTECTS AGAINST EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) where the myelin sheath is destroyed. Myelin insulates axons and is necessary for proper saltatory conduction as well as promoting neuronal survival. No cure exists for MS and its etiology remains unknown. Currently, MS therapies prevent immune cell infiltration and inflammation in the CNS but with varying degrees of efficacy. It is imperative to find superior means by which we can dampen the deleterious effects of the

immune system.
Low density lipoprotein-related protein 1 (LRP1) is a ubiquitous scavenger receptor involved in phagocytosis and cell signaling. Using Cre-Lox recombination, we have deleted LRP1 in Olig1^{+} cells, including oligodendroglia and motor neurons. These mice do not present with any aberrant CNS developmental defects. However, mice lacking LRP1 in Olig1^{+} cells are protected from experimental autoimmune encephalomyelitis (EAE), a murine model of MS. Surprisingly, the autoimmune response in LRP1 deficient mice is diminished, resulting in minimal peripheral cell infiltration into the spinal cord and meninges. We propose here that studying LRP1 in Olig1^{+} cells might provide us with novel ways to modulate the immune system under pathological conditions.

PSM03-10

COMPLEMENT DEPENDENT CYTOTOXICITY AND INTERCELLULAR TOXICITY TO CENTRAL NERVOUS SYSTEM CELLS IN THE NEURONAL-GLIAL NETWORK

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Complement activation has been implicated in the pathogenesis of neurodegenerative and neuroinflammatory diseases. Studies in neuromyelitis optica (NMO), an autoimmune disease of the central nervous system (CNS), have demonstrated that autoantibodies against the water channel aquaporin-4 (AQP4) induce astrocyte damage through complement dependent cytotoxicity (CDC). However, the role of complement in neural and oligodendroglial injury is not clear. Here, we investigated the toxicity of complement on neurons, astrocytes, differentiated oligodendrocytes and oligodendrocyte progenitors in the context of purified primary monocultures, neuro-glial co-cultures and cerebellar slices. Cell death was assessed by IncuCyte live imaging in the cell cultures and by immunohistochemistry in cerebellar slices. We found that neurons, mature oligodendrocytes and oligodendrocyte precursors were sensitive to complement in monocultures. In neuro-glial co-cultures, neurons and differentiated oligodendrocytes showed reduced CDC; however, neuronal and oligodendroglial loss dramatically increased following targeted astrocyte depletion using NMO recombinant antibody and complement. Oligodendrocyte progenitors were resistant to complement toxicity in neuro-glial co-cultures. In organotypic cerebellar slices, damage to neurons and differentiated oligodendrocytes mediated by complement and astrocyte destruction were further reduced, but, in contrast to neuro-glial co-cultures, astrocyte damage sensitized oligodendrocyte progenitors to complement toxicity. Our results indicate that neurons and oligodendrocytes demonstrate variable sensitivity to CDC based on differentiation and culture conditions. In organotypic cultures, astrocyte injury increases the loss of neurons, oligodendrocytes and their progenitors. The mechanisms governing the progression of CNS injury following astrocyte-targeted CDC is currently being investigated.

PSM03-11

DYSREGULATION OF A PRO-INFLAMMATORY RESPONSE AND INFLAMMASOME ACTIVATION IN MICROGLIA, CONSEQUENCES ON PHAGOCYTIC ACTIVITY.

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Microglia initiate innate immune responses to injury or disease, inducing production of pro-inflammatory factors including: interleukin: (IL)-1 alpha (α), IL-1 beta (β), IL-6, and tumor necrosis factor (TNF)α and contribute to the clearance of aberrant material. Alterations in the ability of microglia to perform these normal functions may underlie neurodegenerative progression shifting the relative risk for adverse outcomes following brain injury. Using inorganic arsenic (iAs) as a chemical tool known to disrupt innate immune function of immune cells, we examined its potential immune-dysregulatory effect on microglia. Mice exposed to iAs [42.5 ppm] via drinking water for 6 weeks showed diminished staining for microglia in the hippocampus with no specific change in IL-1α, IL-1β, IL-6, TNFα, or iNOS mRNA levels. A blunting of the cytokine response to LPS [100 μg/kg/ 3 h] challenge was observed. Exposure to LPS [1mg/kg/day for 4 days] showed a similar elevation TNFα and IL-1α with a blunting of arginase 1(Arg-1) and YM-1 induction suggesting a dysregulation of both pro and anti-inflammatory responses. BV-2 cells exposed for 3 weeks to 1 mM iAs showed significant elevation in IL-1β and a blunting of LPS-induced IL-1α, IL-1β, IL-6, and iNOS mRNA elevations. Arg-1 was elevated with iAs and a diminished induction by IL-4/IL-13 was observed for IL-4 and Arg-1. BV-2 cell phagocytosis of 1 uM latex beads was significantly increased by iAs over 3 h. No differences were observed with LPS but activity was diminished with IL-4/IL-13 stimulation. Efferocytosis of apoptotic fragments was stimulated with iAs, LPS, and IL-4/IL-13. The differential effect of IL-4/IL-13 on the engulfment of latex beads versus apoptotic fragments in iAs exposed cells suggests a shift in phagocytic signaling. The blunting of induced pro- and anti-inflammatory cytokines following iAs exposure and the increased phagocytic activity of iAs exposed cells suggests a functional alteration in microglia that may have implications for brain development and repair following injury.
Supported by NIEHS Division of National Toxicology Program and Intramural Research: Z01 ES101623 & ES021164.

PSM03-12

NEUROPATHOLOGICAL CONSEQUENCES OF SUPERWARFARIN POISONING

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Warfarin is an anti-coagulant commonly used to reduce risk of clots in stroke. Warfarin inhibits Vitamin K Epoxide Reductase (VKORC1), which reduces Vitamin K levels, a cofactor for activation of clotting proteins. Warfarin was also used as a rodenticide by causing internal hemorrhage. However, emergence of warfarin-resistant rodents led to development of superwarfarins, which are modified forms of warfarin with greatly increased hydrophobicity, prolonged half-lives (>20 days) and 100-fold greater potency to inhibit VKORC1. Brodifacoum (BDF) was one of the first superwarfarins developed, and its increased use is associated with increased number of accidental poisonings which reach over 16,000 per year. BDF poisoning is treated by Vitamin K supplements, however while those compensate for reduced VKORC1 activity, they do not reduce BDF accumulation which can be maintained for months. Since Vitamin K is a cofactor for some CNS proteins we tested if BDF induced neuropathology. Adult male rats were administered varying doses of BDF (by gavage) and the 4-day LD50 value determined to be 0.225 mg/kg. BDF accumulated throughout the body with low but detectable levels in brain. Immunostaining showed that BDF increased astrocyte and microglial activation, and neuronal damage assessed by fluorojade staining. Analysis of brain lysates showed that BDF increased protein carbonylation, suggesting an increase in oxidative stress. In vitro, BDF induced metabolic stress and reduced viability in SK-N-MC neuronal cells, suggesting disruption of mitochondrial function. BDF had only minor effects in C6 glioma cells, however following cholesterol depletion C6 cells responded similarly to SK-N-MC cells. These findings demonstrate that in addition to anti-clotting actions, superwarfarins have toxic effects on neurons and glial cells involving metabolic dysregulation dependent upon lipid composition. Further knowledge of BDF actions should aid in reducing delayed neurological consequences of superwarfarin poisoning.

PSM03-13

THE ROLE OF TNF SIGNALING IN NEUROPATHIC PAIN AND HIPPOCAMPAL NEUROGENESIS

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Tumor Necrosis Factor (TNF) is a proinflammatory cytokine which is involved in physiological and pathological processes, systemically and within the central nervous system (CNS), and has been found to be crucial in pain development. There are two biologically active forms of TNF, soluble TNF (solTNF) and transmembrane TNF (tmTNF) that preferentially bind to TNFR1 and TNFR2 respectively. In the current study, we are interested in the effects of specifically blocking TNFR1 signaling in neuropathic pain following peripheral nerve injury in a mouse model with the use of both transgenic mice and drug therapy. After undergoing chronic constriction injury (CCI) in the sciatic nerve, knockout mice lacking TNFR1 (TNFR1 -/-) fail to develop a neuropathic pain response in the injured paw compared to wildtype mice. Furthermore, following CCI we observe a decrease in hippocampal neurogenesis following pain onset, but this decrease is not observed in TNFR1-/- mice. To investigate the therapeutic effects of inhibiting TNFR1 signaling after injury, we delivered XPro1595, a novel drug which creates a biologically inactive form of solTNF, to mice following CCI both systemically and within the CNS. Inhibition of solTNF signaling both via peripheral and central delivery of XPro1595 resulted in an accelerated recovery from neuropathic pain which began after 2 weeks of drug delivery. On the other hand, TNFR2 seems to play less of a role in the onset of pain and more so in the alleviation of pain since there is a decrease in TNFR2 levels following injury and an increase in tmTNF prior to pain dissipation. Our in vitro studies on neural stem progenitor cells also demonstrate TNFR1 and TNFR2 have distinct effects on NSPC survival and differentiation. Together, these results suggest that TNFR1 signaling in both the peripheral and central nervous system plays a role in pain induction following CCI, and that maintaining the balance of TNFR1 and TNFR2 signaling is key in regulating pain and secondary consequences in the hippocampus.

PSM03-14

FUNCTION OF PROTEIN KINASE CK2 IN CD4+ T CELLS AND AUTOIMMUNE DISEASE

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$CD4^+$ T cells are the major pathogenic cells in many autoimmune and inflammatory disorders, including Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE), an animal model of MS. Th17 cells are important in the pathogenesis of MS, whereas regulatory T (Treg) cells are crucial in disease resolution. Protein kinase CK2 (Casein Kinase II) is an ubiquitously expressed, constitutively active serine/threonine kinase involved in multiple signaling pathways essential to $CD4^+$ T cell proliferation and differentiation, including PI3K/AKT/mTOR, JAK/STAT and NF-κB. However, little is known about the specific function of CK2 in T cells or the consequences of CK2 inhibition during $CD4^+$ cell differentiation. Our current studies demonstrate that expression of the major catalytic subunit of CK2, CK2α, is elevated in activated $CD4^+$ T cells *in vitro* and *in vivo*. Utilizing the small molecule CK2 inhibitor CX-4945 (Silmitasertib), we find that CK2 is a novel regulator to enhance Th17 cell differentiation and inhibit Treg cell differentiation. Furthermore, CX-4945 treatment attenuates the severity of EAE disease through the inhibition of Th17 cell development at the peak of disease and enhancement of Treg cell development at the resolution stage. Consistent with these results, our data indicate that deletion of the CK2 catalytic subunit, CK2α, inhibits Th17 polarization and promotes Treg polarization *in vitro*. Collectively, our findings suggest that inhibition of CK2 expression and kinase activity in $CD4^+$ T cells suppresses inflammatory Th17 cell

responses and promotes development of anti-inflammatory Treg cells, which may lead to potential new therapies for MS in addition to current immunosuppressive therapies.

PSM03-15

CONTROL OF INFLAMMATION IN CNS REMYELINATION

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Chronic inflammation prevents myelin regeneration (remyelination) and CNS repair. In order for remyelination to occur, inflammation must be controlled, but exactly how this is achieved remains poorly understood. We have found that the immunomodulatory enzyme interleukin-4 induced 1 (IL4I1) is upregulated in remyelinating lesions following lysolecithin demyelination in mice. The IL4I1 gene has previously been mapped to a chromosomal locus of autoimmune disease susceptibility, including MS. However its role in CNS injury/repair has not previously been suggested. We found that IL4I1 is expressed by alternatively activated microglia/macrophages. IL4I1 deficient mice display enhanced inflammation, impaired remyelination and increased axonal injury. Moreover, IL4I1 gain-of-function significantly reduced inflammation in lesions resulting in increased remyelination. We suggest that IL4I1 modulates inflammation to enable CNS remyelination and that targeting IL4I1 has therapeutic potential in myelin repair.

PSM03-16

IS α-SYNUCLEIN A MOLECULAR SWITCH FOR REGULATING MICROGLIAL PHENOTYPE IN THE BRAIN?

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α-Synuclein (Snca) appears to have a significant role in the inflammatory response in the brain. Upon stimulation with lipopolysaccharide (LPS), microglia isolated from Snca gene ablated mice have significantly reduced phagocytic ability as well as increased release of proinflammatory cytokines and eicosanoids. In the brain, 2-arachidonylglycerol (2-AG), a ligand of CB_1 receptors, is the most abundant endogenous cannabinoid and has an important role in the phagocytic ability of microglia. However, it is unknown how Snca expression affects 2-AG biosynthesis within the brain. In this preliminary study, we determined that the level of 2-AG in the brains of Snca gene ablated mice was significantly increased during LPS-induced inflammatory response. However, this increase could result from multiple pathways and/or cell types and current work is assessing the role of primary microglia in this process. In cultured primary microglia, LPS-induced inflammation results in increased release of 2-AG into the medium but reduced Snca expression compared to unstimulated. Our preliminary data supports the hypothesis that Snca expression is linked to 2-AG release in primary microglia and may contribute to regulating the phagocytic phenotype *in vivo*. Experiments are currently underway in Snca gene-ablated mice to further elucidate the role of Snca in 2-AG release in microglia.

PSM04 Neuron-Glial Interactions 1: Metabolism, Signal Transduction and Axon Biology

PSM04-01

FUNCTIONAL IDENTIFICATION OF AN ACTIVITY-INDUCED, CA^{2+}-DEPENDENT GLUTAMINE TRANSPORTER IN HIPPOCAMPAL NEURONS

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Excessive presynaptic glutamatergic transmission is thought to be involved in various human disorders including epilepsy. Seizure activity or intense glutamatergic transmission requires import of glutamine into axon terminals from glia to maintain vesicular glutamate stores for continued release. This concept of glutamate-glutamine cycling for replenishment of the neurotransmitter glutamate pool originated from 'classic' biochemical experiments performed nearly 40 years ago; however, a critical barrier to progress in this field has been the lack of direct functional evidence for activity-induced, Ca^{2+}-dependent glutamine transport activity in hippocampal neurons. Here, I present the functional identification and characterization of a novel activity-induced, Ca^{2+}-dependent glutamine transporter in mature hippocampal neurons. Activity-induced transort is observed in hippocampal neurons and not astrocytes, requires exogenous Ca^{2+}, and is blocked by inhibition of P-type voltage-gated Ca^{2+} channels (verapamil; 20 micromolar). Preferred endogenous substrates likely include alanine, proline, histidine, and glutamine; although glutamine is the most physiologically relevant substrate as it is present at 10-fold higher concentration in extrasynaptic space, compared to all others. This activity-induced, Ca^{2+}-dependent glutamine transport system in neurons saturates at 200 micromolar and it displays a substrate affinity (Km) of 30 +/- 4 micromolar. These results support the concept that a relatively high affinity, activity-induced glutamine transporter operates maximally following Ca^{2+}-dependent exocytosis of this transporter to the plasma membrane. The kinetics of this neuronal glutamine transport system differentiate it from established neuronal glutamine transporters SNAT1 and SNAT2, which display much lower affinity (Km ~0.4 millimolar) under standard conditions. Sarcosine, an effective anti-seizure compound, abolished transport when present at 1 millimolar concentration. This novel activity-induced and Ca^{2+}-dependent glutamine transport system in hippocampal neurons represents a new potential target in disorders of excessive presynaptic glutamatergic transmission and glutamate excitotoxicity.

PSM04-02

ADRENERGIC ATTENUATION OF ASTROCYTE SWELLING: A NEW STRATEGY FOR RESCUING CELLS IN CENTRAL NERVOUS SYSTEM EDEMA

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Edema in the central nervous system develops in many pathologic conditions, rapidly resulting in life-threatening complications. Vasogenic edema is clinically manageable, but there is no established medical treatment for cytotoxic edema, which affects primarily astrocytes and may trigger acute post-traumatic neuronal death. To test the hypothesis that adrenergic receptor agonists, including the stress molecule epinephrine protects neural parenchyma from damage, we characterized its effects on hypotonicity-induced cellular edema in cortical astrocytes by *in vivo* and *in vitro* imaging. After epinephrine administration, hypotonicity-induced swelling of cortical astrocytes was markedly reduced and cytosolic cAMP (3'-5'-cyclic adenosine monophosphate) was increased, as shown by a fluorescence resonance energy transfer nanosensor. Interestingly, in swollen primary cortical astrocytes, the kinetics of cAMP signaling was slowed. To determine the effects on downstream cytoplasmic processes, we analyzed changes in Ca^{2+} concentration. We found that epinephrine, via modifying cAMP-signaling, has reduced hypotonicity-induced cytosolic Ca^{2+} excitability, which may be the key to prevent astrocyte swelling. Furthermore, in a rat model of spinal cord injury, epinephrine applied locally markedly reduced astrocyte swelling around the contusion epicenter. These findings reveal new targets for the treatment of cellular edema in the central nervous system.

PSM04-04

LITHIUM REVERSIBLY INHIBITS SCHWANN CELL PROLIFERATION AND DIFFERENTIATION WITHOUT INDUCING MYELIN LOSS OR DEDIFFERENTIATION

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Lithium has been widely used as in the treatment of bipolar and depressive disorders. Lithium exerts neuroprotective, anti-inflammatory and anti-apoptotic properties. It also controls lineage specification, proliferation and differentiation of varied cell types through the modulation of intracellular signaling systems including PI-3k/Akt and downstream targets such as β-catenin. Because those pathways are required for both Schwann cell (SC) proliferation and differentiation and the role of Lithium in clinical therapy, we performed a comprehensive study of the potential effects of lithium on the proliferation and differentiation of SCs using a variety of *in vitro* systems. We found that lithium specifically and reversibly prevented Schwann cell mitogenesis when administered in conjunction with growth factors such as neuregulin. Prolonged lithium treatment promoted cell enlargement and a growth arrested state without inducing the expression of myelin-related proteins and lipids. When lithium was administered in combination with cAMP, an instructive signal for SC differentiation, it inhibited the expression of Krox-20, a master regulator of the myelinating phenotype, and that of myelin-related markers in a dose dependent, specific and reversible manner. Likewise, lithium suppressed myelin sheath formation in co-cultures of SCs and dorsal root ganglion neurons without inducing myelin loss or dedifferentiation as evidence by levels of immature SC markers. SCs readily responded to lithium by phosphorylating GSK3β on Ser-9 and β-catenin accumulation, and pharmacological inhibition of GSK3β activity was sufficient to mimic lithium's effects on differentiation. In summary, lithium not only halts SC proliferation but also exerts a GSK3β-dependent antagonistic action on the early transcriptional control of differentiation leading to myelin formation.

PSM04-05

EXPRESSION AND FUNCTIONALITY OF THE CYSTINE-GLUTAMATE EXCHANGER (XC) IN BERGMANN GLIA CELLS

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The cysteine-glutamate exchanger, system X^-c, plays a mayor role in maintaining glutathione homeostasis. Recent studies suggested the expression of system X^-c in astrocytes, Müller and retinal pigment epithelial cell cultures. It has also been demonstrated that system X^-c mediates the exchange of cysteine into cells in a Na^+-independent exchange fashion. Nowadays there is not evidence that satisfactory show the expression of the system X^-c in Bergmann glia cells (BGC). Taking into consideration that BGC are a specific cell type of the cerebellar cortex; express proteins involved in metabolic coupling with neurons and actively participate in removing glutamate form the synapse cleft, the aim of the present study was demonstrating the expression and functionality of the X^-c system in BGC primary cultures from 14-day- old chick embryos. To achieve our aim, we employed immunofluorescence to show the expression of X^-c system and confirm our findings by Western blotting. Whereas, [^{3}H]L-Glutamate uptake assays were used to establish the functionality of the X^-c system. Our results demosntrate the expression of X^-c system in BGC. Whereas, glutamate uptake was significantly modified in absence of Na^+ and quisqualic acid. An increase into the X^-c system was appreciated in presence of an oxygen species donor. Same effects were appreciated at protein levels. These results strengthen the notion of the critical involvement of Bergmann glia in synaptic neurotransmission.

PSM04-06

ENDOCYTOSIS OF NEUROFILAMENT PEPTIDE NFL-TBS.40-63 BY OLIGODENDROCYTES PROMOTES THEIR SURVIVAL IN VITRO

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The expression of neurofilaments (NF) is decreased in demyelinated lesions during multiple sclerosis (MS). Moreover NF subunits are released in the cerebrospinal fluid of MS patients, and their concentration correlates with disease severity. However the role of NF in this extra-axonal location is unknown. In vitro we have demonstrated that purified NF fractions and tubulin increase the proliferation, differentiation and maturation of oligodendrocytes (OL). In addition, they protect OL from lysophosphatidyl choline (LPC) toxicity. Similarly, synthetic peptides displaying NFL-tubulin binding site (NFL-TBS.40-63), increase OL differentiation and maturation, and partly protect OL from demyelinating damage induced by LPC in vitro. Thus release of NF, or of NF derived peptides, during demyelination in vivo could regulate OL fate. The mechanism by which these proteins and peptides exert their effects and whether they penetrate into the cells had to be determined since these proteins do not display a sequence signal corresponding to cell penetrating proteins. Using rat OL secondary cultures we localized biotinylated NFL-TBS.40-63 by double immunocytochemistry and confocal microscopy in cytoplasmic processes of cells at different differentiation stages of the OL lineage: A2B5+ progenitors, CNP+ OL, and myelin basic protein (MBP+) expressing OL. Uptake was concentration dependent. A scrambled peptide, with the same amino-acids as NFL-TBS.40-63 but in a random sequence (NFL-SCR), was also used as a control. NFL-SCR has no biological effect on OL, and was not significantly uptaken by OL. This uptake process was further confirmed and characterized, using cholera toxin B and dynasore, an inhibitor of dynamin (involved in clathrin-dependent endocytosis) which inhibited the incorporation of NFL-TBS.40-63 into OL. Thus, the uptake of this peptide occurs mainly through clathrin-dependent endocytosis. This study confirms that

peptide sequences from axon cytoskeleton proteins can be internalized by OL through several mechanisms. This process coud be involved during demyelination, and release of axon proteins might favourize remyelination.

PSM06 Neurodegeneration 1: AD, PD, HD, ALS

PSM06-01

BIOPHYSICAL CHARACTERIZATION OF PATHOLOGICAL TAU OLIGOMERS

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Alzheimer's disease (AD) is the most common form of dementia, and the sixth leading cause of death in the United States. Last year AD cost the US economy 450 billion dollars. By 2050 the inflation-unadjusted Medicare costs *alone* will explode to an estimated 1.2 trillion dollars as the American demographic ages. AD is a neurodegenerative disorder. Symptoms begin with mild memory impairment, progressing to mood and behavior changes, confusion, serious memory loss, and difficulty with motor control and language. At the biomolecular level, AD pathology arises from misfolding and aggregation of two proteins: amyloid-β (Aβ), and tau. The biochemistry of AD is *exceedingly* complex; different aggregation states induce different cellular responses, and therefore influence different aspects of pathology, including direct neuron death, changes in neuron signaling/metabolism, and activation of the neuroinflammatory response. The Aβ aggregation pathway is better understood than tau, yet tau is more closely tied to the cognitive decline in AD.

We have initiated studies to better characterize the tau aggregation process with an emphasis on small soluble oligomers rather than insoluble filaments. Oligomerization of recombinant 2N3R and 2N4R was induced by either a seeded oligomerization method, with pre-formed Aβ oligomers as the seed, or via addition of arachidonic acid. To generate monomeric preparations, recombinant tau pellets were solubilized in 8M urea and dialyzed overnight against PBS buffer. Seeded and unseeded solutions were purified via size exclusion chromatography (SEC) in-line with multi-angle light scattering (MALS). Typically, one SEC-MALS peak was observed with multiple oligomeric species, at 135 and 160 kD, and larger, with little monomeric tau. The trimers and tetramers were also evident in tau immunoblots. Circular dichroism analysis did not show any remarkable structural features for the oligomers nor did conformation-specific dye binding assays. The trimer/tetramer oligomers were also observed in unseeded preparations suggesting that tau may be able to spontaneously oligomerize without seeding. Our work confirms the presence of small tau oligomers but also demonstrates the critical need for obtaining pure monomer solutions when examining the biophysics of tau aggregation.

PSM06-02

MAMMALIAN TARGET OF RAPAMYCIN (MTOR) - MEDIATED MECHANISMS UNDERLYING OLIGODENDROCYTE PROCESS EXTENSION

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Mammalian Target of Rapamycin (mTOR) - Mediated Mechanisms underlying Oligodendrocyte Process Extension

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Oligodendrocytes are macroglial cells of the central nervous system (CNS) whose plasma membrane extends processes to form myelin sheaths that surround axons enabling rapid nerve signal transmission and providing metabolic and trophic support for the axons. Oligodendrocyte development occurs in multiple stages from precursor cells (OPC) to mature myelinating cells. During development, OPCs morphologically transition during differentiation to extend a network of processes through a sequence of events involving cytoskeletal organization. Our prior data demonstrated that oligodendrocyte differentiation is regulated by the mammalian target of rapamycin (mTOR), a downstream target of the of PI3K/Akt pathway (Tyler et al, 2009; Wahl et al, 2014). Pharmacological inhibition of mTOR by rapamycin resulted in a significant reduction in process extension. This reduction in process extension occurred after two days of differentiation during the late progenitor and immature stages of OL development. Moreover, our in vivo studies demonstrated that conditional knockout of mTOR in oligodendrocytes (mTOR cKO) results in deficits in initiation of myelination in the spinal cord. Thus, both in vitro and in vivo studies support the hypothesis that normal mTOR signaling is crucial for proper morphological development of oligodendrocytes.

Morphological differentiation of oligodendrocytes involves protrusion of the plasma membrane initiated by actin filaments positioned immediately beneath the cell membrane. Recently published studies revealed that the activity of actin depolymerizing factors such as cofilin, maintains F-actin turnover which aids in the progression of myelin sheath formation at the onset of myelination (Barres et al, 2015; Simons et al, 2015). Our preliminary data, using immunofluorescent staining of phalloidin indicate a reduction in actin filament polymerization following pharmacological inhibition of mTOR during differentiation in vitro. In addition, western immunoblot analysis revealed that inhibiting mTOR resulted in a reduction in phospho-cofilin and profilin-2 in OPCs during the first two days of differentiation. In vivo, we observe a reduction in phospho-cofilin-positive and total-profilin2-positive oligodendroctyes in mTOR cKO spinal cords, at the peak of differentiation. In contrast, we observed an increase in phospho-cofilin-positive oligodendrocytes in the mTOR cKO during active myelination.

These preliminary data demonstrate a role for mTOR in cytoskeleton reorganization and modulation of cytoskeleton associated protein expression in oligodendrocytes, early during differentiation and later during initiation of myelination. Results from these studies have the potential to contribute to the development of therapeutic strategies to promote remyelination in Multiple Sclerosis and other demyelinating diseases.

PSM06-03

GENETIC DELETION OF JNK3 AMELIORATES HUNTINGTON'S DISEASE PATHOLOGY IN R6/2 MICE

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Huntington's disease (HD) is a fatal adult-onset neurodegenerative disease that results from the expansion of a polyglutamine (polyQ) tract located at the amino terminus of the Huntingtin (Htt) protein. Despite this knowledge, underlying mechanisms of HD pathogenesis remain elusive. Several independent studies have reported the inhibition of fast axonal transport (FAT) in cellular and animal models of HD. Previous work from our lab demonstrated that mutant Htt (mHtt) inhibits FAT through the activation of mixed-lineage kinases (MLKs) which downstream activates c-Jun N-terminal kinase 3 (JNK3). Activated JNK3 directly phosphorylates conventional kinesin, a major motor protein, and inhibits its microtubule-binding activity, resulting in the inhibition of FAT. Furthermore, pharmacological inhibition of JNK rescues mHtt-mediated FAT deficits in isolated squid axoplasm.
In order to address the potential role of JNK3 in HD pathology in a mouse model of HD, we generated R6/2-JNK3$^{-/-}$ mice, combining a widely used mouse model of HD with mice in which JNK3 is genetically deleted. Here, we report that R6/2-JNK3$^{-/-}$ mice show significant improvement in several behavioral, biochemical, and immunohistological HD pathologies seen in R6/2 mice. Importantly, deletion of JNK3 significantly improves survival in R6/2 mice. Collectively, our data suggest that inhibition of JNK3 may represent an effective therapeutic strategy for HD and support the idea that activation of axonal JNK3 by mHtt plays a critical role in HD pathology.

PSM06-04

MIR-124-3P INHIBIT HYPERPHOSPHORYLATION OF TAU VIA REGULATING CAVEOLIN-1- PI3K/AKT/GSK3β PATHWAY IN ALZHEIMER'S DISEASE

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MiR-124-3p belongs to the microRNA (miRNA) which has been increasing concerned and even becomes a hot spot in Alzheimer's disease（AD）. Caveolin-1 is a marker for caveolin protein of cell membrane, and closely related to the AD. However, the relationship between MiR-124-3p and Caveolin-1 has never been reported. Also the mechanisms underlying the involvement of them in AD pathogenes is remain unclear.
Our results showed that the expression of miR-124-3p was significantly decreased in N2a/APPswe group in comparison to wild type N2a (N2a/WT) group, while the mRNA and protein of APP and Caveolin-1 were increased. In addition, the dual luciferasereport experiment showed that the relative luciferase activity in co-transfection with the wild type vector (pGL3-Caveolin-1 3'UTR WT) group were significantly decreased, compared with co-transfection with the mutant vector (pGL3- Caveolin-1 3' UTR MUT) group. When the N2a/APPswe cells were transfected with MiR-124-3p, the expression of Caveolin-1were decreased both at mRNA and protein level. Moreover, electronic microscope showed the number of caveolae on cell membrane was decreased. Caveolin-1 was targeted for negativeregulation by miR-124-3p in AD cell model. The expression of Tau-Ser404/Tau decreased after transfection of miR-124-3p mimics and Caveolin-1-siRNA in N2a/APPswe cells. And the expression levels of PI3K, Akt-Ser473/Akt, GSK-3β-Ser9/GSK-3β increased. On the contrary, the expression of Tau-Ser404/Tau, PI3K, Akt-Ser473/Akt, GSK-3β-Ser9/GSK-3β were decreased in N2a/APPswe cells after pcDNA-Caveolin-1 transfection. Our findings demonstrated that Caveolin-1 could regulate the phosphorylation level of Tau protein via PI3K/AKT/GSK3β signaling pathway, and miR-124-3p can inhibit abnormal hyperphosphorylation of Tau protein by regulating Caveolin-1-PI3K/Akt/GSK3β in AD.

PSM06-05

NEUTRAL SPHINGOMYELINASE-2 DEFICIENCY AMELIORATES ALZHEIMER'S DISEASE PATHOLOGY IN THE 5XFAD MOUSE

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The two major pathological hallmarks of Alzheimer's disease are accumulation of extracellular amyloid-beta (Aβ) and intracellular aggregation of phospho-tau. Recent evidence implicates extracellular vesicles such as exosomes in the aggregation of Aβ and spreading of tau. We previously reported that Aβ-challenged astrocytes release exosomes with apoptotic potential. In this current study, we hypothesized 1) that astrocyte exosomes aggregate Aβ and induce neuronal cell death and 2) that reducing exosomes in the brain of the 5XFAD mouse would ameliorate Alzheimer's pathology and improve cognitive performance. We used differential ultracentrifugation to isolate exosomes from primary glial cultures (~95% astrocytes). Astrocytes treated with $A\beta_{42}$ doubled their ceramide levels and secreted exosomes enriched with ceramide and GM1. These exosomes were able to aggregate Aβ *in vitro* and when preincubated with Aβ prior to addition to glial cultures, decreased Aβ clearance from the medium. Addition of exosomes preincubated with $A\beta_{42}$ to primary cortical neurons increased apoptosis compared to exosomes or $A\beta_{42}$ alone. To prevent exosome-mediated $A\beta_{42}$ aggregation and neuronal apoptosis *in vivo,* we crossed 5XFAD mice into *fro/fro* mice, which lack neutral sphingomyelinase 2 (nSMase2), a key enzyme in formation of ceramide-enriched exosomes. Mice homozygous for the *fro* allele (froFAD) were deficient in total brain ceramide. Compared to 5XFAD mice, male and female froFAD mice showed marked increases in soluble $A\beta_{42}$ levels (e.g. oligomers) while total $A\beta_{42}$ levels were only significantly reduced in males. Likewise, male froFAD mice showed reduced cortical and hippocampal plaque burden as well as reduced

phospho-tau levels (PHF-1 and p262-tau) compared to 5XFAD controls. Additionally, froFAD mice showed reduced GFAP expression in fixed tissue and fewer Fluorojade-B-positive plaques. Moreover, in both contextual and cued fear conditioning tests, froFAD mice performed similarly to wildtype controls while 5XFAD mice showed significant memory deficits. We conclude that nSMase2 deficiency improves Alzheimer's pathology and behavior in the 5XFAD mouse model, likely by reducing exosome-induced $A\beta_{42}$ aggregation.

PSM06-06

A NOVEL ROLE OF TDP-43 IN DNA DOUBLE-STRAND BREAK REPAIR: IMPLICATIONS TO MOTOR NEURON DISEASE, AMYOTROPHIC LATERAL SCLEROSIS

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Tar DNA binding protein, 43KDa (TDP-43) is a multi-functional RNA/DNA binding protein, essential for cell survival. TDP-43 proteinopathy mediated by its nuclear clearance is a hallmark of progressive motor neurodegeneration disorders including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Mutations in TDP-43 has been accounted for 5 – 10% of the familial patients as well as 90-95% in sporadic ALS in the United States. Studies have revealed TDP-43's role in multiple cellular processing for neural functioning mostly related to RNA metabolism, but its DNA binding role has not been investigated till date. Here, we show for the first time that TDP-43's nuclear depletion in motor neurons lead to accumulation of DNA double-strand breaks (DSBs) as analyzed by a dramatic increase in DSB-specific marker proteins' (γH2AX, 53BP1, p-ATM) foci formation. Furthermore, TDP-43 co-IP from neuronal cells contain DNA-PKcs, Ku70, XRCC4/LigaseIV complex and polymerase λ, key proteins in DSB – repair machinery via non-homologous end joining (NHEJ) pathway. These interactions were increased significantly upon DSB induction as confirmed by proximity ligation assay (PLA) and co-IP. These results show that TDP-43 could play role as a scaffold protein for efficient recruitment of the DSB repair machinery at break site. On the other hand, CRISPR mediated conditional TDP-43 knock-out neuronal cell model exhibited prominent increase in endogenous DNA damage along with p-ATM and NF-kB activation indicating TDP-43's probable role in activation of pro-inflammatory pathway following excessive DSB accumulation. In summary, exploration of novel and crucial events of motor neuronal cell death through DNA damage and neuroinflammation could be useful therapeutic interventions in TDP-43-linked neurodegenerative diseases. (*Supported by NIH/NINDS and MDA grants*).

PSM06-08

IN VITRO AND IN CELL PHOSPHORYLATION PROFILE OF TAU AT CDK5-PHOSPHORYLATION SITES ANALYZED BY PHOS-TAG SDS-PAGE

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Tau, a microtubule-associated protein, is a major component of aggregates seen in brains of tauopathies such as Alzheimer's disease (AD) and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Tau is hyperphosphorylated in the aggregates and therefore the mechanism of hyperphosphorylation has been an intense research subject. Here, we applied the Phos-tag SDS-PAGE technique to characterize tau phosphorylation by Cyclin-dependent kinase 5 (Cdk5) in vitro and in cultured cells. Cdk5-p25 phosphorylated tau at Ser404, Ser235, Thr205 and probably Se202 in that order. Among those four major *in vitro* Cdk5 phosphorylation sites, Ser404 was preferentially phosphorylated by Cdk5 in cultured cells, whereas Thr205 was not. Ser202 and Ser235 were phosphorylated by endogenous kinases. Tau existed in 12 different phosphorylation states in COS-7 cells with Ser202, Thr231 and Ser235 as major phosphorylation sites. p35-Cdk5 and p25-Cdk5 phosphorylated tau to a similar extent, when their expression levels were adjusted. Phosphorylation of FTDP-17 tau mutants was also analyzed by this method. While the mutants in the N-terminal or microtubule-binding region were phosphorylated identically as was wild type tau, the C-terminal mutants showed slightly different banding patterns from wild type tau. In particular, it was clear that R406W mutation resulted in loss of Ser404 phosphorylation. These results not only include novel and interesting informations on tau phosphorylation but also indicate usefulness of Phos-tag SDS-PAGE in the analysis of site-specific and cellular phosphorylation pattern of tau.

PSM06-09

CURCUMIN SUBSTRACT THE AGGREGATION OF Aβ40 BY ENHANGCE THE AXONAL TRANSPORTION OF AUTOPHAGOSOME IN AD

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Alzheimer's disease(AD) is a neurodegerative disease characterized with progressive deterioration of memory and cognition. Curcumin is a plant extraction from the herb curcuma rhizome, which have many functions, including anti-inflammatory,

anti-oxidation, anti-tumor, and the neuroprotection in AD. The pathogenesis of AD are very complicated, but only the Tau pathology and Abeta theory are generally accepted by most of the scholars. Glycogen synthase kinase 3beta (glycogen synthase kinase, GSK-3beta) is a multifunctional serine/threonine protein kinase, playing important roles, such as, the protein synthesis, cell proliferation and differentiation, microtubule dynamics change, axonal transport and synaptic plasticity. And its abnormal expression or changes of activity are closely related with the genesis and development of AD. The researches about the effects of curcumin on AD were mostly focused on how to reduce the generation of Abeta and prevent the tau protein phosphorylation, but few studies were about the degradation of Abeta.Our studies in vivo and in vitro have showed that curcumin can enhance autophagy activity, and boost the dynein intermediate chain (DIC) expression; FRAP experiments have showed that autophagosome's axonal transport speed was significantly sped up in the alive cells, these evidences all showed that curcumin could directly enhance the axon retrograde transportion of autophagosome and at last the Abeta were hydrolyzed by lysosomal degradation system. After administration the GSK-3beta agonist wortmannin, the expression level of DIC was restrained, which suggested that the effects of curcumin on axon transportation was dependent on the GSK-3beta. In brief, our experiments confirmed that curcumin could enhance autophagy activity, and accelerate the transshipment of autophagy body resulting into reducing the Abeta aggregation. The potential mechanism may be that curcumin could reduce the expression of DIC and enhance its function by inhibiting GSK-3beta and degradation of Abeta, so as to reduce the AD pathological changes.

PSM06-10

AN IN VIVO MODEL OF BMAA-INDUCED PROTEIN INCLUSIONS OF GUAM ALS/PDC

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Amyotrophic lateral sclerosis/ Parkinsonism Dementia Complex (ALS/PDC) is a neurodegenerative disease suffered by the Chamorro people of Guam. BMAA (β-N-methylamino-l-alanine), a non-protein amino acid produced by cyanobacteria, occurs in traditional dietary items consumed by the Chamorros. BMAA can be misincorporated in place of L-serine in proteins, causing protein misfolding and aggregation. BMAA administered intravenously in mice rapidly crosses the blood brain barrier. In rats, prolonged (30 day) intrathecal infusion of BMAA produces neurodegeneration. Although acute neurotoxicity was previously observed in macaques fed BMAA, the consequences of chronic exposures are unknown. Vervets *(Chlorocebus sabaeus*) in St. Kitts were given fruit dosed with BMAA for 140 days. One cohort of four vervets received BMAA, a second cohort received L-serine, a third cohort received L-serine plus BMAA, and a control cohort received rice flour. This experiment was subsequently replicated with three cohorts of eight adult vervets: one cohort was fed fruit dosed with BMAA, a second cohort received BMAA plus L-serine, and a third cohort received rice flour. All vervets fed BMAA developed neuronal protein inclusions similar to ALS/PDC. Free and protein-bound BMAA was found in brain tissues similar to ALS/PDC. Vervets fed BMAA plus L-serine had few protein inclusions. This replicated experiment suggests that ALS/PDC can be triggered by chronic exposures to BMAA, and may shed light on suggested linkages between cyanobacterial exposures and ALS elsewhere. L-serine is now being evaluated as a possible therapy for ALS in FDA-approved human clinical trials.

PSM06-11

NEUROPROTECTIN D1 ENHANCES THE ACTIVITY OF AUTOPHAGY VIA INHIBITION OF GSK-3β IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is most common neurodegenerative disease characterized by cognitive impairment,formation of amyloid-β (Aβ)-containing plaques and neurofibrillary tangles.Defective autophagy is involved in the pathogenesis of AD.Neuroprotectin D1 (NPD1) the DHA-derived mediator promoted neural survival in AD.However, the effct of NPD1 on autophagy in AD remains unknown.

We observed that NPD1 increased autophagosomes in N2a APP695sw cells detected by transmission on electron microscope.The expression of LC3II/LC3I,Beclin1 in N2a APPsw695 cells after treatment of NPD1 were also upregulated by using western blot, but the ratio of phospho GSK-3β/total GSK-3β was significantly decreased. Realtime PCR also showed increased mRNA of LC3b, Beclin1. NPD1-induced autophagy was compromised by treatment of wortmannin (an activator of GSK-3β). The study indicates that increased activity of autophagy by NPD1 in N2a APP695sw cells may contributes to the neuroprotection of NPD1 via inhibition of GSK-3β in AD which might be a potential therapeutic target for AD.

PSM06-12

SUBSTANTIA NIGRA LIPID COMPOSITION IN PATIENTS WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative movement disorder that involves a selective loss of dopamine-producing neurons in the substantia nigra (SN). No prior studies have evaluated the content and composition of glycolipids, phospholipids, and

cholesterol in SN from PD patients. The content and composition of the following lipid classes were evaluated in 9 PD patients (7M, 2F; 67-95 yrs) and in 15 age-matched, neurologically normal controls (10M, 5F; 57-84 yrs): Total gangliosides, cholesterol, ceramide, cerebrosides, phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SM), cardiolipin (CL), phosphatidic acid, bis-mono(acylglycero)phosphate (BMP), sulfatides, phosphatidylserine (PS), and phosphatidylinositol (PI). Male PD patients were analyzed separately from female PD patients for gangliosides, as no major abnormalities in ganglioside composition were seen in female PD patients. The data from male and female PD patients were combined for analysis of neutral and acidic lipids. Total gangliosides (ug sialic acid/100 mg dry wt) were significantly lower in the male PD patients (195 $\pm$ 9 ug) than in the normal controls (255 $\pm$ 13 ug) ($p < 0.01$). No significant difference was found for GM1 content or distribution, but the content of the neuronal-enriched GD1a and GT1b gangliosides was significantly lower ($p< 0.01$) in the PD patients than in the controls. GD3 distribution was elevated, perhaps reflecting a mild gliosis in PD. The distribution of the myelin-enriched cerebrosides and sulfatides was higher in the PD patients than in the controls, suggesting myelin sparing in the PD patients. The enrichment of GM1 in myelin could mask a possible reduction in neuronal GM1. No abnormalities were found in the PD patients for the distribution of cholesterol, ceramide, SM, PS, CL or BMP, but the distribution of PE, PC, and PI was significantly lower ($p< 0.01$) in the PD patients than in the controls. The lipid results are consistent with selective neuronal loss in SN of PD.

PSM06-13

CASPASE-3 DOWN-REGULATES AMYLOID-DEGRADING ENZYME NEPRILYSIN UNDER HYPOXIA

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The amyloid-degrading neuropeptidase neprilysin (NEP) is one of the major enzymes participating in amyloid β peptide clearance from the brain and an important therapeutic target in Alzheimer's disease. Previously we demonstrated that prenatal hypoxia (E14, 7% O_2, 3 h) resulted in reduced NEP expression and activity in the cortex and hippocampus of rats during postnatal life. Hypoxia (1% O_2, 24 h) also resulted in reduced NEP levels and AICD binding to the NEP gene promoter in human NB7 neuroblastoma cells due to an increased expression of caspases capable to degrade AICD. Incubation of cells under hypoxia in the presence of a caspase-3 inhibitor partially prevented the decrease of AICD and NEP levels and activity. We now report that prenatal hypoxia resulted in significantly increased caspase-3 protein levels and activity in rat cortex, which correlated with reduced AICD and NEP protein levels in rat cortex on P20-P30. Hypoxic animals also demonstrated altered morphology in the cortex and hippocampus and reduced cognitive functions tested in the 8-arm maze and novel object recognition test. Intraventricular injections of the caspase-3 inhibitor Ac-DEVD-CHO on P20 reduced caspase activity 3 days after the injection down to the age matched control values while AICD and NEP levels were significantly increased. Injections of the inhibitor also had beneficial effects on rat performance in cognitive tests. The data obtained suggest that the increase of caspase-3 activity could affect NEP expression via proteolytic degradation of its transcription factor AICD. These data for the first time demonstrate the role of caspases in AICD-dependent regulation of NEP production in the brain of mammals under hypoxic conditions. Supported by RFBR (13-04-00388), ARUK and MRC (UK).

PSM07 Neuron-Glial Interactions 2: Development and Disease

PSM07-01

REGULATION OF HUMAN GLIOMA STEM CELL MIGRATION THROUGH A LOCALLY TRANSLATED LCK PATHWAY

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Migration of tumor propagating glioma stem cells (GSCs) within the brain parenchyma makes glioblastoma one of the most aggressive and lethal human tumors. Studies of the cellular and molecular mechanisms underlying human GSC migration are hindered by the lack of efficient migration models. Here we developed a DRG axon-oligodendrocyte co-culture method to study in real time the migration and interaction of GSCs with axons, which occurs through the extensive formation of pseudopodia. Isolation of pseudopodia-localized RNA followed by eIF4 RNA-IP reveals local transcripts of Lck, Paxillin, Crk-II and Rac1 that undergo eIF4-dependent translation. Inhibition of Lck blocks the activation of this pathway, the formation of pseudopodia and the migration of GSCs. *In vivo* administration of a highly specific Lck inhibitor using an orthotopic xenograft mouse model results in significant inhibition of tumor formation and GSC migration. Targeting this locally translated Lck-dependent pathway constitutes a novel treatment paradigm for human glioblastomas.

PSM07-02

ELUSIVE PERINEURONAL OLIGODENDROCYTE: FROM CAJAL TO PRESENT

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In 1896, Ramón y Cajal described in rabbit cerebellum a wealth of small nuclei apposed to the soma and initial segment of large neurons. He called them "*núcleos satélites perineuronales*". He was struck by such a peculiar arrangement which could not be by chance. He established this satellitosis was perfectly normal, despite former investigators (Golgi, Nissl, Andriezen) had noticed a proliferation in diseases. He hypothesized in 1913, after an ultimate staining did not reveal the cytoplasm, that these small "*third element*" cells must have a symbiotic relationship with neurons. Later in 1921, Río-Hortega successfully stained them and revealed they were small branched oligodendrocytes in perineuronal location, next to astrocytes and occasional microglia. Wilder Penfield made them known in a luminous 1924 Brain article with a beautiful drawing. Penfield marveled that "*oligodendroglia satellites*" have roughly the same morphology than white matter cells, in contrast to astrocyte diversity. Nothing happened until the 1960's when electron microscopy (EM) showed these satellites had various cytoplasmic and nuclear densities (dark to light cells), but were essentially equipped with the same organelles as interfascicular oligodendrocytes. Samuel Ludwin brought the first breakthrough in 1979 showing by EM that perineuronal oligodendrocytes have myelination potential. Spurred by the pioneering studies of Nancy Sternberger using antisera against myelin proteins, Ludwin and Sternberger independently confirmed with MBP and MAG that these cells can make myelin during development and remyelination. The immunohistochemistry era brought confirmation of similar markers for perineuronal and interfascicular oligodendrocytes, both expressing glycerol-3-phosphate dehydrogenase, cyclic nucleotide phosphodiesterase (CNP), transferrin and HNK-1, but also revealed differences, i.e. for glutamine synthetase. CNP still present in cytoplasm of perineuronal cells casted doubt on their myelination, while glutamine synthetase expressed mostly in perineuronal cells (but not in white matter oligodendrocytes) suggested instead involvement with glutamate and GABA neurotransmitters. The 21th century brought the rediscovery they are involved in diseases, but also molecular mechanisms and lineage. Some 150 years after their initial observation, we are beginning to decipher the subpopulations and function of perineuronal oligodendrocytes. This work was supported by NIH/NLM grant G13LM011465.

PSM07-03

NOVEL NEUROFASCIN ISOFORM: POTENTIAL MEDIATOR OF MICROGLIA-AIS INTERACTION

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Multiple Sclerosis (MS) is a debilitating disease of the central nervous system characterized by profound demyelination and inflammation. Axonal injury, another prominent feature of MS, is considered a major contributor of chronic disability in patients. While most axonal injuries associated with MS are thought to be a secondary consequence of demyelination, findings from our lab strongly suggest that disruption of the axon initial segment (AIS), the region of the axon responsible for action potential initiation, is targeted for disruption in an inflammatory mouse model of MS known as Experimental Autoimmune Encephalomyelitis (EAE) through a mechanism independent of myelin loss. This breakdown was, instead, shown to correlate with increased microglial activation associated with microglial-AIS contact. Understanding this disease-associated contact could provide insight into the inflammation-induced AIS disruption. We hypothesize neurofascin, a member of the L1 subgroup of the immunoglobulin superfamily and known mediator of extracellular axolemmal adhesion, to be a potential mediator of this contact. Neurofascin is enriched at the AIS and plays an important role in maintaining stability of the domain via homophilic and heterophilic interactions. Neurofascin has multiple isoforms, and the expression of these alternatively spliced isoforms is cell and tissue specific. To begin to test our hypothesis, we have isolated microglia from the neocortex, the brain region that

exhibited AIS loss, microglial activation, and microglial-AIS interaction. Western blot analysis revealed a neurofascin band of a unique molecular weight indicating the isolation of a novel isoform of the neurofascin gene. Expression of this isoform was increased in microglia isolated from EAE animals. Furthermore, IHC analyses demonstrate neurofascin antibody reactivity in microglia, and this reactivity is enhanced with disease progression. PCR confirmed the presence of neurofascin mRNA transcript in isolated microglia, suggesting this protein is not present as a result of phagocytosis. We, therefore, propose that a novel isoform of neurofascin is present specifically in microglia and mediates contact with the AIS; potentially resulting in disruption of the domain.

PSM07-04

DELETION OF INTEGRIN LINKED KINASE (ILK) IN NEURAL PROGENITOR CELLS DISTURBS NEURON GLIA BALANCE DURING EARLY DEVELOPMENT

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Integrin linked kinase (ILK) is a serine/threonine protein kinase, localized at the inner plasma membrane, where it serves as a scaffolding protein/focal adhesion mediator. It is expressed in neurons, astrocytes and oligodendrocytes in the central nervous system (CNS). However, its role in the commitment to glial or neuronal fates during CNS embryonic development has not been investigated. In studies to investigate its role in oligodendrocyte function, we selectively deleted ILK expression in cells expressing either the transcription factor Olig1 or 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP), typically considered to be expressed primarily during oligodendrocyte development. No viable pups were obtained that were CNP-Cre; ILK fl/fl, and only a few were obtained as Olig1-Cre X ILK fl/fl pups. We therefore investigated whether early deletion of ILK impacted embryonic viability, and established a high death rate of Olig1Cre-cILK fl/fl embryos, with a lower loss of CNPCre-cILK fl/fl embryos. The number of Sox2-positive neural progenitor cells was normal in E12.5 spinal cords, but ILK deletion in either Olig1-Cre or CNP-Cre embryos severely reduced the number of proliferating ($Ki67^{+}$) oligodendrocyte precursor cells (OPCs) in E14.5 embryos. This ultimately resulted in a lower number of OPCs throughout the embryonic spinal cord. Deletion of ILK not only reduced the proliferation potential of OPCs, but also increased the number of apoptotic OPCs, identified by TUNEL staining. At the same time, the number of neuronal progenitor cells in the E14.5 spinal cord was elevated. The number of NeuN, Pax6, GABA, somatostatin, Mash1, ISLET1 or HuCD-expressing cells was increased, indicating that the normal neuron-glia balance shifted towards greater production of neuronal progenitor cells in the absence of ILK. In conclusion, it should be noted that Olig1 and CNP are expressed in early embryonic cells, and that ILK expression in that population of progenitor cells is essential for the normal neuron-glia profile development of the developing spinal cord.

PSM07-05

REGULATION OF ASTROCYTE GLUTAMATE TRANSPORTER-1 (GLT1) AND AQUAPORIN-4 (AQP4) EXPRESSION IN A MODEL OF EPILEPSY

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Astrocytes regulate extracellular glutamate and water homeostasis through the astrocyte-specific membrane proteins glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4), respectively. The role of astrocytes and the regulation of GLT1 and AQP4 in epilepsy are not fully understood. In this study, we investigated the expression of GLT1 and AQP4 in the intrahippocampal kainic acid (IHKA) model of temporal lobe epilepsy (TLE). We used real-time polymerase chain reaction (RT-PCR), Western blot, and immunohistochemical analysis at 1, 4, 7, and 30 days after kainic acid-induced status epilepticus (SE) to determine hippocampal glial fibrillary acidic protein (GFAP, a marker for reactive astrocytes), GLT1, and AQP4 expression changes during the development of epilepsy (epileptogenesis). Following IHKA, all mice had SE and progressive increases in GFAP immunoreactivity and GFAP protein expression out to 30 days post-SE. A significant initial increase in dorsal hippocampal GLT1 immunoreactivity and protein levels were observed 1 day post SE and followed by a marked downregulation at 4 and 7 days post SE. AQP4 dorsal hippocampal protein expression was significantly downregulated at 1 day post SE and was followed by a gradual return to baseline levels with a significant increase in ipsilateral protein levels by 30 days post SE. Our findings suggest that specific molecular changes in astrocyte glutamate transporters and water channels occur during epileptogenesis in this model, and suggest the novel therapeutic strategy of restoring glutamate and water homeostasis.

PSM07-06

EFFECTS OF COMBINATION THERAPY ON BEHAVIORAL RECOVERY AND SUPRASPINAL PLASTICITY FOLLOWING CHRONIC THORACIC SPINAL CORD INJURY

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Severe spinal cord injury (SCI) causes a loss of behavioral function below the level of the injury and can alter the somatotopic organization of supraspinal structures. Rehabilitative therapy, including 5-HT replacement therapy and exercise, has been shown to induce further changes in somatotopic organization associated with behavioral recovery but the mechanisms of these changes are not well understood. Since astrocytes are normally associated with neuronal plasticity, we investigated the effects of rehabilitative therapy on supraspinal neural and glial plasticity in a rat model of

complete mid-thoracic (T8/9) spinal transection. Therapy included bike training and 5-HT replacement therapy paired with trunk supported treadmill training (combination therapy). Results show that this combination therapy improved behavioral recovery as measured by weight supported step cycles on a treadmill and open field testing (BBB) compared to sham therapy. Electrophysiological assessment of cortex sensory and motor organization demonstrated that combination therapy induced greater cortical reorganization than sham therapy. Specifically, trunk motor cortex and forepaw sensory cortex expanded into the deafferented hindlimb sensorimotor cortex of spinalized rats that received therapy compared to spinalized rats that received sham therapy. Western blot analysis suggests this reorganization induced by combination therapy is partly due to synaptic remodeling as demonstrated by increases in protein associated with pre (synaptophysin) and post (PSD95, EphA4) synaptic formation in the cortex. Importantly, increased reorganization was also associated with an increase in the astrocyte related protein GFAP that was confirmed by the astrocyte specific protein GLAST. Finally, increased reorganization was associated with an increase in ephrinA3, a ligand of EphA4, found on astrocytes. The ephrin A3/EphA4 ligand/receptor complex has been suggested to regulate synaptic function and plasticity through modulation of dendritic spines and/or glial glutamate transporters. These data suggest that cortical plasticity associated with functional recovery after SCI is supported by neuron-astrocyte interactions that enhance synaptic plasticity.

PSM07-07

SIGNALING EVENTS ON OLIGODENDROCYTES VIA MYELIN-ASSOCIATED GLYCOPROTEIN: ROLE AGAINST GLUTAMATE-MEDIATED OXIDATIVE STRESS

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Glial cells are engaged in a fluent bidirectional communication with neurons. One molecule that mediates oligodendrocytes (OLs)-neuron interaction is Myelin-associated glycoprotein (MAG), a protein selectively expressed at the periaxonal layer of myelin where an intense neuron-glía communication takes place. A recent body of evidence proposes MAG as a functional receptor in oligodendrocytes (OL), acting as a docking platform for different signal transduction pathways. Previous findings suggest that one of the signaling events triggered by MAG is related to an increased resistance to oxidative stress caused by glutamate overload; nevertheless the molecular mechanisms underlying this effect remain elusive. We used an anti-MAG mAb that allows MAG crosslinking on OL cytoplasmic membrane and triggers a downstream signalization. We assessed the effect of Ab-mediated activation of MAG in OL on two murine models implying glutamate overload: stroke by intrastriatal glutamate administration and Experimental Acute Encephalomyelitis (EAE). We found that MAG activation delayed disease onset, amielorated clinical symptoms and hampered myelinated axon loss in the EAE model while reducing lesion volumes in the stroke model. Further studies in primary OL and organotypic cerebellar cultures showed that MAG crosslinking can increase glutamate uptake on OLs and trigger antioxidant defenses associated with a PKC-dependent erythroid-related factor 2 (Nrf2) activation, resulting in a net increase of glutathione (GSH). GSH increase relies on Xc^- cysteine/glutamate antiporter activity. These results allow us to propose OLs as critical modulators of high extracellular glutamate in white matter. This event results critical toward understanding demyelination-associated glutamate toxicity, opening a new opportunity for therapeutic intervention.

PSM07-08

ASTROCYTE CONNEXIN43 CONTRIBUTES TO MOTOR NEURON TOXICITY IN ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting in progressive degeneration of motor neurons (MN) in the brain and spinal cord leading to weakness and death. Astrocytes play a critical role in ALS influencing disease progression and contributing to MN death. Here we investigate a potential mechanism through which astrocytes lead to MN toxicity. Astrocytes are interconnected through connexins (Cx) and Cx43 is a major astrocyte connexin conducting crucial homeostatic functions in the CNS. Under pathological conditions, connexins are altered and their functions are compromised as observed in spinal cord injury, Alzheimer's disease, ischemia, and others. We hypothesized that abnormal Cx expression serves as a potential mechanism for astrocyte-mediated toxicity in ALS. We first examined Cx43 in the $SOD1^{G93A}$ mouse model of ALS and observed Cx43 protein increases during the disease course and remains significantly elevated in the spinal cord of endstage mice. Notably, this increase in Cx43 was also in motor cortex and spinal cord of post-mortem ALS patients compared to controls patients. We then studied Cx43 expression and function specifically in astrocytes isolated from $SOD1^{G93A}$ and compared them to astrocytes from $SOD1^{WT}$ mice. We observed Cx43 expression was elevated in $SOD1^{G93A}$ astrocytes and further, this increase in Cx43 protein expression resulted in enhanced dye spreading, hemichannel mediated uptake and intracellular calcium levels in $SOD1^{G93A}$ astrocytes compared to $SOD1^{WT}$ astrocytes. Finally, we conducted co-culture experiments between astrocytes and MNs to understand the impact of increased expression of Cx43 on survival of MNs. We tested if blocking Cx43 protects MNs from the toxic effects of $SOD1^{G93A}$ astrocytes and noted significantly better survival of MNs with Cx43 peptide blocker. Current studies are focused on understanding the *in vivo* contribution of Cx43 in the $SOD1^{G93A}$ mouse. These findings are novel and have widespread implications for glial cells as a therapeutic strategy.

PSM07-09

NOVEL ROLE OF NOCICEPTIN AS A REGULATOR OF GLUTAMATE TRANSPORTER EXPRESSION IN DEVELOPING ASTROCYTES

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Previous results from our laboratory showed that the timing of oligodendrocyte development and brain myelination are highly regulated by signaling through the Nociceptin/Orphanin FQ receptor (NOPR). NOPR, a G-protein coupled receptor and the most recently discovered member of the opioid receptor family, is specifically activated by nociceptin, an endogenous heptadecapeptide produced by astrocytes. Interestingly, astrocytes also express NOPR, raising the possibility of an autocrine effect on these cells mediated by nociceptin. In support of this hypothesis, we now found that nociceptin plays a crucial role in regulating the expression of GLAST (EAAT1 in humans), a glutamate/aspartate transporter highly expressed in developing astrocytes and radial glia. Misregulation of GLAST during development is associated with a variety of problems including increased seizure duration and severity, episodic ataxia, and altered gait and motor coordination. Our studies showed that treatment of cultured rat brain astrocytes with nociceptin results in a dramatic increase in the expression levels of GLAST, an effect abrogated by co-incubation of the cells with either BAN-ORL24 or J-113397, two highly specific inhibitors of NOPR. Furthermore, the regulation of GLAST expression by the nociceptin system is also observed *in vivo*. Levels of this transporter were found to be significantly decreased in the brain of rat pups subjected to postnatal administration of a blood brain barrier permeable NOPR inhibitor, as well as in the brain of NOPR knockout mice pups. Importantly, nociceptin effects on GLAST expression are also observed in developing human astrocytes. These findings indicate that, in addition to a role in controlling oligodendrocyte development and myelination, nociceptin also plays a crucial function in maturing astrocytes and their capacity to support glutamate homeostasis in the developing brain. (Supported by VCU CCTR UL1TR000058 and NMSS RG-1501-02891)

PSM08 Neurodegeneration 2: Ischemia, Trauma and Other

PSM08-01 - WITHDRAWN

PSM08-02

CHRONIC TRAUMATIC ENCEPHALOPATHY: ROLE OF ASTROCYTIC THROMBOSPONDIN-1

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Chronic traumatic encephalopathy (CTE) is a neurodegenerative disorder that develops after repetitive episodes of head trauma. It is associated with neuronal injury, leading to neurobehavioral and cognitive impairments. The mechanisms responsible for the neurodegenerative changes in CTE, however, remain largely unknown. Hyperphosphorylation, ubiquitination and aggregation of the transactivating DNA-binding protein (p-TDP-43 proteinopathy), which is known to cause neuronal injury in other neurological conditions, has been identified in CTE. However, it is not known whether the p-TDP-43 proteinopathy contributes to the development of CTE. Since a reduction in thrombospondin-1 (TSP-1) secretion by astrocytes has been implicated in the neuronal dysfunction associated with other neurological conditions, we examined whether astrocytic TSP-1 synthesis/release is affected in CTE, and whether this event contributes to the p-TDP-43 proteinopathy in CTE. An increase in intra− and extracellular levels of TSP−1 were identified in traumatized cultured astrocytes at early stages (24-72 h) post-trauma (3 atms). However, at 15d post−trauma (late stage), intra− and extracellular levels of TSP−1 in astrocytes declined to below normal levels. Trauma to cultured neurons resulted in a time−dependent increase (120−400% over 1−7 d) in cytosolic p-TDP-43 levels, and such increase was exacerbated following multiple traumatic impacts. Further, conditioned media (CM) from traumatized astrocytes at early stage, when added to traumatized cultured neurons, reduced levels of the trauma-induced increase in p−TDP−43. However, the addition of CM from traumatized astrocytes at later stages to traumatized neurons, failed to reverse p−TDP−43 levels. Increases in neuronal TDP-43, as well as a decline in TSP-1 levels in cortical astrocytes were observed following *in vivo* trauma to rats. Additionally, an increase in p-TDP-43 was identified in non-traumatized TSP-1 knock-out mice, indicating that the synthesis/release of astrocytic TSP-1 is required for the regulation of the TDP-43 proteinopathy. These findings suggest that decreased astrocytic thrombospondin-1 contributes to the neuronal TDP-43 proteinopathy associated with chronic traumatic encephalopathy.

PSM08-03

THE GAP JUNCTION NEXUS SIGNALING COMPLEX IN ASTROCYTES

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Gap junctions (GJs) are clusters of channels that bind a variety of membrane components and cytoplasmic proteins to form a specialized cellular signaling complex known as the Nexus. Connexin (Cx) 43 and Cx30 are the main components of GJs in astrocytes. The two connexin isoforms form GJ plaques (orthogonal channel clusters) with very different structural properties and bind to very different sets of cellular proteins. In astrocytes, connexins are specifically localized to subcellular domains including prominent localization to endfeet that enwrap brain vasculature. Kir4.1 and Aquaporin4 are also localized to endfeet. We aimed here to identify the mechanism by which Cx43 GJs are stabilized and how modification of GJ structure alters intercellular communication in astrocytes. We used immortalized Cx43-knockout astrocytes transfected with fluorescent protein tagged connexins and performed high resolution 3D time lapse microscopy (Fluorescence Recovery After Photobleach, FRAP) to show that stability of GJs is controlled

by cysteine residues within the carboxy-terminus of Cx43 and by the cellular redox state. Using 2-color FRAP, we show that GJ stability regulates the localization and mobility of other astrocyte endfoot components including Cx30. To evaluate how the Nexus stability and/or mobility of GJs in astrocytes affects intercellular communication, we used electrophysiological recordings which indicated that fluidly arranged Cx43 GJs transmit current between cells, with ongoing electrophysiology experimentation to test for altered channel activity and/or GJ channel regulation. Computational modeling together with super-resolution imaging are expected to provide further support for the role of Nexus stability in GJ signaling between astrocytes. We conclude that macromolecular structure of the GJ Nexus is regulated by posttranslational modifications that help to determine the mobility and localization of other proteins found in astrocyte endfeet. Supported by: NIH-NINDS grant R01 NS092466.

PSM08-05

BTBD9, A RESTLESS LEG SYNDROME ASSOCIATED PROTEIN, REGULATES MANGANESE-INDUCED TOXICITY IN C. ELEGANS

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Restless Legs Syndrome (RLS) is a common neurological disorders seen in ~10% of the US population. BTBD9 is one of the genetic risk factor for RLS, which is associated with decreased serum iron (Fe) level. Here we present novel data that BTBD9 functions to regulate Mn homeostasis in *Caenorhabditis elegans* (*C. elegans*). A blast search with BTBD9 amino acid sequence identified *hpo-9* as the BTBD9 homolog in *C. elegans*, with ~75% sequence similarity. A mutant strain (tm3719) carrying 761 bp deletion of *hpo-9* was obtained from the National BioResource Project, Tokyo, Japan. We found that tm3719 (*hpo-9*$^{-/-}$) worms were more sensitive to Mn exposure. In the absence of Mn, *hpo-9*$^{-/-}$ animals grow normally. At 10 and 25 mM of Mn treatment, the survival rates of *hpo-9*$^{-/-}$ worms were significantly lower than wild type (WT) N2 control worms; the LD_{50} of *hpo-9*$^{-/-}$ worms is 6.5 mM, compared with 8.4 mM for WT animals. Using ICP-MS, we found that Mn concentrations in *hpo-9*$^{-/-}$ worms were higher than WT worms. Quantitative real time PCR indicated that *hpo-9* expression level was increased at low concentration (0.1 mM) of Mn exposure, but decreased at high concentration (2.5 mM). To better characterize HPO-9 protein, a transcriptional fusion construct was created with green fluorescent protein (GFP) driven under *hpo-9* promoter (~1.5 kb upstream of *hpo-9* start codon), and injected into worms to generate $P_{hpo\text{-}9}$::GFP worms. We found that GFP was present high in the head and pharynx, and low in the intestine and seam cells. Using a confocal microscopy and co-localization study, we found that *hpo-9* was expressed in dopaminergic neurons, indicating that HPO-9 might play a role in dopamine signaling. Our results suggest a novel role for *hpo-9*/BTBD9 in regulating Mn homeostasis and possibly dopamine signaling in *C. elegans.*

PSM08-06

ACIDOTOXICITY VIA ASIC1A MEDIATES CELL DEATH DURING OXYGEN GLUCOSE DEPRIVATION AND ABOLISHES EXCITOTOXICITY

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Ischemia and reperfusion (I/R) injury is associated with a complex and multifactorial cascade of events that includes excitotoxicity, acidotoxicity and ionic imbalance. Acidosis associated with brain ischemia occurs concomitantly with glutamate-mediated excitotoxicity. However, it is still elusive, how extracellular acidosis mediated acidotoxicity interacts with glutamate-mediated excitotoxicity. Here we investigated the effect of acidosis on glutamate-mediated excitotoxicity in adult acute hippocampal slices. We tested the hypothesis that mild acidosis protects against I/R injury via modulation of NMDAR, but produces injury via activation of acid sensing ion channels (ASIC1a). Using a microperfusion approach, we monitored the time course of injury and varied the duration of insult to determine if injury was caused by the primary insult or reperfusion phase. We also manipulated pH in the presence and absence of oxygen glucose deprivation. The role of ASIC1a and NMDAR was deciphered by treating the slices with and without an ASIC or NMDAR antagonist. Our results show that injury due to OGD or low pH occurs during the insult rather than the reperfusion phase. Injury mediated by low pH or low pH OGD requires ASIC1a and is independent of NMDAR activation. These findings point to ASIC1a as a mediator of ischemic cell death caused by stroke and cardiac arrest.

PSM08-08

HIV-1 TAT CAUSES STRUCTURAL DEFICITS IN A CA1 INTERNEURON MICROCIRCUIT, AND DISTURBANCES IN CA1 OUTPUT AND MEMORY FORMATION

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The hippocampus, which contains several subregions each comprised of multiple neuron subtypes that form complex processing networks, is disrupted in neuro-acquired immunodeficiency syndrome (neuroAIDS). To determine whether a subpopulation(s) of hippocampal neurons might be selectively vulnerable to HIV-1, we used inducible transgenic Tat-expressing mice to examine structural and functional changes in the CA1 subfield. We found several subsets of interneurons across CA1 that were selectively vulnerable to HIV-1 Tat. Specifically nNOS+/NPY− interneurons of the stratum pyramidale and stratum radiatum (interneuron specific interneuron type 3 and neurogliaform cells respectively) were found

to be vulnerable. Additionally, parvalbumin+ cells of the stratum pyramidale (putatively bistratified cells), and somatostatin+ cells of the stratum oriens (putatively oriens-lacunosum moleculare projection cells), were preferentially susceptible to Tat. These interneurons form a local feedback and gating circuit known to affect memory. Tat induction resulted in deficits in spatial memory as assessed using the Barnes maze task, and deficits in novel object recognition. An initial electrophysiological analysis of the pyramidal cells in CA1 showed a significant decrease in the firing threshold, and increase in amplitude of action potentials following Tat induction. These morphological and functional deficits provide new insight into the origins of the cellular and molecular pathogenesis underlying neuroAIDS, and may identify new targets for pharmacological intervention. Supported by R01 DA018633 (KH), R01 DA033200 (KH), K02 DA027374 (KH), and T32 DA007027.

PSM09 Neuroprotection and Repair

PSM09-01

INTRANASAL LEUKEMIA INHIBITORY FACTOR REDUCES INJURY AND IMPROVES FUNCTIONAL RECOVERY FROM A CLOSED HEAD INJURY

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Traumatic brain injuries affect over 600,000 children under the age of 14 each year with an estimated financial burden of ~$1 billion/year in hospital costs. Progress is being made in developing neuroprotective strategies; however, there is an unmet need for therapeutics that can both prevent secondary injury and restore neurological function by enhancing neural regeneration. We are testing the hypothesis that Leukemia Inhibitory Factor (LIF) is an essential neuroprotective and regenerative cytokine that is produced in response to brain injuries. Previously, we found that LIF haplo-deficient adolescent mice sustain significantly more gray and white matter damage resulting in worse sensorimotor deficits after a closed head injury. Furthermore, we have established that LIF enhances the growth of neural precursors of the Subventricular Zone. Here we performed experiments to establish whether administering LIF intranasally can reduce the extent of cell death and improve neurological function after a closed head injury. We have established that administering 20 µL of LIF at 2 µg/mL, dissolved in water, applied as 2 µl drops alternately to each nostril, every 2 minutes over 10 minutes, resulted in a 40-80 fold increase in the number of phospho-STAT3+ cells in the olfactory bulbs, neocortex and SVZ 30 minutes later compared to vehicle. The increase in STAT-3 phosphorylation was confirmed by Western blot. Building upon these data we used the same dosing strategy, but initiated LIF delivery 3 days after a closed head injury. Mice that received intranasal LIF sustained less cell death and made fewer foot slips on a beam-walking task at day 5 compared to vehicle treated control mice. Altogether these data support the conclusion that delayed intranasal delivery of LIF is a candidate therapeutic for adolescents who have sustained a traumatic brain injury. Supported by grant # CBIR13IRG017 from the NJ Commission on Brain Injury Research awarded to SWL.

PSM09-02

TARGETING THE JAK/STAT PATHWAY FOR DISEASE MODIFICATION IN EPILEPSY

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Many brain insults including traumatic brain injury, stroke and status epilepticus (SE) cause the later development of epilepsy (epileptogenesis). However, there are currently no treatments that reduce epileptogenesis following brain injuries. Recent data shows that inhibiting the JAK/STAT pathway with the compound WP1066 at the time of brain insult attenuates ensuing epilepsy. Specifically, rats given WP1066 at the time of pilocarpine-induced SE show a significant reduction in the frequency of spontaneous seizures compared to vehicle-injected animals up to 4 weeks following SE. However, unfavorable pharmacokinetic (PK) properties of WP1066 limit its potential for preclinical development.

Methods: We have performed PK studies on an additional JAK/STAT inhibitor, Ruxolitinib, aimed at increasing the efficacy of JAK/STAT inhibition in brain following peripheral administration. Ruxolitinib was also tested for its ability to inhibit STAT3 phosphorylation (pSTAT3) via administration 30 min after onset of SE and again 2.5 hours later. Levels of pSTAT3 were measured via Western blot and video-EEG monitoring was performed to assess the chronic effects of drug injections on the frequency, duration, and severity of spontaneous seizures.

Results: Ruxolitinib improved PK and greater inhibition of pSTAT3 in brain following pilocarpine-induced SE. In our preliminary studies, Ruxolitinib showed an ~30-fold increase in cortex concentrations compared to WP1066 1 hour after SE. In addition, Western blot analysis on rat hippocampi 1 hour after SE showed almost complete inhibition and ~45% reduction of pSTAT3 levels at 3 hours compared to vehicle-injected controls. Ruxolitinib also appears to reduce the frequency and severity of chronic spontaneous seizures compared to vehicle-injected animals.

Conclusions: Due to increased PK properties and pSTAT3 inhibition by Ruxolitinib compared to previously investigated JAK/STAT inhibitors, these findings suggest that Ruxolitinib is a promising candidate for further pre-clinical development as a disease-modifying agent with the potential to reduce development and/or severity of epilepsy following brain insults.

PSM09-03

EVALUATION OF THE NEUROPROTECTIVE EFFECTS OF TNFR2 SPECIFIC STIMULATION IN A SPINAL CORD INJURY MODEL

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Tumor necrosis factor (TNF) has often been thought of as the prototypic pro-inflammatory cytokine due to its association with various inflammatory diseases such as rheumatoid arthritis and multiple sclerosis (Probert et al., 2015, Patejdl, et al 2015). There are two biologically active forms of TNF, soluble (sol) TNF and transmembrane (tm) TNF which preferentially bind to TNFR1, and TNFR2, respectively (Aggarwal et al., 2014). TNFR1 is ubiquitously where as TNFR2 is inducible and expressed primarily on immune cells and cells within the CNS (Probert et al., 2015). Previous work in our lab has shown that blocking soluble TNF using XPro1595, a selective inhibitor of soluble TNF, in spinal cord injury (SCI) and experimental autoimmune encephalomyelitis (EAE) models leads to improved functional recovery, reduction in lesion area, remylination and upregulation of TNFR2 (Novrup et al 2014, Brambilla et al 2011). Based on these data we asked if select activation of TNFR2, through the use of a specific agonist, EHD2-sc-mTNFr2, would improve functional recovery following SCI (Fischer et al., 2011). Immediately after the injury mice received the TNFR2 agonist to the site of the injury through use of an osmotic

pump. We found that animals receiving the drug recovered much quicker than control mice receiving PBS. In an effort to study the mechanism behind this observed neuroprotection we used an *in-vitro* model to evaluate the effect of the TNFR2 agonist on cell death, and neurite extension. We observed that pre incubation with EHD2-sc-mTNFr2 for 12, 18, 24, and 48 hours and found that TNFR2 stimulation was neuroprotective against glutamate-induced excitotoxicity for all time points. We also observed that incubation with TNFR2 after one day in-vitro caused a significant increase in neurite outgrowth within two days. These observations taken together lead us to several paths in which we can explore the neuroprotective mechanisms of TNFR2.

PSM09-04

OLIGODENDROGENESIS VIA A SMALL MOLECULE THERAPY FOR TREATING MULTIPLE SCLEROSIS PATIENTS

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There is an unmet need for remyelinating therapies for diseases like multiple sclerosis (MS). Remyelination depends upon the ability of endogenous oligodendrocyte progenitor cells (OPCs) to differentiate into mature oligodendrocytes that can repair the damaged myelin sheath. NDC-1308 is an analog of estradiol (E2) that was previously shown in culture to cause 3-fold more mouse OPCs to differentiate into mature myelinating oligodendrocytes compared to vehicle. E2 and estriol do not possess this myelinating activity. In addition, side-by-side comparison of NDC-1308 and E2 in the cuprizone mouse model of demyelination showed that only NDC-1308 dramatically increases the level of remyelination (up to 44% in the hippocampus).

In these studies, we investigated how NDC-1308 has gained the function to remyelinate axons, but lost the deleterious side-effects commonly associated with estrogens. *In silico* modeling studies suggest that NDC-1308 interacts with different moieties in the estrogen receptor (ER) ligand binding domain compared to E2, thereby eliciting a distinct pattern of gene expression that is beneficial for myelin repair. Indeed, while NDC-1308 and E2 are both ER agonists, the unique remyelinating activity of NDC-1308 can be traced back to its ability to significantly up-regulate key genes (OLIG2, DNER, MOG and MBP) for oligodendrogenesis and remyelination in several human cell lines. Real-time qPCR analysis showed that these same genes are up-regulated in human PBMCs treated with NDC-1308, demonstrating that they could serve as potential therapeutic biomarkers. Importantly, NDC-1308 was evaluated for estrogenicity, a potential safety concern for treating MS patients. Estrogenicity was directly measured in a mouse uterotrophic assay since E2 treatment is known to cause a rapid and dramatic increase in uterine weight in this assay. Unlike E2, NDC-1308 was not found to be estrogenic in the uterotrophic assay. Further testing revealed that NDC-1308 is not mutagenic and not genotoxic.

Because of its unique mechanism of action, its potent remyelinating activity and its demonstrated lack of harmful side-effects, NDC-1308 possesses many desirable attributes of an effective MS therapy.

PSM09-05

ANTI-GLYCAN ANTIBODIES HALT AXON REGENERATION IN A MODEL OF GUILLAIN BARRè BY INDUCING MICROTUBULE DISORGANIZATION VIA RHOA/ROCK

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Several reports have linked the presence of high titers of anti-Gg Abs with delayed recovery/poor prognosis in GBS. In most cases, failure to recover is associated with halted/deficient axon regeneration. Previous work identified that monoclonal and patient-derived anti-Gg Abs can act as inhibitory factors in an animal model of axon regeneration. Further studies using primary dorsal root ganglion neuron (DRGn) cultures demonstrated that anti-Gg Abs can inhibit neurite outgrowth by targeting gangliosides via activation of the small GTPase RhoA and its associated kinase (ROCK), a signaling pathway common to other established inhibitors of axon regeneration. We aimed to study the molecular basis of the inhibitory effect of anti-Gg abs on neurite outgrowth by dissecting the molecular dynamics of growth cones (GC) cytoskeleton in relation to the spatial-temporal analysis of RhoA activity. We now report that axon growth inhibition in DRGn induced by a well characterized mAb targeting gangliosides GD1a/GT1b involves: i) an early RhoA/ROCK-independent collapse of lamellipodia; ii) a RhoA/ROCK-dependent shrinking of filopodia; and iii) alteration of GCs microtubules organization/ and presumably dynamics via RhoA/ROCK-dependent phosphorylation of CRMP-2 at threonine 555. Also, our results also show that mAb 1B7 inhibits peripheral axon regeneration in an animal model via phosphorylation/inactivation of CRMP-2 at threonine 555. Overall, our data may help to explain the molecular mechanisms underlying impaired nerve repair in GBS. Future work should define RhoA-independent pathway/s and effectors regulating actin cytoskeleton, thus providing an opportunity for the design of a successful therapy to guarantee an efficient target reinnervation.

PSM09-06

TPA PROMOTES AXONAL REGENERATION INTO AND THROUGH A DORSAL COLUMN SPINAL CORD INJURY

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Plasminogen activators tPA and uPA have been shown in our prior studies to promote cerebellar neuronal cell migration, PNS axonal growth in vitro, motor neuron synaptic remodeling following spinal cord injury, and PNS axonal regeneration across prohibitive myelin substrates. Studies on spinal cord axonal regeneration have been initiated by characterizing dorsal column injuries at C4. Longitudinal sections spanning the spinal cord injury have been observed by immunohistochemistry for axonal regeneration following cholera toxin B injections into the sciatic nerve. We compared mice receiving a sciatic nerve injury (“priming or conditioning” event) with uninjured “non-primed” wildtype mice as well as “primed”

tPA-/- mice, and "non-primed" mice receiving exogenous tPA via saturated gelfoam placed over the lesion site. Mice were examined after 75d and extensive axonal re-growth is seen into the filled-in lesion site in "primed" mice, as compared to bulbus terminals with no axonal regrowth in "non-primed" mice or the "primed" tPA-/- mice, which fail to regenerate. Excitingly, "non-primed" mice receiving exogenous tPA soaked gelfoam, overlying the lesion site showed in axonal regeneration into the lesion site and for several hundred micrometers beyond the lesion site, suggesting that tPA can promote axonal regeneration in the SC dorsal column. Further support that tPA induction may be an important part of the PNS "priming" event is our IH observation that tPA levels are dramatically increased in the ipsilateral C4 dorsal column 7d post-sciatic nerve lesion, the time when the "priming" effect is at its peak.

PSM09-07

ESTRIOL TREATMENT PREVENTS AXONAL INJURY, AXONAL LOSS, AND MOTOR NEURON LOSS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Axonal injury, axonal loss, and neuronal loss are all commonly observed pathologies in both multiple sclerosis (MS) and its most commonly used animal model, experimental autoimmune encephalomyelitis (EAE). Estriol treatment has been shown to reduce the clinical severity in mice with EAE, however estriol's effect on axonal injury, axonal transection, and neuronal survival has not been investigated. In this study, female THY1-YFP^{+} mice received subcutaneous pellets containing either estriol or placebo and then EAE was induced. We observed a reduction in clinical disease in estriol-treated compared to placebo-treated EAE mice. 28 days after disease induction, estriol-treated and placebo-treated EAE mice and age-matched healthy controls were sacrificed and Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel (CLARITY) was performed on the tissues. CLARITY is a novel optical clearing technology for the visualization of intact anatomies, such as whole brain and spinal cord, that allows us to image axons along their entire length and count all the neurons in the cerebral cortex without sampling. Using this approach, we observed a reduction in axonal ovoids and end bulbs in estriol-treated compared to placebo-treated EAE mice spinal cords. Furthermore, cortical layer V motor neurons were preserved in estriol-treated, but not in placebo-treated, EAE mice. These results indicate that estriol treatment reduces axonal transection and neuronal loss in experimental autoimmune encephalomyelitis.

PSM09-08

ANTIEPILEPTOGENIC EFFECTS OF 1400W, A SELECTIVE INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR IN A RAT KAINATE MODEL OF EPILEPSY

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First seizure insult initiates the process of epileptogenesis resulting in transformation of normal brain into epileptic brain characterized by spontaneous recurrent seizures. Progressive changes that occur after initial seizure insult include, blood brain barrier (BBB) damage, reactive gliosis, neuronal hyperexcitability, oxidative stress and neurodegeneration. Slowing or halting neurobiological changes during the epileptogenesis is critical to prevent the development of recurrent seizures. In the current study, we tested the effects of 1400W, a highly selective inducible nitric oxide inhibitor in kainate rat (Sprague Dawley) model of epilepsy. We used 6 months continuous video-EEG acquired from durally implanted electrodes along with immunohistochemistry and Western blot at 7d and 6m time points. The 1400W was administered intraperitoneally at 20mg/kg, starting at 2h post-kainate induced status epilepticus (SE), and repeated at 12h intervals for 3 days. Immunohistochemistry revealed a significant reduction in astrogliosis, microgliosis, extracellular serum albumin (a marker for BBB leakage) and neurodegeneration in the hippocampus, amygdala and entorhinal cortex in the 1400W treated rats at 7d post-SE when compared to the control group. Hippocampal western blots revealed downregulation of potassium inward rectifying (Kir4.1) channels in control group, while the 1400W reversed this change which correlated with a reduction in the epileptiform spiking on the EEG during the first week of post-SE. In 6 months study, the 1400W caused a significant reduction in epileptiform spike frequency and convulsive seizures when compared to the vehicle group ($p=0.045$, Mann-Whitney test $n=6$-7). The vehicle treated rats had 319±112 convulsive seizures during the 6m period, while the 1400W treated rats had 22±9 (> 90% reduction) convulsive seizures. There was no significant difference in seizure severity or amount of kainate administered to induce the SE between the groups. These findings demonstrate 1400W as a potential antiepileptogenic drug candidate to intervene epileptogenesis.

PSM10 Demyelination: Pathology, Protection and Repair

PSM10-01

CONDITIONAL DELETION OF L-TYPE CALCIUM CHANNELS IN OLIGODENDROCYTE PROGENITOR CELLS AFFECTS REMYELINATION IN MICE

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Multiple sclerosis (**MS**) is an inflammatory demyelinating disorder of the CNS. Demyelination impairs axon conduction and can lead to deficits in motor, sensory and/or cognitive function depending on the location of the affected axons. Remyelination occurs in many MS lesions, but becomes increasingly less effective over time and eventually fails. Our findings indicate a role for L-type voltage-operated calcium channels (**L-VOCCs**) as potential modulators of oligodendrocyte progenitor cell (**OPC**) development in the postnatal mouse brain. To determine whether **L-VOCCs** are required for oligodendrocyte progenitor cell (**OPC**) development and remyelination, we have generated an inducible conditional knockout mouse in which the L-VOCC isoform Cav1.2 was deleted in NG2 positive OPCs. After causing demyelination with a treatment of 0.2% cuprizone for 7 weeks, the L-VOCC isoform Cav1.2 was deleted in NG2 positive OPCs by tamoxifen injections. The black gold staining for myelin and immunohistochemical experiments for myelin proteins show an inefficient remyelination in the brains of $Cav1.2^{KO}$ mice. This incomplete remyelination was more significant in the cingulate cortex, the lateral corpus callosum and the striatum. Furthermore, this reduced remyelination was accompanied by a decreased number of total oligodendrocytes (Olig2+ cells) as well as mature oligodendrocytes (CC1+ cells) in the corpus callosum and cortex. These results suggest that Ca^{++} influx mediated by L-VOCCs in oligodendroglial progenitor cells is necessary for normal remyelination.

PSM10-02

AUTOPHAGIC MYELIN DESTRUCTION BY SCHWANN CELLS DURING WALLERIAN DEGENERATION AND SEGMENTAL DEMYELINATION

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As lysosomal hydrolysis has long been suggested to be responsible for myelin clearance after peripheral nerve injury, in the present study, we investigated the possible role of autophagolysosome formation in myelin phagocytosis by Schwann cells and its final contribution to nerve regeneration. We found that the canonical formation of autophagolysosomes was induced in demyelinating Schwann cells after injury, and the inhibition of autophagy via Schwann cell-specific knockout of the *atg7* gene or pharmacological intervention of lysosomal function caused a significant delay in myelin clearance. However, Schwann cell dedifferentiation, as demonstrated by extracellular signal-regulated kinase activation and c-Jun induction, and redifferentiation were not significantly affected, and thus the entire repair program progressed normally in *atg7* knockout mice. Finally, autophagic Schwann cells were also found during segmental demyelination in a mouse model of inflammatory peripheral neuropathy. Together, our findings suggest that autophagy is the self-myelin destruction mechanism of Schwann cells, but mechanistically, it is a process distinct from Schwann cell plasticity for nerve repair

PSM10-03

EXPRESSION AND FUNCTION OF MELANOCORTIN RECEPTOR SUBTYPES IN OLIGODENDROCYTES AND OLIGODENDROGLIAL PRECURSORS

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Objective: Investigate expression and function of melanocortin receptors MC1R, MC2R, MC3R and MC4R in oligodendroglia (OL) and oligodendroglial progenitors (OPC).
Background: OL and OPC express MC4R; the MCR agonist ACTH1-39 protects OL *in vitro* from death induced by mechanisms likely involved in OL damage in MS (Benjamins et al. 2013, 2014). Protection of OL and OPC by ACTH1-39 is mimicked by an MC4R agonist and blocked by an MC4R antagonist (Lisak et al. ECTRIMS 2015). The expression and function of other MCR subtypes in OL and OPC is not known.
Methods: Enriched OL or OPC cultures were prepared from neonatal rat brain. MCR expression was assessed by immunocytochemistry with MCR subtype specific antibodies, with blocking peptides as controls. OL and OPC were incubated with: 1) toxic agent; 2) MCR subtype specific agonist; 3) agonist + toxic agent; 4) subtype specific antagonist; 5) antagonist + toxic agent; 6) antagonist + ACTH + toxic agent; 7) ACTH + toxic agent; 8) medium alone. OL and OPC death was assessed by trypan blue uptake.
Results: Both OL and OPC express MC1R, MC3R and MC4R, but do not express MC2R, as expected given its adrenal localization. As reported, both ACTH and MC4R specific agonist THIQ inhibit OL and OPC death induced by staurosporine, glutamate, reactive oxygen species and quinolinic acid, while the MC4R antagonist HS014 blocked ACTH protection of OL and OPC. We now show that MC1R agonist BMS 470539 and MC3R agonist $[D\text{-}trp^3]$g-MSH also inhibit death of OL, while the MC3R antagonist PG 106 blocks ACTH protection of OL.
Conclusion: Both OL and OPC express surface MC1R, MC3R and MC4R. Mimicking ACTH protection by MCR subtype specific agonists and blocking ACTH protection by antagonists suggest that MC1R. MC3R and MC4R on OL are all functional and activate signaling pathways that protect against mechanisms involved in OL

damage in MS and other neurodegenerative diseases.
Support: Investigator Initiated Research Award (RPL), Mallinckrodt Pharmaceuticals Autoimmune and Rare Diseases Division: Parker Webber Chair in Neurology (DMC Foundation).

PSM10-04

INACTIVATION OF ATF6 RENDERS MICE SUSCEPTIBLE TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiples sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) are T cell-mediated autoimmune demyelinating diseases in the central nervous system (CNS). Activation of the unfolded protein response (UPR) in response to endoplasmic reticulum stress aids cell survival under various cytotoxic conditions. The UPR comprises three branches of signaling pathways, pancreatic endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Recent studies demonstrated that activation of the PERK branch of the UPR in oligodendrocytes is cytoprotective, protecting mice against EAE. Evidence suggests activation of the ATF6 branch of the UPR in oligodendrocytes under normal and disease conditions; however, the effects of ATF6 signaling on oligodendrocytes in MS and EAE remain unexplored. Herein, we explore the role of ATF6 signaling in the development of EAE using ATF6 knock-out mice. We found that ATF6 inactivation significantly increased the severity of EAE clinical symptoms and exacerbated EAE-induced oligodendrocyte loss, demyelination, and axon loss in the CNS. Moreover, we found that ATF6 deficiency did not alter the inflammatory response in EAE mice. Thus, these data suggest that ATF6 acts intrinsically to protect oligodendrocytes against inflammation during EAE, resulting in attenuated demyelination, axon degeneration, and disease severity.
Wensheng Lin is supported by grants from the National Multiple Sclerosis Society (RG4813-A-2 and RG5239-A-3) and the National Institutes of Health (NS073132).

PSM10-05

THE DIVALENT METAL TRANSPORTER 1 (DMT1) IS REQUIRED FOR ADEQUATE OLIGODENDROCYTE PROGENITOR CELL MATURATION AND MYELINATION

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The Divalent Metal Transporter 1 (**DMT1**) is a multi-metal transporter with a primary role in iron transport. Even though DMT1 has been previously described in the CNS nothing is known about the role of DMT1 in oligodendrocyte maturation and myelination. We have found that DMT1 is upregulated during the development of the oligodendrocyte. *In vitro*, oligodendrocyte progenitor cells (**OPCs**) showed low levels of DMT1 expression but higher quantities of this metal transporter were found in mature oligodendrocytes. In the postnatal mouse brain, DMT1 was found highly expressed by myelinating oligodendrocytes; DMT1 immunolabeling was located in myelinated fibers and precisely colocalize with myelin proteins in the corpus callosum, cortex and striatum. To determine whether DMT1 is required for OPC maturation, we used siRNAs and the Cre/lox system to knock-down/out DMT1 expression in primary cultures of cortical OPCs. Blocking DMT1 production reduce iron uptake in OPCs and more importantly, significantly delay OPC development. DMT1 knock-down/out induced a decrease in the proportion of oligodendrocytes that expressed myelin proteins, and an increase in cells that retained immature OPC markers. Furthermore, DMT1 knock-down/out does not affect cell viability, but promotes OPC proliferation. In summary, our data suggest that DMT1 is an important multi-metal transporter for proper oligodendrocyte maturation. We have found that DMT1 is upregulated during the development of the OPC and is highly expressed by myelinating oligodendrocytes in the postnatal mouse brain.

PSM10-06

EFFECTS OF HIV-1 TAT ON OLIGODENDROCYTE VIABILITY ARE MEDIATED BY CAMKIIβ-GSK3β INTERACTION

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White matter (WM) injures are frequently reported in human immunodeficiency virus (HIV) patients, and can occur in the CNS very early in the disease process. Previously, we reported that oligodendrocytes (OLs) are direct targets of HIV-1 Tat (transactivator of transcription), a highly conserved lentiviral protein expressed by infected host cells. Interestingly, both immature ($O4^+/MBP^-$) and mature (MBP^+) OLs exhibit increased intracellular Ca^{2+} ($[Ca^{2+}]_i$) and Ca^{2+}/CaM dependent kinase IIβ (CaMKIIβ) activity when exposed to Tat, but only immature OLs showed reduced viability. Since CaMKIIβ has been reported to inhibit glycogen synthase

kinase 3β (GSK3β), and GSK3β activity has been implicated in neuronal and oligodendroglial apoptosis, we hypothesized that the disparity between viabilities of Tat-treated immature and mature OLs may be due to differential CaMKIIβ-GSK3β signaling. Our studies showed that Tat expression *in vivo* leads to increased CaMKIIβ and GSK3β activities in multiple brain regions. *In vitro,* Tat upregulates GSK3β activity in immature, but not mature, OLs. The Tat-induced immature OL death can be rescued by GSK3β inhibitors valproic acid (VPA) or SB415286, indicating involvement of GSK3β signaling. Further, immature OLs express higher levels of GSK3β, and lower levels of CaMKIIβ, than mature OLs. Importantly, inhibiting CaMKIIβ, either pharmacologically or genetically, increases GSK3β activity and promotes cell death in both immature and mature OLs treated with Tat. Together, our results suggest that the effects of Tat on OL viability are mediated through GSK3β activity, and point to CaMKIIβ-GSK3β signaling as a potential therapeutic target for limiting OL injury in HAND patients.

PSM10-07

A XENOPUS LAEVIS TRANSGENIC LINE TO MONITOR REMYELINATION IN RESPONSE TO CONDITIONAL DEMYELINATION

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To facilitate live imaging of demyelination and remyelination, we have generated a *Xenopus laevis* transgenic line, *MBP-GFP-NTR,* allowing conditional ablation of myelinating oligodendrocytes. In this line, the proximal portion of mouse myelin basic protein (MBP) regulatory sequence, specific to mature myelin-forming oligodendrocytes, drives the expression of a transgene protein formed by the GFP reporter fused to the *E. coli* nitroreductase (NTR) selection enzyme. The NTR enzyme converts the innocuous pro-drug metronidazole (MTZ) to a cytotoxin. The demyelination response of *MBP-GFP-NTR* tadpoles to MTZ is followed by spontaneous remyelination after cessation of MTZ treatment. Thanks to the transparency of tadpoles, these events can be monitored *in vivo* by two-photon imaging and quantification on a simple fluorescence macroscope. We have used the *MBP-GFP-NTR* model to screen *in vivo* molecules favoring remyelination. At the end of MTZ-induced demyelination, tadpoles were switch to water containing the compounds to be tested. After 3 days of treatment remyelination was assayed by counting the number of GFP+ oligodendrocytes per optic nerve. Among a battery of molecules tested, we report that the most efficient were, by decreasing order, clemastin, siponimod, benztropin and retinoic acid. Therefore the *Xenopus laevis* transgenic line, *MBP-GFP-NTR,* allowing conditional ablation of myelinating oligodendrocytes, constitutes a new medium-throughput screening platform for myelin repair therapeutics in demyelinating diseases.

PSM10-08

EXCITOTOXICITY: IDENTIFYING NOVEL TARGETS FOR THE PROTECTION OF OLIGODENDROCYTES

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AMPA-type glutamate receptors (AMPAR) induce diverse actions in oligodendrocytes. In the healthy CNS they influences oligodendrocyte development, while under pathological conditions their over-activation causes excitotoxic injury to white matter in the developing and adult CNS. Modulation of AMPAR expression could potentially protect oligodendrocytes from excitotoxic injury, but may also perturb pro-developmental processes required for myelin formation and repair. Therefore, identification of specific mechanisms regulating AMPAR expression during excitotoxic injury has obvious therapeutic value. The transcriptional pathways regulating AMPAR expression in the oligodendrocyte lineage are unknown. Therefore, we conducted an *in silico* analysis to identify transcriptional regulators of the AMPAR subunit GluR4, which is highly enriched in immature and mature oligodendrocytes. Bioinformatic analysis of the transcriptional networks regulating Gria4 (GluR4 gene) identified a number of transcription factors with the potential to influence Gria4 promoter activity. Among these we chose to study NF-Yb because it has established roles in cell growth and apoptosis. The influence of NF-Yb was examined at two levels. First, we measured levels of mRNA and protein for NF-Yb and GluR4 in an *in vitro* model of excitotoxic injury. Second, ChIP assays, and luciferase reporter experiments, were performed to confirm physical and functional interactions between NF-Yb and its binding sites on the Gria4 promoter. These experiments identified NF-Yb as an important regulator of GluR4 expression. Excitotoxic injury induced a down-regulation in NF-Yb expression and binding to its consensus sites on the Gria4 promoter that was associated with reduced levels of GluR4 expression. To determine signaling molecules suitable for therapeutic targeting we are performing microarray analysis and quantitative network modeling to identify transcriptional networks that are exclusively involved in the down-regulation of NF-Yb during excitotoxic injury. This work promises to identify novel targets whose regulation may provide protection to oligodendrocytes and myelin during excitotoxic injury, while preserving development-related actions of AMPAR that may be required for myelin formation and repair.

PSM10-09

INACTIVATION OF NF-κB INCREASES THE SENSITIVITY OF MYELINATING OLIGODENDROCYTES TO INTERFERON-γ

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Oligodendrocyte death is a major hallmark of multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system (CNS). Transcription factor Nuclear Factor κ B (NF-κB) plays a critical role in inflammatory diseases by regulating inflammation and cell viability. Activation of NF-κB has been observed in oligodendrocytes in MS lesions. Although *in vitro* studies suggest that NF-κB activation promotes oligodendrocyte survival in response to inflammatory mediators, the effects of NF-κB activation on oligodendrocytes in MS and its animal models remain unknown. Interferon-γ (IFN-γ) is regarded as a key proinflammatory cytokine in MS. The presence of IFN-γ in the CNS during development results in inflammation, oligodendrocyte death, and myelin loss. Interestingly, our previous study shows that IFN-γ activates NF-κB in oligodendrocytes *in vitro* and *in vivo*. In this study, using a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes, we found that NF-κB inactivation in oligodendrocytes exacerbated IFN-γ-induced cell death and myelin loss in young, developing mice, but did not alter inflammation elicited by this cytokine in the CNS. Thus, this finding implies the cytoprotective effects of NF-κB activation on oligodendrocytes in neuroinflammatory diseases such as MS.

Wensheng Lin is supported by grants from the National Multiple Sclerosis Society (RG4813-A-2 and RG5239-A-3) and the National Institutes of Health (NS073132).

PSM10-10

ELECTROPHYSIOLOGICAL AND HISTOLOGICAL ANALYSIS OF DEMYELINATION AND REMYELINATION IN A CUPRIZONE MOUSE MODEL OF MULTIPLE SCLEROSIS

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Demyelinating diseases, such as multiple sclerosis, result in central white matter loss with consequent loss in axonal function. The cuprizone-induced demyelination model is historically used to study mechanisms of demyelination and remyelination. However, spontaneous endogenous remyelination during demyelination adds unpredictable variability. We used rapamycin to prevent spontaneous remyelination in this model and characterized functional and histological changes in the corpus callosum (CC). Eight-week-old C57BL/6J mice were subjected to 3, 6, and 12-weeks of 0.3% CR-induced demyelination and a 3-, or 6-week recovery. The compound action potential (CAP) was recorded from 350μm thick brain slices containing CC and paraphenylenediamine (PPD) staining was performed on semi-thin sections of CC to quantify myelinated axons. The CAP waveform consists of at least two negative peaks; N1 is elicited by myelinated axons and N2 by non-myelinated axons. CR demyelination for 3, 6 and 12 weeks significantly decreased the average N1 amplitude by 66%, 85%, and 91%, respectively. N1 latency increased by ~30% after 3-weeks of demyelination but no additional increase after 6 weeks. Furthermore, N1 latency returned to control values after 6 weeks of recovery. On the other hand, N1 amplitude showed significant increase after 6 weeks of recovery, but did not recover to control levels. Histological analysis also revealed a 98% increase of myelinated axons after 6-weeks recovery that did not recover to control levels. Analysis along the rostrocaudal axis revealed significantly larger deficits in the middle and caudal CC compared to rostral CC. Remyelination was also more pronounced in rostral CC. We show that CR demyelination for 3, 6 and 12 weeks causes significant functional and histological changes. Spontaneous remyelination for 6 weeks after 12-weeks of demyelination shows significant reversal of functional and histological changes. Therefore, CR model with CAP recordings and histological assessment will be a useful tool to test the efficacy of therapeutics that may affect demyelination/remyelination.

PSM10-11

SOX17 OVEREXPRESSION ALTERS EXTRACELLULAR MATRIX PROTEINS AND HEDGEHOG DYNAMICS IN ADULT WHITE MATTER

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The SRY-box factor Sox17 promotes the oligodendrocyte (OL) development and its overexpression in CNPSox17 mice increases OL regeneration and attenuates demyelination damage in white matter (WM). Cell-intrinsic signaling effects of Sox17 mediating OL regeneration in lysolecithin (LYSO)-induced WM lesions have been ascribed to Sox17-induced GLI2, but it is unknown how Sox17 activates Sonic Hedgehog (SHH) signaling. Furthermore, OL cell-extrinsic mechanisms have not been explored, such as the response of reactive glia which influences tissue integrity. In this study, we investigated a role for Sox17-mediated neuroprotection contributed by outside-in mechanisms. Electron microscopic analysis of LYSO lesions showed that at 3 days post lesion (DPL), before remyelination is allowed to complete, CNPSox17 g-ratios remained unchanged compared with increases in wild-type (WT). This ultrastructural preservation by Sox17 is independent of cellular regeneration, and may arise from changes in injury-induced reactive events. Immunocytochemistry revealed more amoeboid Iba1+ microglia surrounding the WT LYSO lesion, indicating

proinflammatory polarization, whereas microglia in CNPSox17 lesions were more ramified. Since SHH signaling is known to promote immune quiescence, and the extracellular matrix influences SHH signaling and inflammation, we hypothesize that Sox17 alters extracellular matrix components. Western blotting of WM tissue from intact CNPSox17 revealed no change in total SHH protein levels, but showed increased levels of phosphorylated-Integrin linked kinase and autophosphorylated FAK and reduced FAS. Confocal microscopy of CNPSox17 WM showed increased Olig2+ cells colabeled with SHH and laminin, and increased clustering of Smoothened expression, consistent with enhanced ligand interaction and pathway stimulation. Our findings support cell-autonomous and non cell-autonomous roles of Sox17 in WM recovery. We therefore conclude that Sox17 overexpression increases the WM expression of extracellular matrix components, which may modulate reactive responses to injury and stimulate integrin signaling to promote survival signaling.

PSM11 Gene Expression/Regulation

PSM11-01

DIVERSE TRANSCRIPTIONAL REGULATION OF THE SYSTEM XC- LIGHT CHAIN, XCT, IN ASTROCYTES

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System x_c^- (Sx_c^-) is a hetereomeric plasma membrane amino acid transporter known to import cyst(e)ine and export glutamate with a 1:1 ratio. In the astrocyte, cystine import via Sx_c^- is critical for the maintenance of glutathione synthesis, while the export of glutamate contributes greatly to maintenance of its ambient levels in the synaptic cleft. Sx_c^- upregulation occurs as part of cells' adaptive response to various stimuli, in large part, via transcriptional control of its substrate specific light chain, xCT. The purpose of this study was to compare and contrast the signaling pathways involved in xCT upregulation by several stimuli—namely Interleukin 1β (IL-1β), Phorbol-12-Myristate-13-Acetate (PMA), and forskolin —to determine whether they are common or distinct. Toward this end, we demonstrate using RT-qPCR that exposure to IL-1β (3 ng/mL), PMA (30 nM) or forskolin (25 μM) enhances the steady state mRNA levels of xCT in purified astrocyte cultures. The increase mediated by each stimulus was blocked by exposure (30 min pretreatment) to the transcriptional inhibitor actinomycin D (10 μg/mL) or the non-selective protein kinase (PK) inhibitor, H-7 (20 μM). This latter result suggests that some or all of the stimuli may share common signals. Despite this, we found that the PMA-induced increase in xCT mRNA expression was blocked by the pan PKC inhibitor Go 6983 (1 μM), but the IL-1β-mediated increase was unaffected. The IL-1β-mediated increase in xCT mRNA levels was effectively attenuated by an inhibitor of p38 MAPK, SB 203580 (10 μM), however. While ongoing experiments continue to assess the potential cross-talk between IL-1β, PMA and forskolin signaling pathways in mediating an increase in astrocytic xCT, the present results suggest some diversity in regulation, although we can't yet rule out that some pathways may be partially shared. (Supported by grant RO1 NS051445-07 to JAH and SJH)

PSM11-02

NEUROPSIN, A SECRETORY SYNAPTIC PROTEASE IS A NOVEL REGULATOR OF DENDRITE GROWTH AND THERAPEUTIC TARGET OF AMNESIA

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Memory loss or amnesia is a devastating feature of neurodegenerative pathologies, traumatic brain injuries, psychiatric disorders and aging. Understanding the underlying molecular mechanism is crucial for recovery of amnesia, though it is a persistent challenge. We have identified role of a secretory synaptic serine protease, Neuropsin (NP) in amnesia. NP gene expression was drastically reduced in the cerebral cortex and hippocampus of scopolamine hydro bromide induced amnesic mouse model. NP knockdown in primary hippocampal neuronal cells by siRNA and mouse forebrain by antisense oligonucleotides, reduced the cleavage of synaptic adhesion molecule L1CAM, level of dendrite marker MAP2c, dendrite length and branching, and eventually the object recognition memory consolidation. Conversely, increase in NP expression and effector MAP2c by administration of nootropic Ashwagandha leaf extract (i-Extract) markedly enhanced dendrite growth and recovered amnesia. Further investigations revealed that NP function in dendrite growth influences PKA/CREB signaling of memory consolidation. Moreover, NP is regulated upstream by acetylcholine receptor of muscarinic subtype M1. Taken together, our findings suggest a unique mechanism of amnesia and envisage NP as a novel target for recovery of memory disorders.

PSM11-03

SIGNALING AND EXPRESSION OF A TRUNCATED, CONSTITUTIVELY ACTIVE HUMAN INSULIN RECEPTOR

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Insulin signaling is indispensable for key metabolic pathways in the periphery. Several studies have demonstrated that insulin signaling is also important for brain function. Early stage clinical trials report a positive impact of intranasal insulin on memory recall in young subjects and patients with mild cognitive decline or Alzheimer's disease (AD). However, the underlying molecular mechanisms involved are not well understood. Here we sought to investigate the role of insulin in neuronal physiology by overexpressing a constitutively active human insulin receptor (Lebwohl et al., 1991) in rat pheochromocytoma (PC12) and primary hippocampal neurons. Cells were transfected with either pCI-ires-dsRed, a mammalian expression plasmid encoding a red fluorescence protein (ds-Red), or pCI-HA-IRβ-ires-ds-red, the construct with a truncated human insulin receptor beta subunit (IRβ), via either electroporation (PC12 cells) or a targeted lentiviral delivery system (neurons). The expression of IRβ receptor in PC12 cells was corroborated by the expression of the red fluorescent protein. Western blots using pTyr antibodies provide evidence that the truncated IRβ is constitutively active when expressed in PC12 cells. Photomicrographs of mixed primary hippocampal cultures confirmed expression of the lentiviral plasmid in neurons. Confocal microscopy and a fluorescent antibody targeting the HA-tag show expression of IRβ at the plasma membrane. The expression level and effect of IRβ overexpression on insulin signaling was confirmed in both PC12 cells and neurons by performing immunoblots using antibody against HA-tagged IRβ and measuring pAkt/Akt ratio, respectively. Our data show that the overexpression of insulin receptor enhances neurite outgrowth in PC12 cells and increases the pAkt/Akt ratio in both PC12 cells and neurons. Overexpression of the truncated receptor increased insulin signaling compared to control in both cell types. This initial characterization provides insights into future intervention approaches to combat reduced insulin signaling in AD and/or aging.

PSM11-04

GENE EXPRESSION CHANGES IN THE RAT AMYGDALA AND HIPPOCAMPUS ASSOCIATED WITH ACQUISITION OF CONDITIONED TASTE AVERSION

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Differential display of gene expression in the amygdala and hippocampus was used to test the hypothesis that conditioned taste aversion involves engram formation through molecular correlates of different elements of the experience, with no molecular changes unique to the formation of an association between the novel taste and illness. Male rats fasted overnight were given either lab chow followed by a benign injection of saline (CS) or illness-inducing LiCl (CL), or fruit loops, a novel food, followed by injections of saline (FS) or LiCl (FL). Total RNA was extracted 4 h post-injection. mRNA was reverse transcribed and amplified into a series of bands separated by polyacrylamide gel electrophoresis. Of 672 bands expressed in the amygdala in Groups FS, CL, and FL, 99 (14.7%) were differentially expressed relative to the level of transcription in the Controls (Group CS). Combining food novelty with illness induced more differential expression than either stimulus alone, but only 4 bands (1.8%) suggested changes unique to the process of conditioning apart from its modular components. In the hippocampus, fewer changes were seen, and more often involved the coincidence of novel food and illness, but no changes were uniquely associated with formation of the conditioned response. These results suggest that only a small minority of changes in gene expression are devoted specifically to memory formation, as opposed to the processing of its constituent elements. While these quantitative differences in gene expression may be related to engram formation, they provide no support for a unique genomic repository of experiential memory.

PSM12 The Synapse: Signals and Plasticity

PSM12-01

COLLAGEN-DERIVED MATRICRYPTINS PROMOTE INHIBITORY NERVE TERMINAL FORMATION IN THE DEVELOPING NEUROCORTEX

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Inhibitory synapses comprise only a small fraction of the total synapses in the mammalian cerebrum, but play essential roles in controlling neural activity. Perturbing inhibitory synapse assembly or function has been associated with autism, epilepsy and schizophrenia. We recently identified collagen XIX as a synaptic organizing cue that is expressed by cerebral interneurons and is necessary for the proper assembly of inhibitory nerve terminals in hippocampus and cortex. In humans, deletion of the genomic region encoding this unconventional collagen has been linked to familial schizophrenia. Here, we discovered that genetically modified mice lacking collagen XIX exhibit schizophrenia related behaviors, which include defects in pre-pulse inhibition and nest-building activity. These mutants exhibit spontaneous seizures and an increased susceptible to drug-induced seizures. Furthermore we show that these collagen XIX-deficient mice exhibit defects in PV^{+} synapse formation in visual cortex and prefrontal cortex. Interestingly, these synapse are not made by or on collagen XIX-generating interneurons, suggesting this collagen acts in a paracrine fashion. Finally we found that the C-terminal domain of collagen XIX (NC1[XIX]) functions as a matricryptin and it is sufficient to rescue synaptic defects in the absence of full-length collagen XIX. Functional blocking analysis and immunoprecipitation have revealed that this matricryptin binds to and signals through a5b1 integrin to trigger nerve terminal assembly. Take together, these studies not only identify roles for collagen-derived matricrytins in cortical circuit formation, but they also reveal a novel paracrine mechanism that regulates the assembly of these synapse.

PSM12-02

NCAM BINDS THE EPHA3 RECEPTOR AND PROMOTES CLUSTERING AND ACTIVATION IN CORTICAL INTERNEURONS

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Establishment of a proper balance of excitatory and inhibitory connectivity is achieved during development of cortical networks and adjusted through synaptic plasticity. Neural cell adhesion molecule NCAM and the receptor tyrosine kinase EphA3 mediate ephrinA5-induced growth cone collapse and elimination of perisomatic synapses in GABAergic basket interneurons of the postnatal mouse prefrontal cortex (Brennaman et al., 2013, *Cerebral Cortex*). To identify the mode of interaction between NCAM and EphA3, structure-function studies were carried out. The EphA3 binding domain was localized in the NCAM extracellular region by incubating lysates of EphA3-expressing HEK293 cells with Fc fusion proteins consisting of the following NCAM fragments: NCAM immunoglobulin(Ig)1-5/fibronectin III (FN)1-2, Ig1-3, Ig1-2, Ig2, or Ig1, then analyzing associations in pull-down assays. EphA3 was found to associate with the full length extracellular region of NCAM, consisting of Ig1-5/FN1-2, and with truncation fragments of NCAM consisting of Ig1-3, Ig1-2, and Ig2 but not with Ig1 alone. This indicated that EphA3 interacted specifically with the Ig2 domain of NCAM. To identify the interaction domain(s) on EphA3, NCAM Ig1-5/FN1-2 Fc was incubated with lysates expressing full-length EphA3 or EphA3 deletion mutants: ΔLBD (ephrinA ligand binding domain) or ΔLBD/ΔCRD (cysteine-rich domain). Results showed that binding of the NCAM extracellular region to EphA3 required the CRD but not the LBD of EphA3. Molecular modeling and mutagenesis was further used to identify residues at the interface of the NCAM/EphA3 binding site. Co-expression of NCAM with EphA3 enhanced ephrinA5-dependent autophosphorylation of EphA3, suggesting that NCAM-induced clustering activates EphA3 kinase signaling. Using primary cortical neurons from wild-type or NCAM null mutant mice, it was further demonstrated that ephrinA5 promoted NCAM-dependent RhoA GTPase activation. These studies support the interpretation that NCAM promotes ephrinA5-dependent clustering of EphA3, leading to EphA3 autophosphorylation and RhoA activation necessary for growth cone collapse.

PSM12-03

PERIPHERAL VIRAL CHALLENGE DYSREGULATES GLUTAMATE HOMEOSTASIS IN THE HIPPOCAMPUS LEADING TO HYPEREXCITABILITY

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Peripheral infections increase the propensity and severity of seizures in susceptible populations. In a quest to elucidate the underlying mechanisms, we have developed a preclinical model in which intraperitoneal injection of a viral mimic, polyinosinic-polycytidylic acid (PIC), elicits a protracted hypersusceptibility of mice to kainic acid (KA)-induced seizures. The present study was undertaken to determine whether this seizure hypersusceptibility entails alterations in glutamate signaling. Briefly, eight-week old female C57BL/6 mice were intraperitoneally injected with PIC and after 24 hours, glutamate homeostasis in the hippocampus, the ictal region of KA-induced seizures, was monitored using the enzyme-based microelectrode arrays. PIC challenge robustly increased the level of resting extracellular glutamate. While presynaptic potassium-evoked glutamate release was not affected, glutamate uptake was profoundly impaired and non-vesicular glutamate release was augmented. Therefore, it seems that postsynaptic mechanisms are responsible for the elevation of extracellular glutamate.

Electrophysiological examination of hippocampal slices from PIC-challenged mice revealed a several fold increase in the basal synaptic transmission as compared to control slices. Paired-pulse facilitation (PPF), a presynaptic event was not affected. Also, no difference in long term potentiation (LTP) between PIC-challenged and control slices was detected. These results show that PIC challenge increases synaptic transmission through postsynaptic mechanisms, but does not alter synaptic plasticity per se. Altogether, our results implicate a dysregulation of astrocytic glutamate metabolism as the underlying mechanism for seizure hypersusceptibility induced by peripheral PIC challenge. S*upported by NIGMS U54GM104942, NIA R15AG045812, Alzheimer's Association NIRG-12-242187, WVU Faculty Research Senate Grant and WVU PSCOR grant.*

PSM12-04

A TRANSCRIPTOMIC APPROACH FOR INVESTIGATING THE NEUROCHEMISTRY OF SYNAPTIC TRANSMISSION BETWEEN IDENTIFIED MOLLUSCAN NEURONS

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Some “non-model” organisms such as nudibranch molluscs are important for understanding the neural basis of simple behaviors. Until the recent advent of next-generation RNA sequencing, however, research on such exotic species was hampered by a paucity of molecular tools. *Dendronotus iris* and *Melibe leonina* are two nudibranchs that swim by simply flexing their bodies from left to right. In *Dendronotus*, the central pattern generator (CPG) circuit underlying this behavior is composed of only two sets of paired identified neurons. Homologs of these neurons have also been identified in the *Melibe* swim CPG. Although the swim CPGs contain homologous neurons, there are differences in the synaptic wiring. A particular synapse between homologous neurons is excitatory in *Dendronotus* but inhibitory in *Melibe*. The synapse is blocked by nicotinic antagonist, curare in both species suggesting that it is mediated by post-synaptic nicotinic acetylcholine receptor subunits (nAChRsu). To determine whether the corresponding pre-synaptic neuron is cholinergic, and whether there are differences in nAChRsu expression that could account for the species-differences in synaptic transmission, we took a transcriptomic approach. We isolated mRNA from the brains of both species and then Illumina-sequenced and annotated the data. The gene sequence for choline acetyl transferase (ChAT) was identified in both transcriptomes. Whole mount *in situ* hybridization using ChAT gene probes labeled three cells in each pleural ganglion of *Dendronotus*, and up to two cells in each cerebral ganglion. *Melibe* also had 3 cells in each pedal ganglion and 7-8 cells in each pleural ganglion. Additional work will determine if any of these neurons correspond with the known swim CPG neurons. We also annotated the nAChRsu mRNA sequences in the transcriptomes. The relative mRNA expression levels of subunits showed surprising differences. These transcriptomic techniques are providing new insights into the neurochemical organization of simple neural circuits in these exotic species.

PSM13-01

REGULATION OF CHLAMYDOMONAS FLAGELLA AND EPENDYMAL CELL MOTILE CILIA BY CERAMIDE-MEDIATED TRANSLOCATION OF GSK3

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Cilia are important organelles formed by cell membrane protrusions, however, little is known about their regulation by membrane lipids. We characterize a novel activation mechanism for glycogen synthase kinase-3 (GSK3) by the sphingolipids phytoceramide and ceramide, which is critical for ciliogenesis in Chlamydomonas and murine ependymal cells, respectively. We show for the first time that Chlamydomonas expresses serine palmitoyl transferase (SPT), the first enzyme in (phyto)ceramide biosynthesis. Inhibition of SPT in Chlamydomonas by myriocin led to loss of flagella and reduced tubulin acetylation, which was prevented by supplementation with the precursor dihydrosphingosine. Immunocytochemistry showed that (phyto)ceramide was colocalized with phospho-tyr216-GSK3 (pYGSK3) at the base and tip of Chlamydomonas flagella and motile cilia in ependymal cells. The (phyto)ceramide distribution was consistent with that of a bifunctional ceramide analog UV-crosslinked and visualized by click-chemistry mediated fluorescent labeling. Ceramide depletion, by myriocin or neutral sphingomyelinase deficiency (fro/fro mouse), led to GSK3 dephosphorylation and defective flagella and cilia. Motile cilia were rescued and pYGSK3 localization restored by incubation of fro/fro ependymal cells with exogenous C24:1 ceramide, which directly bound to pYGSK3. Our findings suggest that (phyto)ceramide-mediated translocation of pYGSK into flagella and cilia is an evolutionarily conserved mechanism fundamental to the regulation of ciliogenesis. Funded by NSF.

PSM13-02

NECTIN-LIKE 4 AND CHOLINE TRANSPORTER-LIKE PROTEIN-1 REGULATE CHOLINE TRANSPORT, LIPID BIOGENESIS AND MYELINATION IN SCHWANN CELLS

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Oligodendrocytes and Schwann cells are the myelinating glial cells of the Central and Peripheral Nervous Systems (CNS and PNS), respectively. During myelination, glial cells upregulate lipid synthesis to form a mature myelin segment constituting 70-85% lipid. Recently, we and others identified a cell adhesion molecule, Nectin-like 4 (Necl4), that promotes axo-glial interaction along the internode and regulates myelination *in vitro* and *in vivo*. To highlight the mechanisms by which Necl4 regulates peripheral nerve myelination, we performed (reciprocal) co-immunoprecipitation experiments and identified a transmembrane choline transporter, Choline Transporter-Like protein 1 (CTL1), as a putative Necl4 complexing partner. CTL1 and Necl4 are both expressed in myelinating sciatic nerve (P0-P30), and knockdown of Necl4 or CTL1 in Schwann cells significantly inhibited their ability to myelinate neurites *in vitro*. Choline is a cationic amine representing the precursor molecule for many signalling and structural lipids. However, due to its positive charge, extracellular choline cannot passively transit the plasma membrane, instead utilising transport proteins such as CTL1. To investigate whether the impairments in myelination in Necl4-deficient Schwann cells is attributable to (co)regulation of choline transport (presumably through regulation of CTL1), we performed an extensive screen of choline-derived lipid species. Transmembrane choline trafficking, intracellular choline levels and downstream, choline-dependent lipid biogenesis (total phosphatidylcholine and phosphatidylinositol, particularly long acyl chain subspecies) were all disrupted in Necl4-deficicent Schwann cells. Also, disruptions to phosphatidylinositol levels and subspecies translated to disrupted intracellular signalling, where the activation of the pro-myelinating signalling molecule Akt, that requires specific species of phosphatidylinositol for its activation, is significantly repressed in Necl4-deficient Schwann cells. To translate our exciting preliminary *in vitro* data in an *in vivo* system, we have generated a novel CTL1 conditional knock-out mouse. We aim to analyse myelination and nerve ultrastructure, lipid profile and intracellular signalling in the PNS and CNS of CTL1 knockout mice.

Poster session TUESDAY/WEDNESDAY (MAR 22-23)

PTW01 Development, Differentiation and Disorders

PTW01-01

REGULATION OF THE PROTEIN KINASE LKB1 IN THE DEVELOPING BRAIN

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The establishment of long-range connectivity represents one of the greatest challenges faced by the developing nervous system. The axons that will ultimately constitute these neural networks are specified during neuronal differentiation. While the phenomenology of this process has been described in some detail for particular cell types in the embryonic brain, much remains to be learned about the cell biological events that underlie this critical event in neuronal morphogenesis. It is now well established that particular signal transduction pathways are required for axogenesis, but it is less clear how these pathways are regulated and how they cross talk with one another.

One of the requisite signaling elements of axon formation is the tumor suppressor protein kinase LKB1. We, along with others, have shown that LKB1 contributes to multiple stages of axon development including axon specification, branching and synaptogenesis. Here, we focus on LKB1 regulation via its unconventional activation scheme during in the developing cerebral cortex. Specifically, we will describe our genetic and biochemical analysis of the role played by two related pseudokinases in this process. Our data indicate these proteins, STRAD-alpha and STRAD-beta, comprise an essential regulatory node for LKB1 during this early critical step of neural development.

The impact of conditional deletion of LKB1 in the developing mouse cerebral cortex will be compared to the effects of loss of regulatory pseudokinases STRAD-alpha and STRAD-beta. Similarly, loss of STRAD-alpha in a mouse model will be compared with abnormalities found in a rare human genetic syndrome polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE), associated with loss of STRAD-alpha.

PTW01-02

EXPRESSING NON-PRENYLATABLE RAC1 OR RHOA DIFFERENTIALLY ALTERS SIGNALING CASCADES INVOLVED IN NEURITE GROWTH AND CELL CLUSTERING

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The Rho guanine triphosphatases (GTPases) Rac1 and RhoA are highly characterized proteins that act as molecular switches operating between an active guanosine triphosphate (GTP) bound state and an inactive guanosine diphosphate (GDP) bound state. These proteins play a pivotal role in neuronal differentiation and affect neurite outgrowth, axonal guidance and cell migration. Rac1 stimulates assembly of multimolecular focal complexes at plasma membrane, regulates membrane protrusions, membrane ruffling and formation of lamellipodia and filopodia. On the other hand, RhoA promotes assembly of focal adhesion complexes and promotes actin contractility. The interaction between Rac1 and RhoA is not well explored, although they are thought to be antagonist to each other. Both require prenylation for membrane localization; however, active forms of both have been found in other cellular compartments (GTP-bound Rac1 in the cytosol and GTP-RhoA in the cytosol and nucleus). We used non-prenylatable Rac1 and RhoA constructs to test how inhibiting prenylation affects neuronal morphology and the location of active RhoA and Rac1, as well as signal transduction pathways involved in neurite extension. Western blot analysis suggested that expressing non-prenylatable Rac1 leads to an increase in ARP2/3-WAVE complexing within the cytosol and led to the increase in cell clustering and expressing non-prenylatable RhoA increased Arp2/3-WAVE association associated with membranes. We have found transfection of non-prenylatable RhoA increased neurite elongation and transfection of non-prenylatable Rac1 increased neurite initiation in rat cortical neurons). Both constructs retained the ability to be made active independent of membrane targeting by prenylation. With emerging evidence of differential activation of these Rho GTPases based on their subcellular localization, elucidating the signaling cascades affected by subcellular localization may identify novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions.

This research was supported by the TWU Department of Biology, and grants from the TWU Research Enhancement, Summer Research Incentive and Chancellor’s Research Fellows programs. We would like to thank Dr. DiAnna Hynds and laboratory associates for their guidance.

PTW01-03

IMPAIRED MEMORY AND REDUCED HIPPOCAMPAL DENDRITIC SPINE DENSITY IN LCK-/- MICE

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The lymphocyte-specific protein tyrosine kinase (Lck), principally known for its role in T cell activation, may also be involved in memory-related function of the hippocampus. The Lck gene is expressed in neurons throughout the brain, including the hippocampus, and has been shown to be significantly down-regulated in the hippocampus of Alzheimer's disease patients compared to non-demented controls. In C57BL/J6 mice, it has been demonstrated that selective inhibition of Lck impairs hippocampal neurite outgrowth in vitro, and significantly alters spatial learning and memory in vivo. Our laboratory has previously utilized $Lck^{-/-}$ mice to demonstrate that Lck regulates Schwann cell migration and myelination. In the current study, we used $Lck^{-/-}$ mice to investigate the role of Lck in learning and memory. First, we tested adult $Lck^{-/-}$ and control mice using a social transmission of food preference task. In this task, mice use odor cues learned through interaction with a conspecific to acquire food preferences. Long-term retention on this task is critically dependent on the hippocampus. In a separate group of adult mice, brains were harvested and Golgi-stained for the visualization and quantification of hippocampal dendritic spine density. Numerous behavioral studies have demonstrated a relationship between memory and dendritic spine density in the hippocampus. We observed memory deficits in $Lck^{-/-}$ mice compared to controls. In addition, $Lck^{-/-}$ mice exhibited significantly reduced dendritic spine density on hippocampal CA1 pyramidal neurons. Our results support a role of Lck in hippocampal function. Future experiments will assess neurite outgrowth and spine density in cultured hippocampal neurons from $Lck^{-/-}$ mice. Another future endeavor is to elucidate the role of Lck in the structure of the postsynaptic density.

PTW01-04

RETINOIC ACID REGULATES VEGF-A DRIVEN NEURAL PROGENITOR PROLIFERATION

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The ability of the brain to perform complex functions depends on the generation of a wide variety of neural cell types generated by a small population of cells known as neural progenitors. Neural progenitors expand in number and generate neurons and glia that eventually integrate into functional neural circuits. Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are expressed by the progenitors in the embryonic brain and are established positive regulators of progenitor proliferation. What regulates VEGF-driven progenitor proliferation, however, is not well understood.Using *Foxc1* mutant mice we identified Retinoic acid (RA) as a possible regulator of VEGF-A in the embryonic cerebral cortex. *Foxc1* mutants do not form meninges, which are the primary source of RA for embryonic cerebral cortex. We find VEGF-A protein expression is reduced in *Foxc1* mutants and but is restored to control levels when mutants were exposed to exogenous RA via an RA-enriched diet fed to the pregnant dams. To test if upregulation of VEGF-A is a direct effect of RA on cortical progenitors, mouse E14 cortical progenitors were cultured in presence of RA and RA with pan-Retinoic acid Receptor (RAR) antagonist, which inhibits RA signaling. Cell culture supernatants were used to quantify VEGF-A protein levels using an ELISA. RA increased VEGF-A release into the media and this expression was reduced when RA signaling was inhibited. Moreover, RA increased progenitor cell proliferation, an effect that was blocked with the RAR antagonist. Proliferation index was reduced when cells were treated with VEGFR2 inhibitor which blocks VEGF signaling. We next tested if the effect of RA on progenitor proliferation is mediated via increased VEGF-A synthesis by co-treating cells with RA and the VEGFR2 antagonist. When VEGF signaling was inhibited in presence of RA, proliferation index was equivalent to control but significantly higher than VEGFR2 antagonist alone. Taken together, these results support the hypothesis that RA regulates VEGF-A driven progenitor proliferation in the embryonic cerebral cortex.

PTW01-05

INVESTIGATING THE MOLECULAR ROLE OF DOMINANT A-TUBULIN MUTATIONS IN NEURAL DEVELOPMENT

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Mutations in the microtubule cytoskeleton are linked to cognitive and locomotor defects during development, and neurodegeneration in adults. The molecular bases of these pathologies are poorly understood. Using yeast and murine models, we are exploring the molecular effect of dominant negative mutations identified in the α-tubulin gene Tuba1a in human patients. In addition, we have identified a novel missense mutation in Tuba1a a-tubulin that disrupts cortical and motor neuron development. Homozygous mutant mice exhibit cortical dysgenesis reminiscent of tubulinopathies in humans. To directly examine effects on tubulin function, we created analogous missense mutants in one or both of the a-tubulin isotypes in budding yeast. These mutants are sufficient to sensitize yeast cells to microtubule stresses including depolymerizing drugs and low temperatures. Furthermore, we find that the mutation causes selective depletion of the protein from the cell lysate and from microtubules, thereby altering ratios of a-tubulin isotypes. We provide evidence that tubulin-binding cofactors are necessary to suppress the effects of the mutant, indicating an important role for these cofactors in regulating the quality of the a-tubulin pool. Together, our results give new insights into the functions of Tuba1a, mechanisms for regulating tubulin proteostasis, and how these may be compromised in the disease state.

PTW01-06

DOES ESTRADIOL SUPPORT NEURONAL DIFFERENTIATION IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS?

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Human SH-SY5Y neuroblastoma cells maintain their potential for differentiation and regression in culture conditions. The induction of differentiation could serve as a strategy to inhibit cell proliferation and tumor growth. Previous studies have shown that differentiation of SH-SY5Y cells can be induced by all-*trans*-retinoic-acid (RA) and cholesterol (CHOL). However, signaling pathways that lead to terminal differentiation of SH-SY5Y cells are still largely unknown and moreover, clinical trials have demonstrated that treatment with RA is not enough against recurrent neuroblastoma in children. Therefore, new alternative resources for more effective neuronal differentiation are needed. The goal of this study was to examine in the RA and CHOL treated SH-SY5Y cells the additive impacts of estradiol (E_2) and brain-derived neurotrophic factor (BDNF) on cell morphology, cell growth, neurite length and synaptic vesicle recycling. The above features indicate a higher level of neuronal differentiation. Our data show that treatment for 10 days *in vitro* (DIV) with RA alone or when combined with E_2 (RE) or CHOL (RC), but not when combined with BDNF (RB), significantly inhibited the cell population growth. Synaptic vesicle recycling, induced by high-K^+ depolarization, was significantly increased in all treatments where RA was included (RE, RC, RB, RCB), and when all agents were added together (RCBE). Additionally, our results show that treatment with E_2 alone increases neurite length and synaptic vesicle recycling in SH-SY5Y cells. This work contributes to the understanding of the additional ways to improve neuroblastoma cells' maturation and differentiation by estradiol.

PTW01-07

MATERNAL EXPOSURE TO A DDT-LIKE ENVIRONMENTAL CONTAMINANT DURING GESTATION PREDISPOSES ADULT OFFSPRING TO SEIZURES

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Chemical manufacturing processes during the industrial era in Central NY severely polluted the lakebed sediment of Onondaga Lake prompting the Environmental Protection Agency (EPA) to place the lake on its Superfund National Priorities List. Chemical analyses of the heavily contaminated sediments identified a planar aromatic hydrocarbon, 1-phenyl-1-(p-tolyl)-ethane (PTE, CAS #3717-68-8), which has the carbon backbone structure of the insecticide, dichloro-diphenyl-trichloroethane (DDT). DDT is a suspected endocrine disrupting chemical that can affect gestational development, resulting in long-term detrimental consequences to the offspring. In addition to its notorious effects on reproductive capacity, *in utero* exposure to DDT may increase susceptibility to neurological disorders later in life. The structural similarities between DDT and PTE raise the possibility that early life exposure to PTE may have similar consequences in adulthood. The specific hypothesis of the research described herein was that gestational exposure to PTE will increase the risk for seizures in adult offspring. To test this possibility, pregnant mice were exposed to PTE (100mg/kg, daily, PO) or its corn oil vehicle and the effect on seizure susceptibility in adult progeny was assessed by response to the test convulsant, pentylenetetrazol (PTZ, 46mg/kg, IP). Adult offspring exposed to PTE during fetal development (*in utero* day 5-birth) exhibited a higher incidence of PTZ-induced convulsive seizures compared to offspring treated in parallel with corn oil (17/27 vs. 6/23, respectively). Maternal exposure to PTE during the neurogenesis period of *in utero* development (day 10-birth) tended to affect seizure incidence more modestly (3/9 vs. 3/13 PTE and corn oil, respectively). Seizures are the defining characteristics of epilepsy, a brain malady that affects about 1 in 100 individuals. The results from this study raise concern that exposure to PTE (e.g., via consumption of contaminated fish) during gestational development may lower seizure threshold and predispose adults to epilepsy. The mechanism for this effect, however, remains to be determined.

PTW01-08

HIV-1 TAT INHIBITS AUTOTAXIN LYSOPHOSPHOLIPASE D ACTIVITY AND MODULATES OLIGODENDROCYTES DIFFERENTIATION

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White matter injury has been frequently reported in HIV^+ patients. Previous studies showed that HIV-1 Tat (transactivator of transcription), a viral protein that is produced and secreted by HIV-infected cells, is a toxic factor to oligodendrocytes (OLGs). Adding Tat to the culture medium reduced the viability of immature OLGs, and the survived OLGs exhibited reduced process networks. Oligodendroglial lineage cells produce and secrete autotaxin (Atx), an ecto-enzyme containing a lysophospholipase D (lysoPLD) activity that converts lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA). It has been recently reported that this activity modulates OLG gene expression, and promotes the differentiation of OLG precursor cells to early OLGs. Thus, we hypothesized that Tat affects OLG development by interfering with the autotaxin-LPA signaling pathway. Our data show that Tat treatment leads to changes in OLG differentiation gene expression and morphology, both of which can be rescued by the addition of LPA. Tat-treated OLGs do not alter LPA receptor gene expression. However, treating OLGs with Tat, or directly adding Tat to supernatant collected from vehicle-treated OLGs, both resulted in significantly decreased Atx lysoPLD activity, suggesting that Tat blocks Atx lysoPLD activity. These results are supported from co-immunoprecipitation experiments which revealed a physical interaction between Tat and Atx. Together, these data strongly suggest two functional implications of Tat blocking Atx's lysoPLD activities. On one hand

it attenuates OLG differentiation and on the other hand it interferes with the protective effects of LPA on OLG process morphology.

PTW01-09

NOCICEPTIN PRECLUDES PRECOCIOUS BRAIN MYELINATION: IMPLICATIONS FOR MYELIN REGENERATION IN MULTIPLE SCLEROSIS

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Our previous studies showed that perinatal exposure to elevated doses of buprenorphine, an opioid analogue used in the treatment of pregnant opioid addicts, delays rat brain myelination. Unexpectedly, this inhibitory effect was mediated by buprenorphine-dependent activation of the nociceptin receptor (NOPR). This G-protein coupled receptor and its endogenous ligand, the nociceptin/orphanin FQ peptide, are originally known for their role in pain regulation. However, our findings suggest that the nociceptin system could also play a role controlling the timing of developmental brain myelination. Support for this possibility is provided by our recent studies using BAN-ORL24, a blood brain barrier permeable inhibitor of NOPR. Rat pups that were administered this inhibitor from postnatal day 9 indeed showed accelerated brain myelination. Comparison of vehicle-controls and BAN-ORL-treated pups, indicated that at 14 days of age, animals subjected to inhibition of nociceptin signaling exhibited a significant increase in the brain levels of the four major splicing isoforms of myelin basic protein (MBP), myelin proteolipid protein (PLP) and myelin-oligodendrocyte glycoprotein (MOG). This accelerated expression of myelin-specific proteins was also reflected in an increased number of myelinated axons. These observations support the notion that nociceptin secretion by neurons and/or astrocytes during early development may play an important role preventing precocious myelination, a situation that would interfere with axonal outgrowth and connectivity. Importantly, nociceptin levels decrease along brain maturation but elevated concentrations of this peptide are produced by astrocyte exposure to pro-inflammatory cytokines involved in the pathogenesis of multiple sclerosis (MS). Thus, studies are in progress to investigate if increased nociceptin levels within the inflammatory environment of MS may preclude remyelination of damaged tissues in this disease. (Supported by CCTR UL1TR00058 and NMSS grant RG-1501-02891)

PTW01-10

ION CHANNEL FUNCTION CONTROLS BMP/DPP SIGNALING IN DROSOPHILA WING DEVELOPMENT

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Loss of embryonic ion channel function can cause craniofacial and limb abnormalities in mammals, but the underlying reason for these defects has remained elusive. Here, we show that depolarization of epithelial cells governs a novel mechanism of morphogenesis. We show that in *Drosophila,* epithelial cells of the developing wing disc undergo regular bursts of electrical activity, and spontaneous calcium transients. We find that specific inhibition of native inwardly rectifying K^+ (Irk) channels depolarizes cells, changes the period of electrical bursts, and silences calcium activity. Inhibition of Irk channels hinders proper Bone Morphogenetic Protein/Decapentaplegic (BMP/Dpp) signaling to cause wing defects that can be rescued by expression of an orthologous human channel (Kir2.1). Ultra-structural analysis reveals that BMP/Dpp resides in vesicle-like structures within the wing disc. We find that Dpp is released in pulses. We find that inhibition of Irk channels alters the dynamics of pulsatile BMP/Dpp release and leads to a broader distribution of extracellular BMP/Dpp. Our results suggest that Irk channels are required for regulated pulsatile release of BMP/Dpp in the developing fly wing. Thus we propose that the temporal pattern of BMP/Dpp presentation is important for morphogenesis of the fly wing.

PTW01-11

ENDOCANNABINOIDS AND PHYTOCANNABINOIDS EFFECTS IN CHILEAN RESEARCH

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The state of art in Chile respect the use of phytocannabinoids is changing today. The government moved the Cannabis form List 2 to List 1, a list composed by compounds with lower toxicity and medical properties. We are working with childs suffering different kinds of convulsive disorders. The reason for the effects of phytocannabinoids present in Cannabis strains and endocannabinoids of our body is the presence of membrane proteins as the GPCRs CB1R and CB2R and ionotropic receptors as $GABA_A$ receptors or TRPV1, all these proteins widely present at SNC.Here we present evidence that shows that phytocannabinoids are effective to decrease the total number of convulsives crisis. We evaluate between 15-20 chileans childs (8-15 years) and found that some convulsive disorders with wide range of seizures respond in a different way to Cannabis strains with different content of THC and CBD.
In other hand, we are starting to study a model of food intake in

mice that consist in to inject molecules into third ventricle (i.e glucose, leptin and insulin) to modulate synaptic function of hypothalamic neurons producing changes in the animal feeding behavior. Are preliminary data shows that proteins associated with endocannabinoids system signalling are present in different brain regions involved in important cognitive processes (i.e. food intake, decision making, etc).

The endocannabinoid signaling is probably one of the most important signaling system in mammals. The use as pharmaceutical target is widely relevant with a profound impact at the present in the chilean scientific and biomedical research

PTW02 Drugs of Abuse: Alcohol, Cocaine, Methamphetamine

PTW02-01

EFFECTS OF METHYLMERCURY AND ALCOHOL IN DROSOPHILA: IMPLICATIONS IN NEURODEVELOPMENTAL DISORDERS

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Extensive evidence suggests the role of oxidative stress in autism and many other neurodevelopmental disorders. In this study, we investigated whether methylmercury (MeHg) and/or alcohol exposure have deleterious effects in Drosophila melanogaster (fruit fly). A diet containing different concentrations of MeHg in Drosophila induced the free radicals generation in a dose-dependent manner. This effect of MeHg on oxidative stress was further enhanced by the exposure to alcohol. Alcohol alone could also induce free radical generation in flies. After alcohol exposure, MeHg did not affect the immobilization of flies, but it increased the recovery time in a concentration-dependent manner. MeHg also inhibited the activity of alcohol dehydrogenase (ADH) in a dose-dependent manner. Linear regression analysis showed a significant negative correlation between the ADH activity and the recovery time upon alcohol exposure in the flies fed a diet supplemented with MeHg. This relationship between ADH activity and recovery time after alcohol exposure was confirmed by adding 4-methyl pyrazole (an inhibitor of ADH) to the diet for the flies. These results suggest that alcohol consumption in the mothers exposed to MeHg may increase oxidative stress and the time of alcohol clearance by inhibiting ADH activity, which may have direct impact on the neurodevelopment of the fetus, thus increasing the risk of neurodevelopmental disorders.

PTW03 Neuroinflammation

PTW03-01

ALTERED MICROBIOTA COMPOSITION MEDIATES DEPRESSIVE BEHAVIOR IN CHRONIC STRESS

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Family history of depression is one of the main risk factors for developing the disorder. While genetic inheritance may play a role in the etiology of depression, family history may also be viewed in the light of a shared commensal microbiota. Intestinal microbiota dysregulation can cause metabolic dysfunction, leading to altered development and function of other systems in the body.
Therefore, we have investigated the interplay between the nervous system and the gut microbiota in an unpredictable chronic mild stress (UCMS) model for depression. We observed significant alterations in the composition of the intestinal microbiota following UCMS, as well as immune and metabolic disruptions. Restoring the microbiota composition resulted in alleviation of the depression phenotype, as well as of the immune and metabolic dysregulation. Moreover, disturbing the integrity of the gut microbiota directly also increased susceptibility for developing a depression phenotype. Our data indicate that the integrity of the microbiota composition can mediate resiliency behavior. By altering metabolite balance, microbiota dysbioses can shift brain chemistry and contribute to disease outcomes.

PTW03-02

EXACERBATION OF INFLAMMATION, NEURODEGENERATION, AND DELAYED COGNITIVE IMPAIRMENT IN AN AGED MOUSE MODEL OF STROKE

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Up to 30% of stroke patients develop cognitive decline in the first year after stroke, and the etiology of this sub-category of vascular dementia is unknown. We recently developed a model of delayed cognitive dysfunction following stroke (DH stroke), and discovered that chronic inflammation at the site of stroke lesions could be an underlying cause. Our published research was performed in young adult animals, yet stroke is predominantly a disease of the elderly. Little is known about how the inflammatory response to stroke changes with age, despite aging being associated with alterations in the regulation and function of the immune system. Therefore, the goal of the study was to test aged animals to determine if they also experience delayed cognitive decline following our stroke model, if it is more rapid and severe, and if mice have a more pronounced chronic inflammatory response in the brain and systemic circulation. We compared the chronic inflammatory response and development of delayed cognitive deficits using 3-month old and 18-month old stroked mice. With immunostaining, multiplex immunoassays, and behavioral tests, we discovered that 18-month old mice have less glial scarring but more inflammation in the brain at chronic time-points following stroke. Specifically, we discovered that there is decreased GFAP+ immunostaining in the area of the glial scar in 18-month old mice, increased CD3e+ T cell infiltration into the brain, and increased pro-inflammatory cytokine expression in the stroke lesion at 7 weeks post-stroke. This correlates with more brain atrophy, delayed motor recovery, and an accelerated onset of delayed memory dysfunction in 18-month old compared to 3-month old stroked mice. Thus, we posit that poorer compartmentalization of stroke lesions from uninfarcted tissue and more pronounced chronic inflammation following stroke are sequela of age that are promising targets for developing stroke treatments specifically tailored to elderly patients.

PTW03-03

CHARACTERIZATION OF THE INFLAMMATORY AND PATHOLOGICAL PROFILE OF ISCHEMIC INFARCTS IN HUMANS WITH POST-STROKE DEMENTIA

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Each year, ten million people worldwide survive the neurological injury associated with stroke. Importantly, of the 75% of people who survive a stroke, up to 30% develop a progressive decline in memory and cognitive function within the first year. Afflicted patients are grouped with other patients suffering from cognitive dysfunction relating to a vascular mechanism, and are labeled as suffering from vascular dementia. Vascular dementia is the second most common form of dementia in the U.S., and the pathogenesis of delayed dementia after stroke, which affects over 1 million Americans, is unknown. However, there is a clear association between blood-brain-barrier dysfunction and increased inflammation in the blood of patients who develop dementia after stroke, and it has long been suspected that a cause may be the development of a chronic and injurious inflammatory response to necrotic brain tissue. Therefore, the goals of this study are to: (i) advance our understanding of inflammation and pathological markers related to dementia in the human brain after stroke, (ii) investigate the extent of variability between individuals, (iii) determine if some individuals develop an autoimmune response to stroke, and (iv) ascertain whether the astroglial scar that forms in the brain after stroke interferes with the influx and efflux of proteins associated with neurodegeneration. Using immunohistochemistry, scanning electron microscopy, and multiplex immunoassay techniques, we

discovered that for months, and possibly years after stroke, human stroke lesions contain abundant T- and B-lymphocytes, pro-inflammatory cytokines, autoantibodies, and toxic amyloid-beta and tau peptides. We posit that the prolonged presence of these factors associated with neurodegeneration in the stroke lesion, and their leakage into neighboring tissue, is a cause of dementia in some humans diagnosed with stroke related dementia.

PTW03-04

CANNABIDIOL DECREASES SEIZURES IN CNS INFECTION-INDUCED MODEL OF LIMBIC EPILEPSY

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CNS infection can cause epilepsy often refractory to established antiseizure drugs. C57BL/6J mice intracortically infected with Theiler's murine encephalomyelitis virus (TMEV) show acute behavioral seizures between 3-7 days post-infection (dpi), exhibit clinically relevant pathological and physiological changes in the hippocampus, survive the infection, and later develop epilepsy. Therefore, it is an important model to study mechanism(s) underlying epileptogenesis and to identify novel therapeutics. Inflammation, mediated mainly by the cytokines such as TNFα and IL-6, plays a crucial role in neuronal damage and seizure generation in this model. Cannabidiol (CBD) imparts antiseizure effects in several animal models of epilepsy by altering neuronal excitability and inhibiting inflammatory cytokines at doses devoid of adverse motor or psychoactive effects. Therefore, we tested the hypothesis that CBD would be effective against TMEV-induced acute seizures under both prophylactic and therapeutic regimens. One group of mice received 180 mg/kg CBD intraperitoneally every 12 hours until 7 dpi starting at 2 days pre-infection in prophylactic regimen and at 3 days post-infection in therapeutic regimen and the other group received vehicle. Mice were monitored for handling-induced seizures twice daily between 2-8 dpi using treatment-blinded protocol. Seizure intensity was recorded according to modified Racine seizure scale. All mice experienced seizures in the TMEV-vehicle group, whereas 70-80% mice had seizures in the TMEV-CBD group under both regimens. The seizure frequency, intensity and duration were dramatically reduced in the TMEV-CBD group. CBD treatment delayed the development of seizures in the prophylactic regimen. The seizure parameters were similar between the groups at 3 dpi but significantly reduced during 4-8 dpi in the TMEV-CBD group under therapeutic regimen indicating that CBD could be an effective treatment for inflammation-induced seizure activity. Future studies will evaluate safety, efficacy, and mechanisms of actions of CBD in TMEV model.

Funding: Margolis Foundation (KSW, JAW), Epilepsy Foundation TRICE award (MDS), American Epilepsy Society (DCP)

PTW03-05

USING TOXOPLASMA GONDII, A NEUROTROPIC PARASITE, TO UNDERSTAND AMYLOIDOSIS

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Toxoplasma gondii is a eukaryotic parasite that naturally infects most warm-blooded animals, including humans and rodents. In mice and humans, the CNS is the major organ of asymptomatic persistence, suggesting that *Toxoplasma* and the mammalian CNS have co-evolved to tolerate each other. Two studies suggest that this co-evolution may be beneficial. Chronic toxoplasmosis was found to be protective in a stroke model (smaller strokes) and in hAPP mice (decreased beta amyloid (Aβ) deposition and improved cognition). In the hAPP study, *Toxoplasma*-associated neuroprotection was thought to be secondary to increased CNS levels of the anti-inflammatory cytokines TGFβ and IL-10 in infected mice. To test this hypothesis and develop a better mechanistic understanding of *Toxoplasma*-associated neuroprotection, we infected J20 hAPP mice with one of three canonical strains of *Toxoplasma* (type I, type II, or type III) and evaluated the CNS of these mice and uninfected hAPP mice at 6 months post infection (9 months of age) for Aβ deposition, immune cell infiltration, and global cytokine environment. We found that only infection with the type II *Toxoplasma* strain was protective against Aβ deposition (<20% of controls), despite both type II and type III strains establishing a chronic CNS infection. In addition, compared to control mice, both type II and type III showed increased T-cell infiltration and elevated CNS pro-inflammatory cytokines, while neither group showed a > 2-fold elevation of TGFβ or IL-10 compared to uninfected hAPP mice. Type II-infected mice had a 100-fold higher parasite burden and less microglial/macrophage activation than type III-infected mice, suggesting that parasite burden and immune cell polarization maybe factors in driving the protective effect of type II infection. We are currently leveraging these *Toxoplasma* strain-specific differences to identify host pathways and immune cell changes specifically associated with protection against Aβ deposition. Understanding the mechanistic basis for the type II protection against Aβ deposition may offer novel therapeutic targets for treating amyloid-associated neurodegenerative diseases.

PTW03-06

QUANTIFYING CELLS IN CA1 OF THE HIPPOCAMPUS DURING AUTOIMMUNE EPILEPTOGENESIS

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Epilepsy affects 1% of the world population. Epilepsy is characterized by the uncontrolled over-excitation of neuronal populations in the brain. Therefore therapies target neurons. However, 30% of patients do not respond to these treatments and among those treated, many suffer from unwanted side effects. Since astrocytes assist in maintaining homeostatic neuronal function through regulation of ion concentrations and reuptake of neurotransmitters, such as glutamate, research has begun to address their involvement

in epilepsy. Because brain inflammation is one of the greatest risk factors for the development of epilepsy and T cells are found within epileptic tissue, we developed an autoimmune mouse model of progressive epilepsy in which astrocytes are the targets of T cell mediated autoimmunity. Glial fibrillary acidic protein (GFAP) is expressed by astrocytes and increases in GFAP are the stereotypic marker changes in astrocyte function that occurs during the transition from normal brain homeostasis to astrocyte reactions to injury and disease. Diseased brains displayed heterogeneous GFAP immunoreactivity throughout, including in the neocortex and hippocampus, which are the regions that increase in neuronal activity during tonic clonic seizures. To address whether areas of high GFAP immunoreactivity are sites of cell loss, immunohistochemistry and blinded cell counts were performed using hematoxylin staining. No significant differences in cell number at CA1 region of the hippocampus between naïve mice brains and mice brains collected 14 days after disease induction were observed. The heterogeneity is sustained over several time points without cell drop out; therefore, it suggests loss of homeostatic functions without excitotoxic cell death, potentially resulting in aberrant neuronal activity that is the basis of the seizures observed in these animals.

PTW03-07

THE ROLE OF MICROGLIA DURING INFECTION OF THE CENTRAL NERVOUS SYSTEM WITH TOXOPLASMA GONDII

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Microglia are the resident immune cells of the brain. Despite their location in the brain parenchyma and expression of machinery capable of responding to pathogens, little is known about the role microglia play during infections that target the central nervous system (CNS). *Toxoplasma gondii* is an intracellular protozoan parasite that establishes a chronic infection in the brain, persisting in a cyst form for the lifetime of infected host. Deciphering the role of microglia in this infection has been confounded by the inability to separate microglia from monocyte-derived macrophages, which are phenotypically and morphologically similar to one another in the chronically inflamed CNS. Therefore, to explore how microglia respond to *T. gondii in vivo* we developed a system to specifically label microglia. Mice expressing an inducible cre recombinase under the CX3CR1 promoter were crossed to a fluorescent cre-reporter mouse line. Treatment with a single dose of Tamoxifen at weaning labels 99% of microglia, allowing them to be distinguished from infiltrating myeloid cells. Using this system, changes in microglia morphology have been assessed using confocal microscopy and changes in the expression of immune molecules has been monitored by flow cytometry. Preliminary results indicate that microglia increase expression of MHC class II and CD11c. Additionally, microglia upregulate CD45 during infection, an important finding given that microglia and monocyte-derived macrophages have traditionally been defined based on CD45 expression. Together, this system will allow the role of microglia in response to CNS infections to be addressed.

PTW03-08

TARGETING OF SOLUBLE AMYLOID-β PROTOFIBRILS IN ALZHEIMER'S DISEASE-LINKED INFLAMMATION

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Inflammation is a significant pathological component of the Alzheimer's disease (AD) brain. Many findings suggest that accumulation of amyloid-β peptide (Aβ) provokes a glial-mediated inflammatory response, which likely contributes to a deleterious environment for neurons. While monomeric Aβ appears to be benign, oligomers adopt a biologically detrimental structure. This structure can be found in a variety of soluble and insoluble Aβ oligomeric species. Our own *in vitro* studies demonstrate that microglial cells are much more sensitive to soluble Aβ42 protofibrils compared to Aβ42 monomer or insoluble Aβ42 fibrils. These small (<100 nm) curvilinear structures interact with microglia, trigger Toll-like receptor signaling, elicit cytokine transcription and expression, and are rapidly taken up by the cells. Size determination of isolated Aβ42 protofibrils revealed a molecular weight range of roughly 200-2500 kD (45-550 monomers) and a mean hydrodynamic radius of 21 nm indicating these soluble Aβ oligomeric structures are diffusible yet quite large compared to typical proteins. Due to the importance of this Aβ species, we sought to develop an antibody that selectively recognizes protofibrils over other Aβ species. Protofibrils were prepared, isolated, and characterized prior to rabbit immunization of two rabbits. Testing of the collected serum indicated a strong affinity for Aβ protofibrils. Using an indirect ELISA, antibody titers were found to be high initially in rabbit #1 (25,000 dilution) and remained so through multiple immunizations and bleeds. Serum from rabbit #2 initially displayed less affinity, but achieved significant titers over the course of the treatment. The anti-serum was highly selective for Aβ protofibrils over Aβ monomers and also displayed notable selectively over Aβ fibrils. Selectivity was confirmed using both ELISA and dot blot methodologies. The newly-developed antibody may have potential diagnostic and therapeutic uses in AD tissue and patients. The cumulative data indicate that soluble Aβ42 oligomers such as protofibrils serve as robust microglia inflammatory stimuli. Therefore targeting of this biologically active Aβ species in AD may have beneficial effects.

PTW03-09

TYPE I IFN SIGNALING IN ASTROCYTES IS CRITICAL FOR PROTECTION AGAINST VIRUS-INDUCED ENCEPHALOMYELITIS

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IFNα/β signaling is critical to limit virus dissemination throughout the central nervous system (CNS) following neurotropic coronavirus infection. However, the specific role of IFNα/β signaling by distinct CNS resident cells in stemming viral spread is unknown. To better understand the contribution of CNS cell types to the early innate anti-viral response, we evaluated immune

responses and pathology in mice deficient in IFNα/β receptor (IFNAR) specifically in astrocytes (mGFAPcre$^{+/-}$IFNAR$^{fl/fl}$). WT and control IFNAR$^{fl/fl}$ mice infected with the neurotropic coronavirus MHV-A59, which infects glia and neurons, exhibited mild encephalomyelitis and no mortality. By contrast, mGFAPcre$^{+/-}$ IFNAR$^{fl/fl}$ mice developed severe encephalomyelitis, hinb limb paralysis and succumbed between 6 - 7 days postinfection (p.i). High mortality coincided with higher viral replication and uncontrolled viral spread throughout the CNS parenchyma. Immunohistochemistry revealed not only enhanced numbers of infected astrocytes but also neurons within the brain. In astrocytes viral antigen localized to both the cytoplasm as well as along astrocytic protrusions. In spinal cords viral Ag was detected mostly in gray matter at day 6 p.i. Consistent with higher viral load type I IFN mRNAs were significantly increased compared to WT mice, suggesting overall IFNα/β production was intact. Similarly, IFNg mRNA and protein levels within the CNS were not affected, consistent with unaltered T cell infiltration and comparable IFNg production by virus specific CD8 T cells. Surprisingly however, induction of IFNg dependent MHC class II expression on microglia was significantly impaired, suggesting defective IFNg signaling. These results imply an unexpected link between IFNα/β signaling in astrocytes and responsiveness of microglia and potentially other cells to IFNg. Overall the data demonstrate that IFNα/β signaling in astrocytes is not only critical in limiting both early CNS viral spread and tropism, but also promotes lymphocyte derived protective antiviral IFNg responses.

PTW03-10

FRACTALKINE SIGNALING DURING SYSTEMIC ENDOTOXEMIA INHIBITS PERIVASCULAR MICROGLIAL LESION FORMATION IN THE DIABETIC RETINA

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Diabetic retinopathy (DR) is the result of neuronal and vascular damage that leads to vision loss, however the mechanisms that drive DR pathogenesis remain largely unclear. Although infections are highly prevalent in diabetics and there is a strong association between diabetes and chronic inflammation, whether systemic inflammation contributes to retinopathy remains unknown. Moreover, microglial-mediated inflammation has gained recognition as a key contributor to retinal pathology. We have shown that signaling between the neuron-derived chemokine fractalkine (FKN) and CX3CR1 on microglia is important to maintain neuroprotection and that absence of CX3CR1 signaling directs microglial-mediated inflammation and neurotoxicity in the diabetic mouse retina. To extend these studies, we are testing the hypothesis that acute endotoxemia perpetuates microglial activation in the retina, and that CX3CR1-deficent mice will be more susceptible to neuronal and vascular damage in the diabetic host due to dysregulated microglial responses mediated by a proinflammatory phenotype. Systemic endotoxemia was induced in nondiabetic and diabetic CX3CR1-HET and CX3CR1-KO mice by administration of four-daily injections (i.p.) of lipopolysaccharide (1 mg/kg/mouse; *n*=12). Confocal analysis of retinal tissue revealed that in CX3CR1-KO mice, systemic endotoxemia induced a robust-cellular activation represented by morphological changes, IL-1β, iNOS, CD68 expression, and intense microglial clusters near blood vessels. This phenotype was significantly exacerbated in the diabetic CX3CR1-KO retina. These microglial lesions observed in *Cx3cr1*$^{-/-}$ mice coincided with perivascular accumulation of the blood-protein fibrinogen. Lastly, fractalkine treatment (intraocular; 30 ng) into FKN-KO mice mitigated microglial activation, astrogliosis, and ensuing vascular pathology (*n*=5) during acute endotoxemia. These data suggest that systemic inflammation influences microglial activation and breakdown of the blood-retinal barrier, and may provide therapeutic advances by fractalkine treatment to subdue microglial activation and fibrinogen extravasation in DR.

PTW03-11

COMPROMISED AXON INITIAL SEGMENT INTEGRITY AS A CONSEQUENCE OF MICROGLIAL REACTIVITY IN EAE

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Axonal pathology is a key contributor to long-term disability in multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), but the mechanisms that underlie axonal pathology have not been fully clarified. Evidence suggests that axonal pathology can be a direct consequence of demyelination, as we and others have shown that the node of Ranvier disassembles following local loss of myelin. In contrast to the node of Ranvier, we now show that the axon initial segment (AIS) remains intact following cuprizone-induced cortical demyelination. The AIS is the region of the axon, adjacent to the soma, spanning about 20 µm in length. Due to its high density of voltage-gated ion channels, it is the site of action potential initiation and modulation. Maintaining AIS integrity is, therefore, vital to proper neuronal function. Although AIS disruption is independent of demyelination, it is preceded by and associated with cortical microglial reactivity in EAE. Moreover, we have observed an upregulation of neuronal m-calpain, a calcium activated cysteine protease whose substrates include critical AIS proteins. Interestingly, in calpain positive neurons, clustered AIS proteins are not observed, while calpain negative neurons reveal robust AIS protein labeling. Additionally, preliminary data both *in vivo* and *in vitro* suggest that this calpain activity is induced through the increased production of reactive oxygen and nitrogen species by microglia in EAE and MS.

PTW03-12

CD19 DEFICIENCY IMPAIRS HUMORAL IMMUNITY FOLLOWING CORONAVIRUS ENCEPHALOMYELITIS

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The signaling co-receptor CD19 of the B-cell receptor complex drives B-cell differentiation into memory B-cells and antibody (Ab) secreting cells (ASC) by lowering the threshold of antigen-driven activation. CD19$^{-/-}$ mice exhibit diminished germinal center (GC)

formation and, consequently, high-affinity Ab responses to T-cell dependent antigens. However, these effects are dependent on antigen dose and replicative capacity in the case of viral infections. Local Ab production in the central nervous system (CNS) is crucial for control of several neurotropic viruses, including the demyelinating, persisting neurotropic coronavirus designated JHMV. Following JHMV infection, both T-cell and humoral responses are initiated in cervical lymph nodes (CLN) despite minimal replication at this site. To determine how CD19-dependent GC formation affects generation of long-lived protective ASC in the CNS, humoral responses and viral control were monitored in infected $CD19^{-/-}$ mice. Despite impaired CLN GC reactions and early ASC expansion, peak virus-specific serum Ab was only modestly reduced compared to wild type controls. However, CD19 deficiency significantly reduced the longevity of virus-specific serum Ab, diminished CNS accumulation of ASC, and decreased virus-specific Ab in the CNS, resulting in increased levels of infectious virus. The data are consistent with the notion that GC formation imprints virus specific ASC to traffic to the inflamed CNS in a CXCR3-dependent manner. Overall, these results demonstrate that CD19-dependent peripheral GC formation is critical for sustained protective Ab production in the periphery and essential for protective humoral immunity within the CNS following JHMV-induced encephalomyelitis.

PTW03-13

ENDOMT IN THE CNS VASCULATURE DURING REOVIRUS-INDUCED ENCEPHALITIS

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Blood vessels within the brain provide oxygen, nutrients and establish the blood brain barrier (BBB). The BBB maintains an ideal environment for neurons by limiting the passage of molecules or pathogens into the neural tissue. The BBB is disrupted in many CNS diseases. BBB breakdown exacerbates neuronal damage therefore identifying mechanisms underlying BBB breakdown in disease pathology is an important area of research. Encephalitis is characterized by inflammatory cell infiltration, neuronal death and BBB breakdown however very few studies have focused on blood vessel pathology. Reovirus-induced encephalitis is a well-established mouse model of encephalitis and we have discovered blood vessel integrity is severely affected 8 days post infection (dpi). Endothelial cells are not infected by Reovirus indicating that signals from the inflamed neural environment causes the vascular damage. To determine how the blood vessels become disrupted we first looked at the Wnt signaling pathway which is essential for establishing and maintaining the BBB. We found, however, that Wnt signaling and Wnt driven BBB targets were not significantly altered in Reovirus-induced encephalitis. Surprisingly we found significant up-regulation of known TGFb/BMP driven endothelial to mesenchymal transitional (EndoMT) transcripts, S100A4, KLF4 and Id1, in whole brains at 8dpi. Additionally we found by immunohistochemistry that some of these mesenchymal proteins, such as S100A4, KLF4 and a-smooth muscle actin are up-regulated within the endothelial cells and this correlates with the presence of downstream TGFb/BMP effectors, such as pSMAD1/5. Together our evidence suggests that the CNS blood vessels undergo EndoMT during Reovirus-induced encephalitis resulting in a disruption of the BBB and possibly mediated by TGFb/BMP signaling. Although further investigation is needed, several studies suggest that the TGFb/BMP signaling pathway intersects with the Wnt signaling pathway to promote a mesenchymal transition within many cell types.

PTW03-14

PROTEOLIPID PROTEIN REGULATES MICROGLIA ACTIVATION AND INFLAMMATION

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Mutations in the proteolipid protein gene (PLP1/Plp1) are clinically classified as Pelizaeus-Merzbacher Disease (PMD). Behavioral deficits and shortened lifespan in PMD patients have been attributed to proteolipid protein's (PLP) localization in CNS myelin. However, a host of 2° abnormalities include abnormal oxidative phosphorylation, a reactive microglial response, pro-inflammatory cytokine/chemokine up-regulation and neuronal death, all of which may contribute to host death. The mechanisms that lead to these 2° events are unclear. We show that PLP is inserted into mitochondria via the Mia40/Tom40-Erv1 pathway not only when the Plp1 gene is duplicated, but also in diseased states of normal mammals. Mitochondrial function and inflammation are interdependent and may occur under various stress conditions. We hypothesize that PLP insertion into mitochondria mediates inflammation. Western blotting and immunohistochemistry have been performed on brain sections from PLP over-expressing, PLP null, and neonatal hypoxia mice compared to control mice. Preliminary data indicates that these conditions lead to an increased microglia cell activity, as well as differential regulation of specific chemokines and cytokines including TNFα and IL-6. In both blastocyst cells and in hypoxic neonatal mouse brains we see an hypoxia induced increase in PLP expression. Concomitant with increased PLP expression is an increase in inflammation. The PLP promoter contains several oxygen responsive elements (ORE's) and an HIF-1 element which is activated in hypoxia conditions. This could partially explain the secondary inflammation events that occur during over-expression of PLP and create a link between PLP's insertion into mitochondria and the role it plays there.

PTW03-15

IFN-γ SIGNALING ON ASTROCYTES REGULATES TNF MEDIATED DISABILITY DURING PROGRESSIVE AUTOIMMUNE ENCEPHALOMYELITIS

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Tumor necrosis factor (TNF) is linked to the pathobiology of several neurodegenerative disorders including MS. Anti-TNF therapies have been successful in treating several autoimmune diseases including rheumatoid arthritis, Crohn's disease, and psoriatic arthritis. However, therapies for progressive forms of multiple sclerosis (MS) are limited. Induction of EAE in mice impaired in IFN-γ signaling in astrocytes results in persistent CNS inflammation as well as sustained demyelination and TNF, resembling features of progressive MS. Anti TNF therapy via neutralization of both soluble and membrane bound TNF during progressive EAE in these mice ameliorated disease progression and decreased demyelination. TNF blockade further reduced the overall infiltration of immune cells into the CNS consistent with restoration of the blood brain barrier integrity. Moreover, a decrease in both Th1 and Th17 cells in the CNS correlated with reduced macrophage and microglial activation. Conversely, anti-TNF therapy promoted anti-inflammatory responses within the CNS exemplified by both increased IL-10 and IL-27. Overall, these data implicate TNF is an important mediator of the disability, inflammation, and pathology during progressive EAE and support TNF blockade as a potential treatment to mitigate progressive forms of MS.

PTW03-16

PERIPHERALLY DERIVED B CELLS ARE SUSTAINED WITHIN THE INFECTED CNS INDEPENDENT OF ECTOPIC FOLLICLES

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Central nervous system (CNS) inflammation triggered by infection and autoimmune responses results in the accumulation of various B cell subsets, including naïve, activated, memory B cells (Bmem), and antibody secreting cells (ASC). While ASC are well studied, signals driving recruitment of other B cells, their relationship to peripheral activation, and role within the CNS remain largely unknown. Encephalomyelitis induced by gliatropic coronavirus resolves into a persistent infection associated with chronic demyelination and is characterized by changes in B cell subsets throughout infection. Early accumulating B cells are largely undifferentiated, but more differentiated, isotype switched Bmem and ASC progressively prevail. While phenotypically naïve IgD+IgM+ B cells decline over time, IgDintIgM+ and IgD-IgM+ B cells displaying activation and proliferation markers remain stable. Immunohistochemistry revealed unswitched B cells predominately localize to the meninges and perivascular space with little evidence for ectopic follicles, although occasional interactions between B and T cells were evident. Mice lacking CXCL13, a chemokine promoting germinal centers in lymphoid tissues and upregulated during CNS inflammation, showed no alterations in activated B cell accumulation, suggesting CNS B cell maintenance during infection is independent of ectopic follicles. Nevertheless, CD4 T cell depletion at days 5 and 7 post infection decreased B cell numbers and reduced activation within the CNS, although peripheral activation was initially unaffected. Overall, the results suggest the kinetics of B cell differentiation in the CNS reflects peripheral activation, with CD4 help potentially promoting local B cell differentiation within the CNS independent of ectopic follicle formation.

PTW03-17

MICRORNA-155 ENHANCES T CELL TRAFFICKING AND ANTIVIRAL EFFECTOR FUNCTION IN A MODEL OF CORONAVIRUS-INDUCED NEUROLOGIC DISEASE

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Micro-RNAs (miRNAs) are non-coding RNAs that modulate cellular gene expression at the post-transcriptional level. These molecules are becoming increasingly recognized as important in helping tailor T-cell responses following viral infection. We therefore sought to examine the functional role of miR-155 in a model of viral-induced demyelination. Intracranial injection with the neurotropic JHM strain of mouse hepatitis virus (JHMV) results in an acute encephalomyelitis followed by an immune-mediated demyelinating disease. Compared to wild-type mice, JHMV-infected mice deficient in miR-155 (*miR-155*$^{-/-}$) developed delayed but exacerbated disease concomitant with increased morbidity/mortality, weight loss, and spinal cord demyelination. In addition, *miR-155*$^{-/-}$ mice had decreased total and virus-specific CD4^{+} and CD8^{+} T-cell accumulation within the CNS during the acute phase of disease. Furthermore, IFN-γ and TNF-α production, as well as and cytolytic function, were impaired in CD8^{+} cells from *miR-155*$^{-/-}$ mice. These results identify miR-155 as a key mediator of disease development and recovery in a model of virus-induced neurological disease.

PTW04 Neuron-Glial Interactions 1: Metabolism, Signal Transduction and Axon Biology

PTW04-01

GLUCOSE TRANSPORTER 2 INHIBITION IN TANYCYTES AFFECTS FEEDING BEHAVIOR

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Glucose is a key modulator of feeding behavior both at peripheral tissues and the central nervous system, where it is known to modify neuropeptide expression involved in hunger/satiety signals. In the brain, glucosensing occurs in the hypothalamus and relies on the presence of glucose transporter 2 (GLUT2), whose expression has been detected on glial cells lining the third ventricle, known as tanycytes. These cells are in contact with cerebrospinal fluid and in close proximity with arcuate nucleus neurons that regulates food intake. This study aims to clarify the role of tanycytes in feeding behavior using injections of adenovirus encoding a shRNA against GLUT2 (Ad-shGLUT2) into the third ventricle of rats. This method allows specific transduction of tanycytes and GLUT2 inhibition. Through real time PCR and Western blot we determined a decrease in GLUT2 expression levels on both tanycytes cultures and animals treated with Ad-shGLUT2, compared with control groups treated with adenovirus encoding a shRNA for β-galactosidase (Ad-shβgal). Neuropeptide expression in response to intracerebroventricular (icv) glucose after adenovirus injection was measured using real time PCR, observing a loss of response. Feeding behavior in GLUT2 knock-down rats was evaluated in fast-refed cycles (24 h/24 h) as two separated aspects: (i) amount of food intake and changes on body weight, and (ii) frequency of feeding events. Animals receiving Ad-shGLUT2 icv injections showed an increase on both food intake and body weight, compared with the control group (Ad-shβgal). On the other hand, feeding frequency significantly increased on the dark phase of the first cycle, showing no differences on the second cycle. In summary, our results show GLUT2 inhibition on tanycytes produces a disruption of the hypothalamic glucosensing mechanism that alters feeding behavior.

PTW04-02

DOUBLE KNOCK-DOWN OF MONOCARBOXYLATE TRANSPORTERS MCT1 AND MCT4 IN TANYCYTES BY A SH-RNA ALTERS THE BRAIN GLUCOSENSING MECHANISM

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Tanycytes are glial hypothalamic cells lining the third ventricle that contact the cerebrospinal fluid and are also in close proximity with arcuate nucleus (AN) neurons that regulates food intake. These cells expresses glucose transporter 2 (GLUT2) and monocarboxylate transporters 1 and 4 (MCT1 and 4), not detected in hypothalamic neurons, which in addition to their morphological features allows their involvement on brain glucose sensing mechanism, a process based on glial-neuronal interactions. This study aims to provide new evidence on tanycytes contribution to glucosensing, proposing lactate as a key mediator of feeding behavior response. We use injections of two adenovirus encoding a shRNA against MCT1 (AdshMCT1) and MCT4 (AdshMCT4) or a shRNA against *E. coli* ß-galactosidase (control) into the third ventricle of rats, allowing specific transduction of tanycytes. We evaluated their effect on neuropeptides expression and feeding behavior. Through real time PCR, Western blot and ^{14}C-lactate uptake and efflux by HPLC we demonstrated loss of function for MCT1 and MCT4 in tanycytes cultures and MCT double knock-down animals. After adenovirus injection, neuropeptide expression were measured in the AN in response to intracerebroventricular (ICV) glucose using real time PCR, finding a loss of response, however, normal glucose circulating levels were detected. Feeding behavior in MCT double knock-down rats was evaluated in fast-refed cycles (24 h/24 h), showing an increase on food intake but not body weight compared with the control group (Ad-shβgal). On the other hand, a significantly higher feeding frequency on both cycles was detected. In summary, our results show MCTs inhibition on tanycytes alters neuropeptide mRNA levels involved in feeding behavior, disrupting hypothalamic glucosensing mechanism. This supports the notion that tanycytes are metabolically coupled with neurons from the arcuate nucleus and the involvement of lactate on glucosensing and feeding behavior.

PTW04-03

ACETATE METABOLISM DOES NOT REFLECT ASTROCYTIC ACTIVITY AND IS INCREASED BY SILENT INFORMATION REGULATOR 1 ACTIVATION

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Acetate metabolism in the brain has been thought to be confined largely to astrocytes due to limited neuronal uptake of acetate. Recently, we showed that the neuronal monocarboxylate transporter was capable of taking up acetate at levels sufficient to support metabolism and suggested that the limiting step for neuronal acetate metabolism was the down regulation of acetylCoA synthetase by post-translational acetylation. Here, we studied the cortical brain tissue slice under a range of conditions using [1,2-^{13}C]acetate and [1-^{13}C]glucose to better understand the regulation of acetate metabolism. Using a range of [1,2-^{13}C]acetate concentrations we found acetate incorporation was not proportional to concentration, and was suggestive of at least two compartments, with the most efficient use of acetate occurring at relatively low (0.4 mM) concentrations. Activating astrocytes by depolarization resulted in gagging of acetate metabolism at citrate, with increased incorporation into Cit C1,2 but greatly decreased use of [1,2-^{13}C]acetate to make Gln C4,5

and GABA C1,2. Instead, use of [1-^{13}C]glucose was significantly favored. Incubation of slices with the SIRT1 activator SRT1720 did increase the amount of [1,2-^{13}C]acetate incorporated into Cit C1,2, Glu C4,5 and Gln C4,5 but not GABA C1,2. In conclusion, the data suggest that measurement of acetate utilization is not a reliable marker of astrocytic metabolism both because it does not accurately reflect astrocytic activity and because it samples only a subset of the astrocytic pool. Acetate is metabolized to a degree in neurons but this metabolism is subject to post-translational modification control at the level of acetyl-CoA synthetase.

PTW04-04

CANNABINOID RECEPTORS IN BERGMANN GLIA: GLUTAMATE UPTAKE REGULATION

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Glutamate is the major excitatory transmitter in the vertebrate brain. It exerts its actions through the activation of specific membrane receptors that are present both in neurons and in glial cells. Over-activation of glutamate receptors has been linked to neuronal death in a phenomena known as excitotoxicity, therefore the extracellular levels of this neurotransmitter have to be tightly and efficiently regulated after periods of intense electrical activity. A family of glial sodium-dependent glutamate transporters play a key role in the prevention of excitotoxic insults. Within the cerebellum, Bergmann glia completely surrounds glutamatergic synapses and harbors glutamate receptors and transporters that presumably are involved in synaptic transmission regulation. The
The endocannabinoid system is present in the cerebellum and apparently is involved in the prevention of glutamate-mediated toxicity, although the molecular mechanism associated to these events are not fully characterized. In order to gain insight into this problematic, in this contribution we sought to characterize a plausible effect of the activation of cannabinoid receptors in the glutamate uptake process. To this end, we took advantage of the well-characterized system of cultured Bergmann glia cells from chick cerebellum.
An anandamide-dependent increase in [^{3}H]-D-aspartate uptake activity (a measure of glutamate uptake) was detected. In order to characterize the canabinnoid receptors involved in this regulation, we undertook a Western blot strategy. Both CB1 and CB2 proteins were detected in whole Bergmann glia extracts. Next we decided to ask ourselves if both or these receptors are present in intact cerebellar slices. Our immunohistochemical results indicated that indeed *in situ* both receptors are expressed in cerebellar tissue sections, precisely in Bergmann glia. These results strength the notion of glial cells as targets of endocannabinoids and support the idea of a critical involvement of glia cells in the function and dysfunction of the brain.

PTW04-05

CONFLICTING BIOENERGETIC EFFECTS OF IDEBENONE ON RAT CORTICAL ASTROCYTES AND NEURONS: THE ROLE OF NQO1

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Idebenone is a synthetic short-chain Coenzyme Q_{10} analogue initially developed for the treatment of neurodegenerative disorders. In its reduced form idebenol, it can act as an antioxidant and electron carrier for cellular respiration. Idebenone would therefore seem like a promising drug for the treatment of many disorders characterized by mitochondrial dysfunction and oxidative stress such as Friedreich's Ataxia and Alzheimer's disease. Unfortunately, clinical trials for these disorders failed to show a convincing improvement in neurological outcome. In addition to the predicted beneficial effects of idebenone, an inhibitory effect of idebenone on Complex I of the mitochondrial respiratory chain has been reported. NAD(P)H:quinone acceptor oxidoreductase (NQO1) was recently found to reduce idebenone to idebenol. We tested the hypothesis that astrocytes, which express NQO1 in abundance, but not low NQO1-expressing neurons, will demonstrate NQO1-dependent resistance to respiratory impairment by idebenone, as well as the ability to use idebenone as a mitochondrial electron carrier. We found that idebenone stimulated oxygen consumption in rat cortical astrocytes and also reduced the ability of the mitochondrial Complex I inhibitor piercidin A to impair respiration. Both of these effects were attenuated by the NQO1 inhibitor dicoumarol. In contrast, rat cortical neurons treated with idebenone demonstrated impaired Complex I-dependent respiration and reduced respiratory capacity. Importantly, supplementation of neurons with exogenous recombinant NQO1 and its substrate NADPH, delivered via plasma membrane saponin permeabilization, prevented inhibition of Complex I-dependent respiration by idebenone. Overall, results suggest that targeted induction of neuronal NQO1 may unlock the therapeutic potential of idebenone in neurological disorders.

PTW04-06

TWO MODES OF GLIOTRANSMISSION IN THE ENTERIC NERVOUS SYSTEM AFFECT MOUSE COLONIC MIGRATING MOTOR COMPLEXES AND GUT PHYSIOLOGY

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Local gut reflexes are controlled by the enteric nervous system, which contains networks of neurons and glia residing in the gut wall. We hypothesized that enteric glia can modulate gut reflexes otherwise known to be regulated by neurons. We propose that this glial task would be accomplished by the release of gliotransmitters

through two distinct pathways: Cx43 hemichannels and Ca^{2+}-dependent exocytosis. To address this issue, we used four inducible and glia specific mouse models to increase ATP release through mutated Cx43 hemichannels or to reduce ATP release by the reduction of Cx43 expression, as well as to inhibit exocytotic release machinery or IP_3-dependent calcium signaling known to trigger exocytosis release. All the models reported on the cell specificity of the genetic manipulation in glial fibrillary acidic protein (GFAP)-positive enteric glia. Gut function was tested *in vivo* by pellet output/composition analysis and assays of gastrointestinal transit. Gut motility *in vivo* was increased or decreased in response to enhanced or decreased Cx43 hemichannel activity, respectively. Inhibition of exocytotic release, however, did not affect gut motility but increased pellet fluid content. More precise investigation of gut function was performed on isolated and perfused colon and by the analysis of colonic migrating motor complexes (CMMCs). Tampering with Cx43 hemichannel expression and Ca^{2+}-dependent exocytosis caused a decrease in velocity and frequency of contractions, while Cx43 hemichannel activity also affected the relative CMMC duration. Taken together**,** enteric glial cells can modulate gut physiology through the use of two distinct mechanisms, functional Cx43 hemichannels and Ca^{2+}-dependent exocytosis, of gliotransmitter release.

PTW05 Axon Biology and Pathobiology

PTW05-01

HIGH FAT DIET IMPACTS AXONAL DAMAGE BY MODULATING DNA METHYLATION IN MONOCYTES AND OLIGODENDROCYTES

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Multiple Sclerosis (MS) is a debilitating neurological disease that can lead to long-term disability as a consequence of axonal damage. At least two mechanisms of axonal damage have been proposed: direct cytotoxicity by peripheral immune cells, such as monocytes, and loss of myelin support. Lifestyle choices, such as overconsumption of saturated fat, may modulate disease course by limiting or exacerbating damage to the axons. Here we revealed through Magnetic Resonance Imaging (MRI) that MS patients with a high Body Mass Index (BMI) (≥25) and diet high in saturated fat have increased neuronal damage and decreased gray matter volume compared to patients with a low BMI (<25), which we validated in a progressive Experimental Autoimmune Encephalitis (EAE) mouse model. We also detected a positive correlation between BMI and peripheral monocyte counts in this cohort, thus we conducted a genome-wide analysis of DNA methylation in these cells to study the epigenetic changes induced by high fat diet. Monocytes in high BMI MS patients showed hypermethylated DNA compared to those from low, specifically on genes negatively regulating pro-inflammatory cytokine production and cell migration, and these changes correlated with increased monocyte infiltration into the CNS in preclinical mouse models. Since axonal damage can also occur through loss of trophic support by myelin, we checked the DNA methylation status of oligodendrocytes under high fat diet conditions and detected hypermethylation of genes regulating myelin formation, which resulted in impaired remyelination in a model of demyelination. Taken together, our results suggest that high fat diet increases axonal damage by favoring a pro-inflammatory, migratory phenotype in blood monocytes while impairing myelin repair. Future studies will address the causal link between diet, DNA methylation, and functional outcome using lineage specific ablation of DNA methyltransferases.

PTW06 Neurodegeneration 1: AD, PD, HD, ALS

PTW06-01

MIR-124-3P INHIBITS CELLULAR CALCIUM ION CONCENTRATION AND CELL APOPTOSIS VIA DOWN-REGULATING CAVEOLIN-1 IN ALZHEIMER'S DISEASES

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Alzheimer's disease (AD) is a chronic progressive neurological degenerative disease. Whose etiology and pathogenesis is still not fully elucidated so far. Micro RNA plays important role in AD. Caveolin-1 is a marker for caveole protein of cell membrane and has close relationship with micro RNA and AD. The specific high expression of MiR-124-3p is associated with the development of a variety of neurological disorders, including AD, but whether MiR-124-3p can regulate Caveolin-1 in AD is still unclear.
Our studies in vitro showed that APP mRNA and protein were significantly increased, while miR-124-3p expression was obviously decreased in N2a/APPswe cell group, compared with those of N2a/WT cell group. Dual luciferase report experiment showed that compared with those of co-transfection with the mutant vector(pGL3-Caveolin-1 3'UTR MUT) group, relative luciferase activities in co-transfection with the wild type vector (pGL3-Caveolin-1 3'UTR WT) group were significantly decreased. After the transfection of miR-124-3p mimics, Caveolin-1 mRNA and protein were significantly decreased in transfection group than in control group byreal-time PCR and Western blot or immunohistochemistry. Furthermore, the number of caveolae on cell membrane was decreased by electronic microscope. In addition, after the transfection of miR-124-3p mimics and Caveolin-1-siRNA, the cell apoptosis rate and free calcium concentration were decreased in transfection group than in control group. At the same time, the opposite results were obtained after the transfection of pcDNA-Caveolin-1. In summary, miR-124-3p can inhibit the apoptosis of cell and reduce the concentration of free calcium in cell by targeting down-regulation of Caveolin-1 expression, which plays a neuroprotective role in prevention and cure of AD and provides new ideas and targets for AD.

PTW06-02

ROLE OF E1 DOMAIN IN THE ALPHA SECRETASE PROCESSING OF APP AND ITS HOMOLOGUS PROTEIN APLP2

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Alzheimer's disease (AD) is the leading cause of dementia worldwide. It is characterized by the accumulation of amyloid beta (Abeta) peptide within the brain along with hyperphosphorylation of tau, which may lead to increased formation of neurofibrillary tangles. The role of amyloid-beta precursor protein (APP) in AD is being studied extensively as sequential cleavage of APP leads to the secretion of Abeta peptide. APP can be proteolytically processed in two different pathways i.e. amyloidgenic pathway initiated by beta-secretases and non-amyloidgenic pathway initiated by alpha-secretases. As the name suggests the non-amyloidgenic pathway precludes the formation of Abeta and instead generate fragments, which are neuroprotective. There are also two homologus proteins the APP –like protein1 and 2 (APLP1 and APLP2). Although they are processed in a similar way, it was previously shown from our group that insulin-like factor-1 (IGF-1) and retinoic acid (RA) induced alpha secretase processing of APP and APLP2 is mediated by different enzymes in which APP is cleaved by ADAM10 in a PI3-K dependent manner and APLP2 is cleaved by ADAM 17 (TACE) in a PKC dependent manner. Therefore, it appears important to characterize how substrate specificity is determined. We have made different chimeric constructs in which the entire E1 domain of APP and APLP2 has been switched. Our results demonstrate that these chimeric constructs are successfully expressed and proteolytically processed in transiently transfected SH-SY5Y cells. We have analyzed the signaling pathways involved in regulated cleavage of the chimeric proteins. Our data suggest that the E1 domain is important for regulation of alpha-secretase cleavage of APP.

PTW06-03

TARGETING THE PERIPHERAL IMMUNE SYSTEM TO MODULATE ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is typically associated with age-associated central nervous system degeneration and dementia. This correlates with increased Aβ peptide containing plaque deposition and associated reactive gliosis. The inflammatory phenotype of microglia, in particular, is often considered detrimental to cognitive function in AD. Moreover, the immune-related communication between the periphery and the brain is increasingly recognized as critical for brain physiology. Based upon this idea, we hypothesized that modulating the peripheral immune system may alter the proinflammatory gliosis associated with AD. Therapeutic antibodies against the a4/b1 integrin receptor have been used clinically to attenuate the ability of various immune cells to adhere to endothelium to migrate into target tissues such as the intestines (Crohn's disease) or brain (multiple sclerosis). We hypothesized that a similar peripheral antibody-based therapy would attenuate gliosis by altering immune cell infiltration/phenotype in peripheral organs and the brain using and APP/PS1 mouse model of Alzheimer's disease. For this purpose, we tail-vein injected littermate control wild type and APP/PS1 mice with an antibody recognizing α4-integrin. As expected, antibody therapy was able to reduce microgliosis in the APP/PS1 mice compared to isotype control injections. Interestingly, the antibody therapy also reduced overall plaque load in these mice. This preliminary data suggests that it is indeed feasible to alter the immune component of AD brain changes using a clinically available strategy that targets the peripheral immune system.

PTW06-04

OPTOGENETIC MODULATION OF THE HYPERDIRECT PATHWAY IN NON-TRANSGENIC MICE

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During the 2015 ASN annual meeting, we reported preliminary results on a study of optical high frequency stimulation (HFS) of subthalamic nucleus (STN) and primary motor cortex (M1) in layer V projections to STN in non-transgenic mice unilaterally lesioned with 6-OHDA. The main goal of the study was to determine whether optogenetic HFS stimulation in these descending fibers might effectively reverse parkinsonian behavior, since such a method might have translational potential for ameliorating PD symptoms in humans. The results showed electrophysiological changes in cortico-subthalamic projections in freely moving 6-OHDA mice with opsins expressed using a WGA-Cre retrograde transfection approach. However, initial results indicated that, although electrophysiological changes were present, behavior was not significantly changed in these non-transgenic mice.
We now report that, in the past year statistically significant behavioral results were found in 6 non-transgenic mice transfected using the above approach. The new study was performed with mice expressing ChR2 ET/TC opsins over a period of 3 months or more. Additionally, the new study used 473 nm laser light stimulation with longer pulse-widths than used in the previous study. Histological analyses of the opsin transfection patterns suggest that the behavioral effects were induced through stimulation of projections and may also involve the activation of collaterals. We present behavioral, electrophysiological, and histological analyses from the 6 parkinsonian mice and 3 controls.

PTW06-05

DENDRITIC REMODELING AND SYNAPTIC ALTERATIONS IN C9ORF72 NEURONS

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the loss of cortical and spinal motor neurons. The discovery of the hexanucleotide repeat expansion GGGGCC (G4C2) in the *C9orf72* gene represents up to 10% of sporadic and up to 40% of the familial ALS cases. *In vitro* and *in vivo* disease models revealed several disease mechanisms: protein loss of function, toxic RNA gain of function, generation of dipeptide repeat proteins via non-ATG initiated translation, and impairment of the nuclear-cytoplasmic transport. Studies from different laboratories have identified RNA interacting proteins that are sequestered to the G4C2 repeat expansion, including proteins involved in synaptic neurotransmission. In addition, human C9 patient-derived induced pluripotent stem cells differentiated into neurons (iPSNs) show changes in neuronal excitability and increased susceptibility to cellular stressors. Based on these phenotypes *we hypothesize that synaptic deficits are a consequence of the sequestration of proteins involved in synapse formation and function to the G4C2 repeat expansion.* Here we present preliminary data showing significant changes in the expression of synaptic proteins when using C9 iPSNs. Morphological analysis of the iPSNs further confirmed alterations of dendritic branching, spine density and spine morphology. To validate the *in vitro* synaptic alterations *in vivo*, we injected AAV9 viral construct overexpressing G4C2 repeats of different lengths into wild-type mice. Loss of expression of synaptic proteins were observed in selected brain regions of AAV9 $(G4C2)_{66}$ infected mice when compared with AAV9 $(G4C2)_2$ controls. Lastly, to address glutamate receptor function, we measured RNA editing efficiencies of the GluA2 AMPA receptor subunit and found significant loss of GluA2 R-G editing due to a deficient nuclear-cytoplasmic trafficking of RNA editing enzyme ADARB1. Our data suggest that synaptic dysfunction plays a role in C9ORF72 pathogenesis and may explain the observed changes in neuronal excitability and increased susceptibility to stressors, which are likely to lead to cognitive impairment and neuronal cell death, as observed in C9ORF72 ALS patients.

PTW06-06

CURCUMIN IMPROVED COGNITIVE FUNCTION OF AD TRANSGENIC MICE VIA REGULATING THE EXPRESSION OF α-SYNUCLEIN AND MAP2

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Alzheimer'sdisease (AD) is the main type of dementia with classic clinical symptoms of progressive cognitive dysfunction and memory loss. Cognition and memory are the most advanced functions of the brain, and the neural basis may be associated with the high plasticity of the central nervous system network. α-synuclein and MAP2 are mainly protein components of the synaptic plasticity. Previously, it has been reported that curcumin, a kind of components of herb curcuma rhizome, can improve the cognition and memory function of mice. But the exact mechanisms are not fully clear. In the present study, Morris water maze test was used to detect the effects of curcumin on spatial learning and memory of transgenic AD mice with different dosage of curcumin; IHC was carried out for evaluating the expression of α-synuclein and MAP2 in hippocampus. Our results showed that curcumin could improve learning and memory of AD mice.The expression of α-synuclein in the hippocampus were significantly reduced, while the expression of MAP2 were significantly increased in curcumin treatment group. All these data suggested that cucurmin could dramatically improve the ability of learning and memory of mice, the potential mechanism of which may due to reducing the expression of α-synuclein and enhancing the expression of the MAP2.

PTW06-07 - WITHDRAWN

PTW06-08

MITOCHONDRIAL ACCUMULATION OF α-SYNUCLEIN IN PARKINSON'S DISEASE CAUSES IMPAIRED PROTEIN IMPORT VIA TOM40 DEGRADATION

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α-Synuclein is a presynaptic protein, whose intracellular aggregation in brain has been etiologically implicated in Parkinson's disease (PD), a progressive neurodegenerative disorder, affecting 4.9-19 per 100,000 people per year worldwide. Accumulation of misfolded α-synuclein in the substantia nigra of the brain leads to the formation of Lewy body deposits, one of the histopathological features of PD, which also involves oxidative stress, pro-oxidant metal toxicity and increased nuclear genome damage. More recent studies have implicated significant mitochondrial pathology in PD brain, including accumulation of dysfunctional mitochondria with high levels of mitochondrial DNA (mtDNA) deletions/damage, and altered outer mitochondrial membrane (OMM) proteins, which also correlates with increased α-synuclein in mitochondria. However, the role of α-synuclein toxicity in mitochondria is unclear. Here we demonstrate that α-synuclein stably interacts/co-localizes with mitochondrial membrane translocases involved in the import of nuclear-coded proteins into mitochondria, TOM40 (outer membrane) and TOM20 (inner membrane) in normal neurons. Furthermore, we made surprising observation that in Neural Precursor Stem Cells (NPSC) from PD patient-derived iPSC with α-synuclein gene *(SNCA*) triplication, TOM40 is distinctly depleted in mitochondria, while TOM20 is not affected. Interestingly, exposure to PD associated agents 6-hydroxydopamine and pro-oxidant metals enhance TOM40 loss in α-synuclein overexpressing cells. These noteworthy observations are accompanied with reduced mitochondrial membrane potential ($\Delta\psi m$), whose optimal level is critical for the initial step of mitochondrial protein import. Thus α-synuclein pathology in PD causes defective protein import via TOM40 depletion in mitochondria of dopaminergic neurons leading to imbalance in mtDNA damage/repair and anti-oxidant machinery. These data are consistent with evidence of increased mitochondrial DNA damage in PD affected human brain. Comprehensive molecular events involved in this phenomenon and potential prevention strategies will be discussed.

PTW06-09

ABERRANT AXONAL OUTGROWTH IN PRIMARY NEURONS EXPRESSING PATHOGENIC HUNTINGTIN INVOLVES INCREASED ACTIVATION OF THE JNK PATHWAY

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Huntington's disease (HD), the most common hereditary neurodegenerative disease, exhibits a progressive decline in cognitive and motor functions due to cortical and striatal neurons undergoing a dying-back pattern of degeneration. HD is caused by a polyglutamine (polyQ) repeat expansion at the N-terminus of the huntingtin protein, where non-pathogenic Htt contains <30 polyQ repeats and pathogenic mutant huntingtin (mHtt) is comprised of >30 to 180 polyQ repeats. In HD patients and animal models of HD, axonal degeneration long precedes neuronal cell death and the onset of symptoms. We, and others, have identified a pathogenic mechanism linking mHtt to abnormal activation of the JNK pathway, which regulates gene transcription, synaptic plasticity, and axonal transport. However, it is unknown whether this mechanism relates to mHtt-induced axonal degeneration within cortical and striatal neuron populations, or causes neuronal subpopulation vulnerability. The current study optimized a live cell axonal outgrowth measurement assay using novel microfluidic chambers to examine primary neurons expressing mHtt exon 1. Embryonic

cortical neurons were either from transgenic mice co-expressing tdTomato and mHtt (R6/2), eliciting similar pathogenic features observed in HD patients, or from wild-type mice and transfected with mHtt. Axonal outgrowth lengths for individual primary cortical neuron axons were measured using live cell fluorescent microscopy over a 21-day time course. The results revealed four subgroups of cortical neurons defined by specific alterations in axonal outgrowth patterns, an observation consistent with differential vulnerability of neuronal subpopulations in R6/2 mice and HD brains. In addition, R6/2 mHtt cortical neurons exhibited increased activation of JNK. Furthermore, application of the SP600125 JNK inhibitor to R6/2 mHtt cortical neurons reduced the number of axons that underwent degeneration and promoted elongation of other axons, thereby promoting neuroprotective effects. Cortical neurons transfected with pathogenic mHtt showed a reduction of continued axonal elongation following transfection. This study further supports the notion that mHtt expression enhances detrimental JNK pathway activation in susceptible neuronal populations.

PTW06-10

INHIBITION OF THE ASTROCYTIC NFAT4 QUELLS GLUTAMATERGIC HYPERACTIVITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Calcineurin (CN) and its transcription factor substrate, Nuclear Factor of Activated T-cells (NFAT), are associated with cognitive decline in Alzheimer's disease (AD). Several recent studies have reported that the NFAT4 isoform is specifically associated with activated astrocytes, but the role(s) of this isoform in AD remains unclear. Here, we found an increase in nuclear localization of NFAT4 in astrocytes from postmortem human AD brain tissue and in a common mouse model of AD (i.e. 5xFAD). Direct targeting of astrocytic CN/NFAT signaling, using AAV vectors expressing the NFAT inhibitor VIVIT under the control of a GFAP promoter (Gfa2), led to a reduction in nuclear NFAT4 levels in intact 5xFAD mice in parallel with a reduction in dendritic degeneration, elevated synaptic strength, and improved memory function. Whole-cell voltage clamp analyses of CA1 pyramidal neurons indicated that the astrocytic CN/NFAT pathway modulates the balance of AMPA/NMDA receptor mediated signaling in 5xFAD mice, but does not significantly contribute to other synaptic changes (*e.g.* the appearance of silent synapses). Strikingly, AAV-Gfa2-VIVIT reduced the frequency of spontaneous AMPA receptor-mediated currents and spontaneous glutamate spikes in AD mice, suggesting that astrocytic CN/NFAT signaling may drive glutamate-mediated hyperactivity. Together, our findings reveal a novel modulatory role of astrocytic NFAT4 signaling in astrocyte-neuron interactions, synaptic transmission and cognitive function. Inhibition of astrocytic NFATs, especially the NFAT4 isoform, may provide a unique way to prevent or treat a complex neuronal disease such as AD.

PTW06-11

EXOGENOUS FIBRILLATED α-SYNUCLEIN STIMULATES RELEASE OF GLUTAMATE FROM SYNAPTONEUROSOMES AND PENETRATES VESICULAR MEMBRANES

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The α-synuclein protein exists *in vivo* in a variety of covalently modified and aggregated forms associated with Parkinson's Disease (PD) pathology. However, the specific proteoform structures involved with neuropathological disease mechanisms are not clearly defined. Since α-synuclein is known to play a role in presynaptic vesicle dynamics, an *in vitro* assay was developed to measure glutamate neurotransmitter release using mouse forebrain synaptoneurosomes (SNs) enriched in synaptic endings. Overall glutamate measurements for SNs from endogenous mouse genotypes (over-expressers, knock-outs) and SNs reconstituted with purified α-synuclein proteoforms (monomers, oligomers, fibrils) pointed to the requirement for a threshold amount of α-synuclein in both steady-state and calcium/depolarization-dependent glutamate release from forebrain terminals. Notably a brief exogenous application of fibrillated forms of α-synuclein enhanced synaptic glutamate release. Consistent with membrane penetration, immunogold/transmission electron microscopy detected exogenously applied fibrillated α-synuclein in multiple membranous vesicularized entities including synaptic terminals and myelin ensheathed structures. Overall excitotoxicity due to enhanced glutamate release in the face of either excessive monomeric or fibrillated α-synuclein should be considered as a potential neuropathological pathway during the progression of PD and other synucleinopathies such as Multiple Systems Atrophy.

PTW06-12

IN VIVO CHARACTERIZATION OF THE EFFECTS OF AMYLOID-BETA OVEREXPRESSION ON GLUCOSE UTILIZATION

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Epidemiological connections between type 2 diabetes (T2DM) and Alzheimer's disease (AD) have not been mechanistically elucidated. AD mouse models that overexpress familial mutations have been assessed for changes in glucose metabolism and insulin

responses. These models however do not specifically address the role of Aβ, the principle component of plaque pathology, because they rely on overexpression of the entire amyloid precursor protein (APP). Full-length APP itself regulates metabolism and contains several other bioactive components when cleaved, such as secreted APP and the APP intracellular domain. We used the BRI-Aβ42 model to study Aβ's effects in the absence of APP overexpression. Male BRI-Aβ42 mice and wild-type littermates were fed either a western diet (high-fat, high sucrose) or the normal chow diet, and blood glucose responses to peripheral insulin and glucose administration were measured. Aβ expression impaired glycemic control on both diets and created significantly higher fasting blood glucose levels on the western diet. Differences in insulin response were only seen under the western diet. Further metabolic testing was performed using a Comprehensive Lab Animal Monitoring System to measure food consumption, respiration, sleep, and activity. While male mice showed no distinguishable phenotype at three months of age, female mice displayed a depressed respiratory rate. Analysis of cortical tissue using 2-deoxyglucose via liquid scintillation counting shows decreased glucose uptake. These results confirm metabolic perturbations as a consequence of Aβ accumulation. This may impact hypotheses about the role of peripheral metabolism in cognition, as well as the etiology of T2DM. Supported by the Sturgis Charitable Trust, NIH P01AG012411, and the Center for Translational Neuroscience (NCRR P20 RR020146).

PTW06-13

CURCUMIN IMPROVED METABOLIC DISORDER OF LIPID BY ABCA1 TRANSMEMBRANE-TRANSPORT SYSTEM IN ALZHEIMER'S DISEASE

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The disorder of cholesterol metabolism is an important pathogenic factor of Alzheimer's disease (AD). ABCA1 plays a pivotal role in cholesterol transport and the deposit of amyloid-beta (Aβ),whose overexpression,is one of the major pathogenesis of AD. Curcumin has been used for the treatment of lipid metabolism disorder. But how curcumin regulate ABCA1 transmembrane-transport system in AD has never been reported. In the present study, APP/PS1 double transgenic AD mice Tg2576 were fed for 6 months at different concentration of curcumin diet, namely, dementia group（0g/kg）, low-dose group(0.16g/kg) and high-dose group(1.0g/kg). The special cognitive and memorial capability of transgenic mice of each group were investigated using Morris water maze. And the contents of apoA1, total cholesterol (TC) and high-density lipoprotein (HDL) in each group of transgenic mice were tested. Aβwas detected to show the effect of curcumin on pathological changes of transgenic mice's brain. Finally, ABCA1, liver X receptor (LXR) and retinoid X receptor (RXR) were analyzed to address the possible mechanism of curcumin on improving metebolism disorder of lipid by ABCA1 tranport system. The result showed that the treatment of curcumin could significantly improve the special cognitive and memorial capability of transgenic mice ($P<0.05$). And both low-dose and high-dose of curcumin improved the pathological changes of transgenic mice induced by Aβ. Compared with dementia group, the content of TC in blood serum of transgenic mice fed with curcumin diet decreased ($P<0.05$), while the level of HDL increased ($P<0.05$). More interestingly, the treatment of curcumin could increase LXR-β, RXR-α, ABCA1 and apoA1 dramatically ($P<0.05$) in the transgenic mice in dose dependent manner. Our data suggests that the effects of curcumin on improving metabolic disorder of lipid in AD may due to increasing ABCA1 transmembrane-transport system via LXR-β and RXR-α.

PTW07 Neuron-Glial Interactions 2: Development and Disease

PTW07-01

PROTEOLIPID PROTEIN NULL MICE HAVE UNIQUE BEHAVIORAL CHANGES AND ALTERED NUMBERS OF OLIGODENDROCYTES

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Myelin is required for proper nerve conduction and normal axonal function. However, little is known about the behavioral consequences of myelin disruption alone. To address the contribution of myelin function to behavioral and cognitive deficits, we used a mouse line lacking the myelin proteolipid protein (PLP). These mice generate myelin but exhibit progressive myelin dysfunction and eventual axonal degeneration. No gross motor deficits are observed in PLP(-/Y) at 3 and 8 months of age. However, 3 mo PLP(-/Y) mice exhibit subtle motor deficits, with altered gait and they overstep the forelimb, creating a narrower stride (0.47 cm vs 0.33 cm, $p<.01$), although stride length was not significantly different. Intriguingly, a lack of coordinated swimming was observed when 8 month PLP(-/Y) mice were placed in water (distance moved = 140 ± 5 vs 339 ±11 in 30 second swimming trial, $p<0.01$). PLP(-Y) mice demonstrated a decrease in the motivation to bury marbles in marble burying task (mean marbles buried: 3 month = 2.9±0.3 vs. 6.1±0.6; 8 month = 0.3±0.2 vs. 4.2±0.9, $p<0.001$). Performance on the Y maze, a test of spatial memory and hippocampal function, was normal. In the Puzzle Box, a test of problem-solving and executive function, young PLP(-/Y) mice displayed longer latency to reach the goal box when presented with a new challenge (repeated measure ANOVA, $p=0.02$), indicating deficits in higher cognition. The number of oligodendrocytes in 2 month PLP(-/Y) mouse corpus callosum, striatum, olfactory bulb, and cerebellum increased, relative to controls, while oligodendrocyte numbers in motor cortex were unaltered, and a decrease was observed in the hippocampus ($p<.01$). In conclusion, myelin dysfunction in PLP(-/Y) mice results in targeted behavioral deficits before significant axonal degeneration is observed, and an increase in the number of oligodendrocytes in specific brain regions. Ongoing investigation will characterize the pathophysiological process that results in altered oligodendrocyte numbers and altered behavior. Supported by NS25304, T32DC 12280-2 and NMSS.

PTW07-02

CUPRIZONE-INDUCED INFLAMMATORY DEMYELINATION PROMOTES VISUAL SYSTEM EXCITATORY/INHIBITORY IMBALANCE AND AXONAL SPROUTING

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Inflammatory demyelination is a pathological event present in different central nervous system disorders, such as multiple sclerosis, that is commonly followed by neuroplasticity. However, the mechanisms underlying this plasticity are not well elucidated. Using the cuprizone feeding animal model and focusing on connectivity of subcortical visual system, the present work aimed to study, in- vivo, inflammatory demyelination-driven axonal and synaptic remodeling. Combining mRNA, immunohistochemistry and Western blot analysis, our findings showed cuprizone-induced demyelination of dorsal lateral geniculate nucleus (dLGN) followed by micro- and astrogliosis, firstly associated to a pro-inflammatory (higher levels of TNF-α, iNOS, COX-2 mRNA) and later to a non-inflammatory profile. Inflammatory demyelination induced synaptic changes in the dLGN: while excitatory synapses density decreased (54%), inhibitory ones increased (54%). Additionally, a higher number of GluN2B subunit (54%) on inhibitory neurons was found. Field potential recordings in the presence of GluN2B antagonist also supported this increment on inhibitory cells. In parallel, using anterograde neuronal tracer, we showed that retinogeniculate projections increase the percentage of binocular overlapped territories on dLGN, suggesting a decrease of the eye-specific segregation. Our data suggest an impact of inflammatory demyelination on excitation/inhibition balance, favoring an increase of inhibition. This imbalance might destabilize synaptic sites, inducing axon remodeling. These findings unravel relevant mechanisms, which might be useful to the development of interventions in demyelinating diseases in the near future.

PTW07-03

TRANSCRIPTION FACTOR REGULATION BY MTOR DURING OLIGODENDROCYTE DIFFERENTIATION

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Oligodendrocytes are generated from oligodendrocyte progenitor cells (OPCs) that divide and migrate throughout the CNS. Differentiation of OPCs into mature oligodendrocytes requires extensive changes in gene expression. Several transcription factors play a role in the control of oligodendrocyte differentiation; these transcription factors can be intrinsically and extrinsically regulated. We have shown that mTOR is critical for normal oligodendrocyte differentiation in vitro and in vivo (Tyler et al, 2009, 2011; Wahl et al., 2014). Here we extended that analysis to investigate how mTOR controls the transcription factor machinery that is essential for the OPC progression through the oligodendrocyte lineage.

We found a significant increase in the mRNA levels of the inhibitor of DNA binding-2 (Id2) in the spinal cord of mice lacking mTOR in the oligodendrocyte lineage (CNP-Cre, floxed-mTOR) at postnatal days (PND) 7 and 14. Id2 is a negative regulator of transcription and differentiation in the oligodendrocyte lineage and is regulated by the bone morphogenetic protein (BMP)/Smad pathway (Samanta and Kessler, 2004). OPC cultures differentiated for 3 days in the presence of the mTOR inhibitor, rapamycin, showed increased levels of Smad1/5/8 bound to the Id2 promoter compared to untreated control cells. Upon mTOR inhibition *in vitro* and genetic deletion *in vivo*, we also observed a decrease in total levels of Sip1, which is a negative regulator of the Smads. Surprisingly, the levels of Sip1 bound to Id2 promoter were higher in differentiating OPC cultures treated with rapamycin. Our results suggest that mTOR modulates Id2 expression through a complex regulation of the transcriptional machinery at its promoter, which is essential for OPCs to differentiate into mature oligodendrocytes.

PTW07-04

GLIAL RESPONSES IN JUVENILE ISCHEMIA

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White matter injury is a crucial component of ischemic stroke pathophysiology. In addition to local neuronal death, ischemia damages oligodendrocytes, the myelin-producing cells of the CNS. This causes secondary damage to myelinated axons connecting brain regions, effectively increasing the scope of the injury. Stroke susceptibility varies with age, and neonatal and aged white matter are particularly vulnerable. Late juvenile ischemic stroke is characterized by greater functional recovery than is observed at other ages. Interestingly, the juvenile period is the peak of CNS myelination, which is particularly relevant to white matter responses. However, the cellular and molecular responses to ischemia during this time remain understudied. Direct comparison of juvenile and adult mouse tissue following middle cerebral artery occlusion demonstrated comparable neuronal injury. Conversely, while adult striatal oligodendrocytes were highly susceptible to ischemia, juvenile oligodendrocytes were resistant to injury, which mitigated secondary axonal damage and long-term tissue loss. The progression of glial responses also differed, with juvenile tissue exhibiting reduced astrogliosis, fibrosis, and NG2 cell loss, and increased vascular preservation. These findings suggest that tissue preservation in juvenile mice may be a consequence of the actively myelinating state of oligodendrocytes during this developmental period, and we are therefore investigating the effect of ischemia in an animal that continues active myelination through adulthood. The dramatic difference in physiological responses to ischemia between juvenile and adult animals corroborates clinical observations of greater recovery of juveniles following stroke. This study provide novel insight into the cellular responses that underlie the progression of ischemic stroke pathology, as well as the cellular interactions involved in white matter development and myelination.
Supported by the Bugher Foundation.

PTW07-05

INVESTIGATION OF THE LEVELS OF GLUTAMATE ASPARTATE TRANSPORTER (GLAST) IN A MOUSE MODEL OF EPILEPSY

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Epilepsy, estimated to effect 2% of the population, is a neurological disorder characterized by spontaneous recurrent seizures. Excess glutamate levels in the synaptic cleft are known to overexcite neurons and play a role in seizure initiation. Glutamate aspartate transporter (GLAST), a protein found on astrocytes is responsible for removing glutamate. Despite its importance in maintaining normal glutamate levels, the role of GLAST in epilepsy is not well understood. We studied the regulation of GLAST in epileptogenesis (development of epilepsy) using an intrahippocampal kainic acid (IHKA) mouse model at 1, 4, 7, and 30 days. Kainic acid is injected into the hippocampus to induce seizures via glutamate receptor activation. Using polymerase chain reaction (PCR) and immunohistochemical techniques, we found that 1) there was no significant difference in GLAST mRNA levels post kainic acid injection and post saline control; and 2) there was upregulation of GLAST immunoreactivity after 1 day followed by a persistent downregulation. These results may lead us to glial cell-specific seizure therapeutics to avoid the development of epilepsy

PTW07-06

MOLECULAR CONTROL OF SCHWANN CELL DEVELOPMENT BY FBXW7

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Myelin is the multilamellar sheath generated by specialized glia that iteratively spiral their plasma membranes around axon segments. Myelinating glia provide trophic support essential for neuronal survival, and myelin enables rapid propagation of action potentials. In the peripheral nervous system (PNS), myelin is made by Schwann cells (SCs), and disruptions in SC development and myelination lead to devastating symptoms in many neurological disorders. To develop therapies for these patients, we must understand the mechanisms that govern SC development, myelination, and myelin maintenance.

In a genetic screen in zebrafish to define new regulators of myelinating glial cell development, we recovered *stl64* mutants, which display overexpression of myelin-related genes and hypermyelination in both the central and peripheral nervous systems. Using whole genome sequencing, we determined that *stl64* disrupts *fbxw7,* which encodes the substrate recognition component of E3 ubiquitin ligase complexes. Notable Fbxw7 targets are master regulators of transcription and cell cycle including: mTOR, Notch, and cyclin E. Thus, Fbxw7 is required for critical cellular processes such as proliferation and differentiation.

Ultrastructural analyses of *stl64* mutants revealed increased SC numbers as well as thicker myelin in the PNS. Further, examination of SC-specific knockout mice ($Dhh^{Cre};Fbxw7^{fl/fl}$) suggest that Fbxw7 functions cell autonomously in SCs. Given that mTOR levels must be tightly regulated to achieve proper myelin thickness, we are testing whether loss of Fbxw7 regulation on mTOR is the primary cause of the SC defects observed in zebrafish and mouse *Fbxw7* mutants. Preliminary analyses indicate that pharmacological inhibition of mTOR partially suppresses hypermyelination in zebrafish mutants, suggesting that mTOR is indeed an important Fbxw7 target in SCs. Our current mechanistic and behavioral studies will be discussed. This work provides the first evidence of a role for Fbxw7 in SCs that is conserved in zebrafish and mammals.

PTW07-07

ROLES OF PERINEURIAL GLIA AND THEIR PRECURSORS IN MOTOR AXON PATHFINDING AND NEURAL CREST STREAMING

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Motor nerves play critical roles of sending information out of the central nervous system (CNS). Efficient motor function is dependent on the proper assembly of spinal motor nerves. During spinal motor nerve formation, motor neurons project axons out of the CNS to their targets in periphery by navigating through specialized CNS/PNS transition zones known as motor exit points (MEP). These unique positions in the nervous system have highly stereotyped placement along the anterior-posterior axis of the spinal cord, with a single MEP being found within each muscle segment. However, the mechanisms that direct their precise location are not known. Previously, studies from our lab have discovered that establishment of MEPs requires the coordinated interactions of several distinct cell types, including neural-crest derived Schwann cells, MEP glia, perineurial glia and motor axons. Prior works have found that perineurial glia are derived from the CNS, migrate out of the spinal cord through the MEP and ultimately form the mature spinal motor nerve perineurium. However, very little is known about whether these cells help establish the MEP earlier in development or guide motor axons into the periphery. In the absence of perineurial glia, we observed the ectopic exit of motor axons from the CNS and a failure of Schwann cells to associate with nascent motor nerves. Therefore, we hypothesize that CNS-derived perineurial glia play a very early role in MEP establishment. Using in vivo, time-lapse imaging and photoablation in zebrafish, we found that perineurial glia and their precursors guide motor axon outgrowth from the spinal cord and direct neural crest migration to the MEP and along motor nerves.

PTW07-08

REPOPULATION AND BEHAVIORAL PATHOLOGY AFTER PARTIAL MICROGLIA DEPLETION AND RESTORATION IN ADULT MICE USING A GENETIC MOUSE MODEL

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Although it is now firmly established that microglia normally self-maintain without contribution of peripheral monocytes, the role of microglia in CNS homeostatic function remains unresolved. Multiple models of microglia depletion have been used to study both the kinetics of microglia repopulation and the role of microglia in CNS function. While some have reported that microglia depletion results in no significant behavioral deficits, others have reported that microglia-derived BDNF plays an important role in learning. We sought to further elucidate the role of microglia in CNS function via a new model of postnatal genetic microglia depletion. Partial microglia depletion resulted in no obvious cognitive impairment. Interestingly, however, restoration of microglia, in part through peripheral cells, resulted in anxiety behavior and impaired contextual fear conditioning, supporting a role for microglia in homeostatic CNS function. In summary, our work thus far suggests that partial microglia depletion or deactivation may result in repopulation of microglia through peripheral immune cells, which has devastating effects on higher brain function.

PTW07-09

EXTRACELLULAR ADENOSINE REGULATES OLIGODENDROCYTE PROGENITOR CELL MIGRATION IN THE DEVELOPING NERVOUS SYSTEM

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During spinal cord development, a barrier is established separating the spinal cord (CNS) from peripheral nerves (PNS). Axons are permitted to cross this barrier at transition zones, but other cell types, including oligodendrocyte progenitor cells (OPCs), are prevented from following axons into the PNS. Recently, we discovered that OPCs not only exhibit dynamic migration throughout the spinal cord, they also extend processes short distances into the periphery, where they are repelled by peripheral glia. Previous studies in the lab have implicated the existence of a repulsive signal generated by these peripheral glial cells as a mechanism for repelling OPCs, but the identity of that signal is unknown. Using a screen of pharmacologically active compounds and *in vivo,* time-lapse imaging, we have identified several candidate regulators of OPC migration. One of these mechanisms utilizes extracellular adenosine, signaling through the A2a receptor. This is a novel mechanism regulating OPC migration during development, and disruptions to this signaling pathway result in ectopic OPC migration into the PNS. Currently, we are investigating the source of extracellular adenosine and the signaling pathway downstream of A2a activation that results in OPC repulsion via peripheral glia.

PTW08 Neurodegeneration 2: Ischemia, Trauma and Other

PTW08-01

ALTERED CNS EXCITABILITY IN SYSTEM XC- NULL MICE UNCOVERED USING THE KAINIC ACID MODEL OF STATUS EPILEPTICUS

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System x_c^- (Sx_c^-) is a cellular antiporter that links the import of L-cystine with the export of L-glutamate. In the CNS, this export contributes to the ambient glutamate levels found in the synaptic cleft. To wit, a 50% reduction in extracellular glutamate has been demonstrated in animals null for the substrate-specific light chain, xCT. This reduction has been hypothesized to result in alterations in synaptic strength and neuronal excitability. This idea was explored herein by assessing seizure behavior in mice wild-type or null for the *SLC7A11* gene, that encodes for xCT, using the kainic acid (KA) model of status epilepticus (SE) Acute seizure activity was generated in wild-type and *SLC7A11* null mutant littermate mice derived from heterozygous breeders (JAX; 001310) using a repeated dosing paradigm. Animals were administered an initial dose of 10 mg/kg KA (i.p.) followed by five booster doses of 2.5 mg/kg KA at 30 min intervals. All mice received diazepam (5mg/kg, i.p.) 90 min after the final KA dose to arrest seizure activity. KA-induced behavioral seizure activity was scored at 10 min intervals using a nine-point scoring system modified from Claycomb et al., *Neurobiology of Disease*, 45: 234-242, 2012 by an investigator blind to genotype. Irrespective of genotype, mice attained a median maximal seizure score of 5 (rearing with clonic forelimb movements) within the 150 min dosing period. However, during the 90 min following the last dose, the median wild-type seizure score increased to 7 (ferocious jumping), whereas the median score in the *SLC7A11* null group decreased to 1 (hypomobility) (n = 8-13; p = 0.028, Mann-Whitney U test). Furthermore, mortality associated with the paradigm was enhanced in wild-type mice as compared to *SLC7A11* null littermates (4/13 vs. 0/8, respectively). Thus, our data demonstrate that mice null for *SLC7A11* have alterations in neuronal excitability, an effect uncovered using the KA-paradigm of SE. Supported by NS051445-07 to SJH and JAH.

PTW08-02

INHIBITION OF ASTROCYTIC CALCINEURIN/NFAT SIGNALING IN A MOUSE MODEL OF VASCULAR COGNITIVE IMPAIRMENT AND DEMENTIA

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Astrocytes are one of the most abundant cell types in the brain, and play a vital role in maintaining healthy nervous tissue. Calcineurin (CN), an exquisitely Ca^{2+}-sensitive protein phosphatase that has been associated with several neurodegenerative diseases, appears at elevated levels in activated astrocytes associated with aging, injury, and disease. The signaling between CN and the transcription factor NFAT (Nuclear Factor of Activated T-cells) regulates several critical pathways in astrocytes, including those involved in excitotoxicity, inflammation, and neuronal death. Inhibition of the astrocytic CN/NFAT pathway in a mouse model of Alzheimer's disease was associated with reduced glial activation and improved synaptic and cognitive function (Furman et al. 2012). However, no studies that we know of have investigated the role of astrocytic CN/NFAT signaling in vascular cognitive impairment and dementia (VCID). Here, we used adeno-associated virus (AAV 2/5) vectors containing an astrocyte-specific promotor, Gfa2, and VIVIT, a potent NFAT inhibitor, to selectively inhibit astrocytic NFAT signaling in a diet-induced mouse model of VCID. AAV-treated mice were maintained on either control or methionine-enriched and folate-deficient diet for a minimum of 11 weeks to induce hyperhomocysteinemia (HHcy) associated with vascular pathology. HHcy diet was associated with a significant reduction in hippocampal synaptic strength and long-term potentiation (LTP). Both of these deficits were ameliorated by AAV-Gfa2-VIVIT, suggesting that astrocytic CN/NFAT signaling contributes to synaptic dysfunction during VCID. Other hallmarks of VCID, including cognitive decline and cerebral hypoperfusion, are being assessed in a second cohort of HHcy mice using the radial-arm water maze (RAWM) task and MRI. Histochemical analyses will also be performed to characterize the relationship between the astrocytic CN/NFAT pathway and a variety of vascular abnormalities, including microhemorrhages and microinfarcts.

PTW08-03

TRANSGENIC MOUSE MODEL TO SELECTIVELY IDENTIFY α3 NA,K-ATPASE EXPRESSING CELLS IN THE NERVOUS SYSTEM

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The α_3 Na^+,K^+-ATPase (α_3 NKA) is one of four known α isoforms of the mammalian transporter. A deficiency in the α_3 NKA associates with severe impairment of movement control, and in some cases, seizure disorder. Understanding the pathogenesis of these disorders is limited by incomplete knowledge of the expression of the various isoforms in brain tissue, as well as the challenges in exploring the functional alterations of these isoforms in electrophysiological studies. To address this problem the promoter of the mouse α_3 subunit gene (*Atp1a3*) for Na^+,K^+-ATPase was fused to a promoterless ZsGreen1 reporter gene, and α_3 NKA-ZsGreen1 transgenic mice were generated. Founder mice were bred to C57BL/6 wild-type (WT) mice, and three transgenic lines were established. Expression of ZsGreen1 does not interfere with expression of the endogenous α_3 subunit of NKA as evidenced by normal growth, phenotype and breeding capacity of α_3 NKA-ZsGreen1 mice in our colonies. Transverse and sagittal sections of whole brain from five adult transgenic mice were analyzed. Consistent with published results on α_3 NKA distribution, the display of ZsGreen1-labeled neurons varied considerably (non-uniform), with highest density observed in hypothalamic, midbrain, pontine, brain stem, deep cerebellar and select thalamic nuclei. Intensively labeled neurons were also present in the cerebellar cortex, neocortex, and hippocampus. ZsGreen1-labeling was not observed in glial cells or white matter-enriched brain regions. In electrophysiological experiments, discharges of ZsGreen1-labeled hippocampal interneurons were sensitive to 1 mM ouabain (a concentration too small to inhibit rodent α_1 NKA), while non-fluorescent interneurons did not show this sensitivity. Thus, the α_3 NKA-ZsGreen1 transgenic mice model constitutes a novel and versatile tool to elucidate the properties, regulation, and functional significance of α_3 NKA in its native environment.

PTW08-04

FOLLOWING STROKE, FACTORS LEAK FROM NEWLY FORMED BLOOD VESSELS, BUT ARE PREVENTED FROM ENTERING THE BRAIN BY ENDOCYTIC GLIAL SCARS

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Stroke is the leading cause of long-term disability in the United States, affecting 795,000 people annually. Following stroke, the blood brain barrier (BBB) is breached in the area of the lesion. One method of repair involves the formation of new blood vessels in the damaged region to deliver immune cells to participate in the clearance of dead tissue. Another method of repair involves reactive astrocytes joining together to seal off the damaged region to protect the surviving neuropil. Both of these processes are important components of the healing response to stroke. However, there have been no studies on the possibility that the formation of the glial scar and its segregation of the lesion from the rest of the brain, prevents the blood vessels that form in the lesion from developing into mature brain blood vessels that have tight junctions and astrocytic end-feet, which are key components of the BBB. Therefore, to extend our knowledge about restoration of the BBB following stroke, C57BL/6J mice underwent a distal hypoxic (DH) model of stroke and the structure and function of blood vessels within the lesion, and the structure and function of the glial scar, were evaluated at multiple time points for seven weeks following stroke.

PTW08-05

ROLE OF GLIAL CCR5 IN MEDIATING HIV-TAT AND OPIATE NEUROTOXICITY

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Despite the success of antiretroviral therapy, human immunodeficiency virus type 1 (HIV-1) persists in viral reservoirs harbored by certain CNS cell populations, including microglia. These infected and/or activated cells release viral proteins, such as transactivator of transcription (Tat) and a variety of pro-inflammatory factors such as CCL5, creating a positive loop of neuro-inflammation and resulting in sublethal and lethal neuropathology. These effects, synaptodendritic injury in particular, are thought to be the basis of a constellation of mild to moderate HIV-mediated CNS impairments, collectively known as HIV-associated neurocognitive disorders (HAND). Previous work from our lab and others has shown that opiates exacerbate these neurological effects, largely through actions at mu-opioid receptor (MOR)-expressing glia. The CCL5-CCR5 system may be critical in HIV neuropathology. CCR5 is a co-receptor for HIV entry, but also may be independently involved in promoting glial activation and migration to create a damaging environment for nearby neurons. Here we employ in vitro and in vivo HIV-1 Tat models to test the hypothesis that CCR5 is a point of convergence for interactive Tat and opiate-induced neurotoxicity. We performed repeated measure studies over a period of 72 hours on co-cultures of mixed glia and neurons obtained from

C57Bl/6 and/or CCR5 global knockout mice, treated with Tat ± morphine. Our studies confirmed that morphine worsened Tat-induced toxicity in wild-type co-cultures; substitution of CCR5-null glia reduced the loss of wild-type neurons in all treatment groups, implying a net neuroprotective effect. Neuroprotection was not seen in cultures of wild-type glia and CCR5-null neurons, suggesting that the presence of CCR5 on glia, but not on neurons is critical for neurotoxic Tat-morphine interactions. Parallel in vivo studies are ongoing in CCR5-null mice crossed with inducible Tat transgenic mice. These utilize a variety of behavioral paradigms to measure anxiety, motor skills and cognition - three areas of neurologic decline seen in HAND - and are designed to support our in vitro evidence of the importance of CCR5 in driving Tat/morphine-mediated neuronal damage. Support by DA034231 (KFH/PEK)

PTW08-06

TARGETING NEURON SPECIFIC ENOLASE IN REDUCING SECONDARY DAMAGE IN RAT SPINAL CORD INJURY MODEL

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Spinal cord injury (SCI) is a complex debilitating condition leading to neurological deficit and requiring a concerted multi-target approach to improve function. Spinal cord damage is complicated by an increase in damage from secondary injury mediated by activated microglia/macrophages and astrocytes. Recent studies suggest that elevation of Neuron Specific Enolase (NSE) in glial and neuronal cells triggers inflammatory cascades in acute SCI and induces secondary damages. Thus, there is an urgent need for a therapy that reverses secondary damages after SCI. The present study investigated the role of a novel enolase inhibitor ENOblock in a male Sprague-Dawley (SD) rat SCI model. Serum cytokines, chemokines, and NSE levels were evaluated in injured animals following treatment with vehicle alone or ENOblock. Spinal cord samples (S-2, S-3, S-4) were also analyzed for NSE and MMPs 2 and 9 in S-4 by Western blotting. The results indicated a significant decrease in serum inflammatory cytokines/chemokines and NSE levels after treatment with ENOblock. Data also showed that ENOblock treatment decreased MMPs 2 and 9 protein expression as determined by Western blot analysis. These results support the hypothesis that activation of glial cells and inflammation status can be modulated by regulation of NSE expression. Studies are underway to determine if ENOblock may prevent further damage through reduction of NSE-mediated effects in rats. Overall, cell surface expression of NSE is deleterious as it promotes extracellular degradation and production of inflammatory cytokines and chemokines, which damage neurons. Thus, reduction of NSE by ENOblock may have potential therapeutic implications in SCI.

PTW08-07

PERIVASCULAR STROMAL CELLS AND CNS INJURY

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Stroke is a leading cause of death in the United States and costs $34 billion yearly. Current treatments for stroke are few and require application in less than 3 hours following onset of symptoms. The development of novel therapies with broader application times that treat acute and chronic stages of stroke is an active area of pre-clinical research. Neuroprotective agents are the primary focus of new stroke therapies for the acute phase, however no therapies exist for the chronic, regenerative stage following stroke. CNS regeneration following injury is impaired by fibrosis. Perivascular stromal cells (PSC) are a major component of the fibrotic scar following brain injury like stroke. Prior to injury, PSCs surround large diameter blood vessels and occupy the same perivascular niche as vascular smooth muscle cells and pericytes. PSCs uniquely express the RA synthesis enzymes Raldh1 and 2 and collagen 1a1 (Col1a1). We find PSCs along with activated inflammatory cells synthesize RA in the stroke lesion and activate signaling in adjacent neurons and astrocytes. This sets up the possibility that PSCs have a role beyond fibrosis and may be important for RA-mediated regeneration following injury. The signal(s) in the injury niche that activates PSCs following injury has not been identified and no signaling pathways that underlie their fibrotic activity have been described. We are currently utilizing *Col1a1-GFP* mice to identify the cellular source of activation signals in the ischemic core and an *in vivo* model of stroke injury to obtain “activated” PSCs for unbiased gene profiling experiments.

PTW09 Neuroprotection and Repair

PTW09-01

NORADRENALINE PROTECTS NEURO2A AND C6 CELLS FROM BENZO[A]PYRENE INDUCED CYTOTOXICITY

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Benzo[a]pyrene (B[a]P) is a cytotoxic chemical pollutant known to cause neurobehavioral abnormalities by affecting neurons and glia in the brain. Their molecular resposes upon exposure to stressors, oxidative insults including by B[a]P and physiologically available antioxidant, noradrenaline (NA), are likely to differ, which we have explored in this study. We have evaluated the B[a]P-induced oxidative DNA damage and the role of NA in preventing it on Neuro2a and C6 cell lines *in vitro.* The cultured cells were treated with either B[a]P or NA alone or in combination; their viabilities were evaulated by methyl thiazolyl tetrazolium (MTT) assay, while their morphology were evaluated under phase constrast microscopy. The effect of B[a]P induced oxidative stress on the cell cycle status was analysed using flow cytometry, DNA damage was estimated by comet assay as well as by estimating the level of 8-OxodG and the intracellular Ca^{2+}-concentration was assessed using Fura 2AM. We observed that the B[a]P treatment reduced the cell viability and affected cytomorphology. It also affected the cell cycle, caused DNA strand breaks, increased 8-OxodG levels and intracellular Ca^{2+}-levels in both cell types although Neuro2a was more vulnerable; however all the effects were largely reduced by NA. Thus, B[a]P induces neuronal damage by increasing oxidative stress and that was prevented by NA possibly by reducing Ca^{2+}-influx. The findings suggest that the NA may be considered a potential protective agent against neurotoxicity induced by environmental contaminant, B[a]P in particular.

PTW09-03

MELATONIN AND QUERCETIN IN MITIGATES TITANIUM DIOXIDE NANOPARTICLES INDUCED NEUROTOXICITY: ROLE OF MITOCHONDRIA

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Nanotoxicology is the study of interactions and toxicological impact of nanomaterials (size: 1-100 nm) with biological systems that emphasis on illustrating the physical and chemical properties of the nanomaterials. Smaller size metal based Nanoparticles (NPs) have been shown to induce higher levels of cellular oxidative stress and can easily pass through the Blood Brain Barrier (BBB). Titanium dioxide nanoparticles (TNPs) have been widely used in day-to-day life in form of cosmetics, paints, sterilisation and have become a part of an industrial transformation developing strong materials used in many purposes due to their remarkable properties and there has been raising concern regarding the inimical effects of TNPs on central nervous system. Mitochondria, an important origin for generation of energy as well as free radicals and these free radicals can lead to mitochondrial damage and finally lead to apoptosis. This study investigates possible pathways by which titanium dioxide nanoparticles (TiO2) when intravenously administered to Wistar Rats could cross the BBB by employing both toxicity and mechanistic studies and also attenuation of TNPs in combination with Quercetin and Melatonin in hippocampus, striatum and cortex of brain of Rats under in vivo conditions. In our study, treatment with melatonin (5 mg/kg, orally) and quercetin (5mg/kg, orally) improved the toxicity effect caused by the TNPs (5mg/kg, i.v) in different sections of the brain. Findings indicate that TNPs may pose adverse health risk to mitochondrial brain with the generation of reactive oxygen species and lead to neuronal cell death; therefore it is in need to consider the proper usage of titanium dioxide nanoparticles.

PTW09-04

BRANCHED CHAIN FATTY ACID INDUCES NITRIC OXIDE-DEPENDENT CELL DEATH IN SH-SY5Y CELLS: PROTECTIVE ROLE OF MELATONIN

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Phytanic acid (PA) is a saturated branched chain fatty acid (BCFA) and major constituent of the human diet, predominantly found in dairy products, meat and fish. It is a degradation product from the phytol side chain of chlorophyll. Degradation of PA is known to occur mainly in peroxisomes via α-oxidation and in mitochondria via β-oxidation. Due to its β-methyl group present at the 3-position of the carbon atoms, PA cannot be β-oxidized. Alteration in the metabolism of PA may play an important role in the neurodegeneration but the exact mechanism behind it remains to be evaluated. In this study, we described the potential of BCFA such as PA to induce neurotoxicity in an *in vitro* model. In the present study, our data confirmed that PA markedly increased both the intracellular ROS and RNS levels. Moreover, we examined whether PA induced oxidative stress resulted in cell death. We confirmed that PA treatment did not induce cell death by cleavage of caspase-3/PARP-1 mediated by mitochondria through intrinsic pathways, but PA induced nitric oxide-dependent apoptosis in SH-SY5Y cells. However, we found that Mel pre-treatment reduced the cell death in SH-SY5Y cells. These results suggested that Mel acted as an antioxidative and antiapoptotic agent by modulating ROS, apoptotic proteins and inflammatory responses under BCFA-induced neurotoxic conditions. Although the precise mechanism involved in the protective effects of Mel against BCFA-induced damage to SH-SY5Y cells, is not understood, but the potential antioxidant and anti-apoptotic activities of Mel is useful for the suppression of oxidative stress-mediated neurotoxicity.

PTW09-05

CRYOPRESERVED DOPAMINE NEURONS DERIVED FROM HUMAN IPSCS REVERSE FUNCTIONAL DEFICITS IN A RODENT MODEL OF PARKINSON'S DISEASE

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Cryopreservation of post-mitotic, induced pluripotent stem cell derived midbrain lineage dopamine neurons (iPSC-mDA) is a significant advancement for cell therapy in Parkinson's disease. Here, we demonstrate that cryopreserved iPSC-mDA neurons are reliably thawed with excellent viability and maintain biochemical and physiological signatures indicative of human midbrain dopamine neurons. We also examined the engraftment potential of iPSC-mDA neurons after transplantation into both the rodent brain up to 6-months post-grafting and the nonhuman primate brain up to 3-months post-transplantation. Immunohistochemical analysis demonstrated robust graft survival and maintenance of the midbrain dopaminergic phenotype with extensive fiber innervation into the host. A long-term functional study revealed significant reversal in motor deficits in the 6-OHDA-lesioned rat model of Parkinson's disease that persisted for up to 6-months post-transplantation. Moreover, we found no evidence of cell proliferation, indicating safety in our initial studies. IND-enabling studies are currently underway to ascertain whether cryopreserved iPSC-mDA neurons are both safe and efficacious at longer time-points in both rodent and nonhuman primate models of Parkinson's disease. These results indicate considerable promise for the development of pluripotent cell-based therapies in Parkinson's disease.

PTW09-06

INTERLEUKIN-1 MAY FUNCTION AS A NEUROMODULATORY PATHWAY TO RESTRAIN EXCESSIVE EXCITATORY NEURONAL ACTIVITY

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Interleukin-1β (IL-1β) is a well-characterized cytokine of the innate and adaptive immune systems and is an important mediator in the communication between the peripheral immune and central nervous systems (CNS). Within the CNS, while it contributes to the pathogenesis of various neuro-inflammatory and neurodegenerative maladies, it also modulates certain physiological functions under normal conditions. Thus, IL-1β is a neuromodulator in both the normal and dysfunctional CNS. IL-1β is expressed constitutively in the hippocampus and this may be coupled with changes in excitatory neuronal activity. For example, its expression can be increased by acute seizure activity induced experimentally by the $GABA_A$ receptor antagonist, pentylenetetrazol (PTZ), or by the glutamate receptor and muscarinic receptor agonists, kainic acid (KA) and pilocarpine, respectively. However, the nature of its role as a neuromodulator of neuronal activity remains controversial. Hence, while some evidence suggests that IL-1β may enhance excitation in the brain, other results suggest otherwise, that IL-1β suppresses neuronal excitation. This paradox is likely due at least in part to the context in which it functions. The overall hypothesis of our research is that IL-1 signaling is an endogenous neuromodulatory pathway that contributes to the homeostatic balance between excitation and inhibition in the normal brain. The observation demonstrating that mice lacking IL-1 signaling are more prone to seizure activity raising the possibility that IL-1 signaling may serve to restrain excessive neuronal excitation. However, the molecular mechanisms involved in IL-1 signaling in the brain remain to be characterized fully. Therefore, the goal of our research is to explore the mechanisms that link this pathway to neuronal excitation. We have confirmed the basal expression of IL-1β in the hippocampus and demonstrated that its expression is linked to excitatory neuronal activity. We are currently exploring the localization and dynamics of IL-1 signaling in the context of hyper-excitation. This research will contribute to a better understanding of the role of this cytokine in the brain.

PTW09-07

A POTENTIAL ROLE OF PERICYTE IN REGULATING TJ FORMATION AND BBB INTEGRITY DURING STROKE-INDUCED VASCULAR REMODELING

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Angiogenesis and re-vascularization are the main repair processes activated following an ischemic stroke. However, angiogenesis-induced new vessels have a higher than normal permeability, which may exacerbate outcome in ischemic brain. Tight junction proteins (TJP) that form the tight junction (TJ) between endothelial cells (EC) play a key role in regulating the blood-brain barrier (BBB) permeability. Here, we demonstrated that microvascular pericytes play a critical role in the TJ formation and BBB restoration during stroke recovery.

Adult rats had a transient middle cerebral artery occlusion with reperfusion up to 8 weeks. At 3 weeks after stroke, the newly formed vessels induced by spontaneous angiogenesis in peri-infarct regions have abnormally high BBB permeability due to a lack of TJPs, occludin and zonula occludens-1 (ZO-1) in ECs. Along with the new vessels, pericytes expressed ZO-1, astrocytes expressed ZO-1, occludin, while ECs expressed claudin-5. The pericytes also overexpressed neural-glial antigen 2 (NG2) and other angiogenic factors. NG2 expressed by microvascular pericytes in angiogenic vessels plays a key role in EC migration and morphogenesis during neo-vascularization. Increased NG2 in pericytes surrounding the new vessels was also detected at 8 weeks after stroke. When expression of NG2 in human brain pericytes was down-regulated with siRNA targeted to NG2 in an *in vitro* 3D BBB model, the distribution of occludin and ZO-1 around and between ECs that tightly connects these ECs, seen in siRNA-free co-culture, are significantly attenuated in ECs co-cultured with the NG2-deficient pericytes. Rats treated with pro-angiogenic reagent resulted in increase of NG2 expression in pericytes at 8 weeks. TJPs, which was found in pericytes surrounding the new vessels in vehicle, was seen in ECs forming continuous linear strands in treated brain as the intact TJPs in vessels.

Our findings suggest a potential role of vascular pericytes and NG2 in TJ formation and BBB maturation in angiogenic vessels after stroke.

PTW10 Demyelination: Pathology, Protection and Repair

PTW10-01

MORPHOLOGICAL AND FUNCTIONAL CONSEQUENCES OF HIPPOCAMPAL DEMYELINATION AND REMYELINATION-INDUCED RECOVERY

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Background: Multiple Sclerosis (MS) is a chronic, demyelinating disease of the central nervous system that results in a variety of symptoms in patients. Cognitive impairments are reported in up to 50% of patients, with learning and memory deficits being among the most prevalent. The hippocampus has a well-documented role in memory formation and hippocampal demyelination is frequent and extensive in MS. To better understand the progression of demyelinated, hippocampal related, morphological and functional deficits, we exploited the chronic cuprizone-diet mouse model of MS and established pathological and behavioral deficits related to the CA1 region of the hippocampus.

Methods: 8-week-old C57BL/6 mice were fed a diet containing 0.2% cuprizone for 12 weeks. Some groups were switched to normal diet for 3-weeks to induce remyelination in the presence of vehicle or estrogen receptorβ ligand treatment. Assessment of spatial learning and memory was performed using Barnes maze. Golgi staining and immunohistochemistry were performed in hippocampus-containing brain slices.

Results: Barnes maze testing showed alterations in spatial learning and memory in demyelinated groups when compared to normal groups. Golgi analysis revealed significant decreases in CA1, stratum radiatum dendrite arborizations and significant recovery in remyelinated groups. Immunohistochemistry analysis revealed significant demyelination and alterations in pre- and post-synaptic proteins as well as atrophy of the CA1 pyramidal cell layer. Marked recovery in all stains was observed in remyelination groups. Electrophysiology analysis is underway to assess functional changes.

Conclusion: These preliminary data clarify that chronic demyelination has a significant effect on hippocampus related behavior and structure and support the idea that remyelination can repair the associated cogntivie deficits.

Grants supporting research: NMSS and NIH

PTW10-02

ANTIBODIES FROM MULTIPLE SCLEROSIS AND NEUROMYELITIS OPTICA CAUSE DEMYELINATION THROUGH DISTINCT TARGET-DIRECTED MECHANISMS

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Multiple sclerosis (MS) and neuromyelitis optica (NMO) are inflammatory immune-mediated diseases of the CNS characterized by myelin loss. Most NMO patients have a pathologic autoantibody response against aquaporin-4 (AQP4) that induces astrocyte-targeted complement-mediated cytotoxicity (CDC), oligodendrocyte loss and demyelination. The target of intrathecal oligoclonal IgG in the CSF of MS patients remains unknown. To understand the role of antibody in MS, we compared the effects of recombinant monoclonal antibodies (rAbs) derived from CSF clonal plasma cells isolated from MS and NMO patients using primary cortical cell cultures and organotypic cerebellar slices. Cell death in cell cultures was assessed by IncuCyte live imaging. Demyelination, gliosis, and cell death in slices were measured by immunohistochemistry. Cytokine levels were measured by multiplexed immunoassays. In cell cultures, NMO AQP4-specific rAbs #53 and #186 caused complement-dependent destruction of astrocytes. MS rAb#38, which binds to astrocytes and neurons, induced neuronal death; whereas, rAb#30, which targets myelin, caused complement-dependent mature oligodendrocyte death. In cerebellar slices, NMO rAb#53 induced rapid complement-dependent destruction of astrocytes, followed by sequential oligodendrocyte and neuronal death. In contrast, the MS rAbs demonstrated distinct patterns of injury. rAb#38 induced demyelination, oligodendrocyte loss and neuron death without complement. rAb#30 induced oligodendrocyte loss and demyelination without neuronal death in the presence of complement. Neither MS rAb resulted in a significant loss of astrocytes. NMO and MS rAbs induced distinct pro-inflammatory cytokine production. Media from cerebellar slices exposed to NMO rAb#53 and complement had increased levels of IL-1β and IL-6, while MS rAbs did not have this effect. Increases in TNF-α often accompanied demyelination. Our results demonstrated that recombinant antibodies generated from NMO and MS CSF mediate distinct target-directed injury.

PTW10-03

IN VIVO IMMUNOGLOBULIN-G DRIVEN MODEL OF MULTIPLE SCLEROSIS RECAPITULATES THE HISTOPATHOLOGICAL FEATURES OF MS LESIONS

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Background: Intrathecal IgG synthesis and oligoclonal bands (OCBs) are biochemical hallmarks of Multiple Sclerosis (MS) and are hypothesized to represent a pathologically relevant targeted B cell response. We have generated monoclonal recombinant antibodies (rAbs) that reproduce the *in vivo* specificities of expanded MS cerebrospinal fluid (CSF) plasma cell clones producing OCBs. We previously demonstrated that MS rAbs targeting both myelin and neuronal/astrocyte antigens mediate demyelination in ex vivo spinal cord cultures. Herein, we have examined the CNS pathology produced by MS rAbs in vivo following microinjection into mouse brain in the presence of human complement.

Methods: We microinjected MS and neuroinflammatory control rAbs into the brains of wildtype and PLP-eGFP mice in the presence of human complement. Mice were perfused with 4% paraformaldehyde (PFA) at days 1, 2, 3, 7, and 14, and brains removed, cryoprotected, and sectioned for histologic analyses. Myelin integrity, oligodendroglial cell death, neuronal injury, immune cell influx, complement activation, and repair were assayed by immunofluorescence microscopy.

Results: Injection of MS rAbs led to loss of oligodendrocytes followed by focal demyelination and influx of phagocytes containing MOG+ debris that continued until at least 14 days post-injection. Damage was accompanied by terminal complement activation and axonal damage. On day 7, immature NG2+/eGFP dull oligodendrocyte precursors begin to infiltrate lesions suggesting ongoing attempts at lesion repair.

Conclusions: Recombinant antibodies derived from MS CSF plasma cell clones produce CNS demyelinating lesions that bear hallmarks of human MS pathology when injected into mouse brain. MS rAbs binding to distinct antigenic and cellular targets likely vary in their capacity to produce disease and in the mechanisms used to initiate CNS damage. Intracerebral microinjection provides a novel IgG-driven model of MS pathology that may be used to define disease mechanisms and serve as a platform to test reparative therapies.

Supported by the NEI, NINDS, Mallinckrodt Pharmaceuticals, and the Guthy-Jackson Charitable Foundation.

PTW10-04

VISUAL DYSFUNCTION IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Optic neuritis (ON) is an inflammatory disease of the optic nerve that affects 80% of MS patients during disease progression. Similarly to MS, experimental autoimmune encephalomyelitis (EAE) has been observed to cause inflammation, demyelination, and axonal damage in the brain and spinal cord. Our lab has demonstrated that EAE is also associated with retinal ganglion cell loss, decreased retinal nerve fiber layer thickness, and demyelination in the optic nerve: hallmarks of ON. Although ON is a focus in MS pathology, many MS patients that do not report ON still demonstrate loss of visual function suggesting that other areas of the visual pathway are affected. In fact, similar to pathology in the brain and spinal cord, we saw inflammation, demyelination, and axon degeneration in the optic tract, lateral geniculate nucleus, and visual cortex of mouse EAE models. Our lab has been investigating the therapeutic effects of indazole chloride (Ind-Cl), a highly specific estrogen receptor β agonist which has been demonstrated to improve remyelination and mitigate inflammation in the brain and spinal cord. The therapeutic effect of Ind-Cl was assesed in the visual pathway. Results indicated oligodendrocyte and myelin loss, and increased inflammation and axonal damage in the optic tract after EAE induction. Oligodendrocyte and myelin loss were attenuated by Ind-Cl however inflammation and axonal damage remained. In the ventral lateral geniculate nucleus and visual cortex, a decrease in parvalbumin positive interneurons in the presence of EAE that was not mitigated by Ind-Cl was observed. These data suggest that it may be possible to measure EAE progression and drug therapeutic effects using the visual pathway. However, further studies are needed to confirm behavioral and functional recovery in the presence of Ind-Cl.

PTW10-05

AZETIDINE-2-CARBOXYLIC ACID (AZE)-INDUCED OLIGODENDROGLIOPATHY

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Rubenstein (2008) hypothesized that early life dietary exposure to the non-protein imino acid Aze results in its substitution for proline in CNS myelin proteins, (which normally are stable throughout life), and that the resultant myelin protein instability contributes to MS pathogenesis. Aze can be misincorporated in place of proline in

myelin proteins and induce their misfolding *in vitro*. Juvenile CD1 mice given Aze i.p. or p.o. (gavage) develop dose-dependent clinical signs, (weight loss, hind limb gait ataxia, tremors, ruffled fur and dyspnea but no paralysis), and a distinct oligodendrogliopathy. White matter (WM) oligodendrocytes (OGC) show watery swelling of nuclei and undergo apoptosis (TUNEL, caspase-3 IHC). There is a multifocal microglial reaction (Iba-1 IHC), increased OGC MHC I expression, and nuclear translocation of the transcription factor XBP1, (indicating ER stress/unfolded protein response [UPR] upregulation), and of NFBkB (a key factor in CNS autoimmunity). There is no detectable astrocytosis (GFAP IHC), myelin degeneration (LFB, MBP IHC), axonal injury (Bielschowsky, β-APP IHC), or leukocyte infiltration following acute exposure. Pregnant females and newborn CD-1 mice pups given Aze in drinking water showed no detectable clinical effects but Aze-exposed pups had more OGC apoptosis than their dams or age-matched, non-Aze-treated controls (5-7 pups/group; 3 dams/group), indicating that mice exposed in utero, (i.e. during CNS myelinogenesis), are more vulnerable to Aze effects. In summary, Aze-induces a non-inflammatory metabolic OGC injury that may result from ER stress/UPR activation and leads to apoptosis. This unique model has features not found in other OGC toxin or inflammatory MS models but that are reported in MS normal-appearing WM (Prineas, 2012; Bonetti, 2008; Mháille, 2008). Thus, in addition to determining effects of this specific dietary agent *in vivo*, this model will be useful for elucidating mechanisms of OGC alterations and myelin protein instability that may contribute to the lesion growth, failure of remyelination and WM degeneration that underlie clinical progression in MS patients. (Supported by Progressive MS Alliance PA 0082, SPARK and Gift Funds)

PTW10-06

UNWRAPPING IT: USING ZEBRAFISH TO INVESTIGATE DEMYELINATION IN VIVO

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Multiple Sclerosis (MS), a chronic, neurodegenerative disease, results from the immune system inappropriately attacking the insulating myelin sheath surrounding axons of the central nervous system (CNS). This attack leads to myelin destruction, axonal degeneration and a life often dependent upon walking aids and assistance. Oligodendrocytes, the myelinating glial cell population in the CNS, are targeted during MS attacks, leaving axons exposed and vulnerable to their environment. Unfortunately, the cellular mechanics underlying demyelination, which eventually lead to the exposure of naked axons, are not well understood. Mammalian models commonly used to study MS, including experimental autoimmune encephalomyelitis (EAE) and drug-induced demyelination, as well as patient data or clinical samples, do not allow us to investigate the processes underlying myelin destruction and repair *in vivo*. Although a generalized demyelination model exists in zebrafish that utilizes the nitroreductase-mediated ablation system, a major limitation of this model is the inability to create focal lesions, which are a hallmark of MS pathology. Therefore, we have created a novel, drug-induced focal demyelination model in zebrafish. Using microinjection, we deliver a demyelinating lysolecithin cocktail solution focally and precisely into the spinal cord of zebrafish larvae. Preliminary characterization of this model demonstrates that the lysolecithin cocktail has an affect on oligodendrocytes as we observe a significant reduction in their number 8 hours post injection within the lysolecithin cocktail dispersal region. Furthermore, *in vivo,* time-lapse imaging of the *mbp:EGFP-CAAX* transgenic zebrafish line demonstrates changes in *mbp*$^{+}$ membrane, as evidenced by the appearance of myelin ovoid-like structures and other myelin defects. Using this focal drug-induced demyelination model, we will investigate cellular mechanics and molecular mechanisms underlying myelin destruction as it occurs in real time, in a living vertebrate organism, a feat that is currently not possible in mammalian models.

PTW10-07

OLIGODENDROCYTE MATURATION THROUGH GESTATIONAL IRON DEPRIVATION: THE ROAD NOT TAKEN

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Iron deficiency (ID) is, according to the WHO, the most common nutritional deficiency in the world and the second one in economic impact. This deficiency affects oligodendrocyte (OL) maturation, causing hypomyelination which continues in adulthood even after normal iron diet reinstatement. In this context, our fundamental aim is to elucidate the role of iron during OL maturation and myelination processes. We have previously demonstrated that gestational ID produces alterations in myelin composition, abnormal migration and maturation of oligodendroglial precursor cells (OPC) and altered myelin structure. To further describe the whole population of OL, we have now focused on four aspects: a) OL morphological architecture, which reflects OL maturation, b) the timing of dysmyelination in different brain areas, c) the expression pattern of markers for different stages of OL lineage along ontogenetic myelination and d) OL interaction with different cell types within normal CNS cytoarchitecture, such as relative abundance of GAP junction proteins and astrocyte distribution pattern. To this end, we used an eGFP::CNPase transgenic mouse experimental model, whose green-fluorescent OL-lineage-committed cells (CNPase-positive cells) allow the visualization of the entire myelin structure, as well as the analysis of single OL morphology and population features. Pregnant mice were fed an iron-deficient diet (4mg/g/kg) as from gestational day 5 until pup weaning (post-natal day 21, P21). Plates within the anterior-posterior axis were evaluated at P15 and 30, and CNPase-positive cell distribution was quantified in prefrontal cortex, corpus striatum and corpus callosum. Control animals evidenced an increase in OL complexity both during ontogenetic development and along the anterior-posterior axis; in particular, OL population exhibited the greatest mature proportion at posterior position. In turn, ID animals exhibited fewer CNPase-positive cells, with prevalence of immature OL, as tested by specific markers and confirmed by a decrease in MBP expression. We conclude that low iron availability does not affect cell lineage

specification but expands an arrested OPC population, which is responsible for hypomyelination.

PTW10-08

REGULATION OF LIPID SYNTHESIS IN OLIGODENDROCYTES AFFECTS PROCESS FORMATION AND MYELIN PROTEIN EXPRESSION

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The myelin membrane is as much as 70% lipid, including cholesterol, glycosphingolipids and saturated long chain fatty acids. Although it has been shown that cholesterol is essential for myelin membrane formation, the control of lipids synthesis in oligodendrocytes, the myelinating cells of the CNS, are poorly understood. In liver, adipose tissue and other tissues where lipid synthesis is crucial, major intrinsic factor necessary to regulate are sterol regulator element binding proteins, SREBPs. SREBP1 and SREBP2 and their associated regulatory proteins function as sterol sensors to increase sterol production if levels fall too low. A role for SREBPs have been identified in Schwann cells but remarkably, not in oligodendrocytes, even though these cells increase their membrane production 6,500 fold during maturation, most of which is cholesterol. Using well-characterized purified cultures of oligodendrocyte lineage cells, we found that both SREBP1 and SREBP2 are present in oligodendrocyte precursor cells and oligodendrocytes. To inhibit SREBP signaling, we used inhibitor of site-1 protease (S1P) that normally cleaves SREBP to allow the mature form of the protein to enter the nucleus and activate transcription of target genes. Inhibition of SREBP processing blocked oligodendrocyte process growth and extension and reduced levels of myelin basic protein but not proteolipid protein or galactocerebroside. The effect was reversible upon removing the inhibitor. Blocking SREBP processing also down-regulated key target genes involved in both the fatty acid and cholesterol synthetic pathways; fatty acid synthase, hydroxyl-methyl-glutaryl CoA reductase and acetyl CoA carboxylase. De novo cholesterol synthesis was also dramatically decreased as measured by mass spectrometry. These results suggest that SREBPs function in oligodendrocytes to regulate myelin formation as well as lipid synthesis.

PTW10-09

CHRONIC DEMYELINATION INDUCED SEIZURES

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Importance: Multiple sclerosis (MS) patients are three to six times more likely to develop epilepsy compared to the wider population. Seizures are more common in patients with early onset or progressive forms of the disease and prognosticate rapid progression to disability and death. Grey matter atrophy, hippocampal lesion, and elevated juxtacortical lesion burden have been identified in MS patients with seizures, however translational studies aimed at the pathophysiological processes underlying MS epileptogenesis remain extremely limited. We utilized electroencephalography (EEG) and confocal imaging to correlate hippocampal recordings to histopathology findings in chronically demyelinated C57BL/6 mice with recurrent seizure activity.

Methods: Chronic demyelination was induced for 9 to 12 weeks by cuprizone (CPZ) diet. Bipolar stainless steel wire electrodes were chronically implanted in the right dorsal hippocampus 8 weeks after CPZ treatment and longitudinal EEG recordings were obtained at 9 and 12 weeks. Brain tissue was collected to visualize myelination, oligodendrocyte (OL) health, microgliosis, and CA1 neuron density by confocal microscopy.

Results: Spontaneous seizures were observed in a subset of mice, and depth hippocampal EEG analysis demonstrated significant increases in EEG power between 9 and 12 weeks of CPZ diet. Demyelination and fraction of caspase 3+olig2+ apoptotic OLs in the corpus callosum plateaued by 9 weeks, accompanied by increased Iba1+ microglia/monocytes present. Significant decrease in olig2+ nuclei in CA1 and decrease in NeuN+ pyramidal cell layer thickness was observed at both 9 and 12 weeks. Microglia/monocytes remained low in CA1 from 9 weeks CPZ mice, but were dramatically elevated at 12 weeks.

Conclusion: Chronic demyelination is sufficient to induce seizures that are characterized by diminished OL/OL precursor viability in the corpus callosum, progressive contraction of pyramidal cell soma, and pronounced infiltration of microglia/macrophages into the epileptic CA1.

PTW10-10

ENHANCED ERK1/2 SIGNALING IN OLIGODENDROCYTES OF ADULT MICE LEADS TO HYPERMYELINATION

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Multiple sclerosis (MS) is a disease characterized by chronic demyelination in the central nervous system (CNS). Demyelinated lesions often remyelinate during early stages of the disease; however, over time oligodendrocyte precursor cells (OPCs) in and around chronically demyelinated lesions fail to differentiate and remyelinate axons, resulting in devastating physical disabilities. We have previously shown that sustained activation of the ERK1/2 pathway is sufficient to drive increased myelination during development. Here, we examined whether oligodendrocytes (OLs) in the adult mouse CNS can reinitiate myelination after forced activation of the ERK1/2 pathway. We generated mice that express a constitutively active variant of the ERK1/2 upstream kinase *Mek1* (*Mek1*DD) following tamoxifen inducible Cre-expression specifically in PLP+ OLs. Sustained activation of ERK1/2 was confirmed in *PLP-CreERT2; Mek1DD/+* mice 2 months following injection of tamoxifen in adult mice. Co-staining with CC1 (to mark mature OLs) and pERK1/2 showed that ERK1/2 activation more than doubled in the corpus callosum and the spinal cord white matter of mutant mice. Analysis of myelin ultrastructure using electron microscopy revealed significantly thicker myelin in the corpus callosum, spinal cord, and optic nerve, a remarkable phenotype given the evidence that mature OLs normally only myelinate shortly after their differentiation. The thicker myelin was a result of excess myelin production by each OL, as the number of mature OLs did not change with sustained ERK1/2 activation. Since changes in myelin thickness could lead to inferior neural function, we next performed a battery of behavioral assays to specifically assess motor and cognitive behaviors. No major differences were identified in mutant mice compared to control littermates during all of the behavioral tests performed. Downstream targets of the ERK1/2 pathway that mediate this myelin growth phenotype are now being actively pursued as they may represent suitable targets for the treatment of demyelinating diseases.

PTW10-11

GENOME-WIDE FUNCTIONAL SCREENING IDENTIFIES MIRNA REGULATORY NETWORK OF OLIGODENDROCYTE PROGENITOR CELL DIFFERENTIATION

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Oligodendrocyte progenitor cells (OPCs) represent the largest stem cell population in the central nervous system and are the principal source of myelinating oligodendrocytes. In demyelinating disorders such as multiple sclerosis (MS), enhancing the generation of new oligodendrocytes from native OPCs is a highly sought after treatment strategy to regenerate myelin. However, precise control of OPC differentiation in vivo remains a challenge. Towards this goal, we recently developed a method to generate pure populations of OPCs in large numbers from mouse pluripotent stem cells. Here, we leveraged this scalable resource to perform a high-throughput phenotypic screen using high content imaging analysis to evaluate the ability of all known microRNAs (miRNAs) to modulate the differentiation of OPCs. Testing of a miRNA mimic and a miRNA inhibitor for all 1,309 miRNAs (as of miRBase 19) enabled discovery of a novel set of miRNAs that function to control the differentiation of OPCs into mature oligodendrocytes. Combining small-RNA and mRNA sequencing with the functional screening data, we define the global miRNA-mRNA interaction network that regulates oligodendroglial cell fate and function. Given the ability of miRNA mimics and inhibitors to be delivered to the CNS, top hits from our screen provide promising candidates to be tested as RNA therapeutics in demyelinating diseases like MS.

PTW11 Gene Expression/Regulation

PTW11-01

EARLY EPIGENETIC CHANGES IN BLADDER SENSORY AND SPINAL NEURONS CONTRIBUTE TO VISCERAL HYPERALGESIA AND CHRONIC BLADDER PAIN

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Introduction and objectives: Chronic bladder pain involves epigenetic regulation of genes in sensory and spinal neurons. Our previous studies identified changes in a number of nociception-related genes (*e.g.* calcitonin gene-related peptide, CGRP) which activity can be modulated by chromatin-modifying repressor, Methyl-CpG-binding protein 2 (MeCP2). The objective of this work was to examine if prolonged up-regulation of CGRP expression by MeCP2 in bladder sensory and spinal neurons contribute to chronic bladder pain syndrome.
Methods: Neurogenic bladder pain in rats was induced by transient colonic inflammation due to cross-sensitization in pelvic neural pathways. Bladder-projecting sensory neurons were identified by retrograde labeling with a fluorescent dye. Cellular distribution and expression levels of MeCP2 and pMeCP2 in bladder sensory neurons and lumbosacral (LS) spinal cord were evaluated under control conditions and after experimental intervention.
Results: Neurogenic bladder pain was associated with an up-regulation of CGRP gene in bladder sensory neurons and its regulator, pMeCP2, was maximal at 1h in LS spinal cord. These changes occurred in parallel with an increase in phosphorylation of cAMP response element-binding protein (CREB) in the same neurons. MeCP2 expression in bladder DRG neurons was increased 2h after transient colitis when compared with control ganglia. MeCP2 positive neurons were co-labeled with CREB in the spinal cord sections. pMeCP2 positive cells were predominantly located in the dorsal horn of LS spinal cord confirming their activation by nociceptive signaling.
Conclusions: These results suggest that the changes in chromatin compaction regulated by the binding of MeCP2 complexes to methylated DNA are involved in modulation of CGRP gene expression in the spinal cord and sensory ganglia during pelvic organ cross-sensitization. These early epigenetic changes may contribute to visceral hyperalgesia and chronic pain in functional pelvic pain disorders such as bladder pain syndrome.

PTW11-02

THE WMN1 ENHANCER REGION IN INTRON 1 IS REQUIRED FOR EXPRESSION OF HUMAN PLP1

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The myelin proteolipid protein gene (*PLP1*) encodes the most abundant protein present in myelin from the central nervous system (CNS). Its expression must be tightly controlled as evidenced by mutations that alter *PLP1* dosage; both overexpression (elevated*PLP1* copy number) and lack thereof (*PLP1* deletion) result in X-linked genetic disorders in man. However not much is known about the mechanisms that govern expression of the human gene. To address this, transgenic mice were generated which utilizes human *PLP1* (*hPLP1*) sequences (spans proximal 6.2 kb of 5'-flanking DNA to the first 38 bp of exon 2) to drive expression of a *lacZ* reporter cassette. *Lox*P sites were incorporated around a 1.5 kb section of *hPLP1* intron 1 because the orthologous sequence in mouse (wmN1) was shown to augment expression of a minimally promoted transgene, coincident with the active myelination period of CNS development. Eight transgenic lines were generated with the 6.2hPLP(+)Z/FL ('parental') transgene. All lines expressed the transgene appropriately in brain as determined by X-gal staining of white matter regions and olfactory bulb. Deletion of the sequence orthologous to the mouse wmN1 enhancer region by Cre recombinase caused a marked decrease in transgene activity compared with the parental line. These results demonstrate for the first time that the wmN1 enhancer: (i) is functional in *hPLP1*; (ii); works in collaboration with its native promoter, not just a basal heterologous promoter; (iii) is required for high levels of *hPLP1* gene activity; (iv) has a broader effect, both spatially and temporally, than initially predicted.

PTW11-03

HIGHLY PURIFIED ADULT ASTROCYTES DISPLAY DISTINCT STIMULUS-DEPENDENT ACTIVATION AND CYTOKINE SECRETION PATTERNS

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Astrocytes are the most abundant cell type in the mammalian brain. However, little is known about their functions in inflammatory diseases such as multiple sclerosis. This is partly due to technical difficulties in isolating highly purified astrocytes from adult brains. In the past, we have set up an automated procedure for dissociation of neonatal brain using the gentleMACS™ Octo Dissociator with an optimized enzymatic treatment. For isolation and culture of adult neural cells, we have further improved the method and included a novel protocol for removal of cell debris and erythrocytes, yielding 2–4×106 living neural cells per adult mouse brain. To assess and compare neonatal and adult astrocyte diversity, we isolated astrocytes using MACS® MicroBeads coupled to a specific pan-astrocyte marker, Anti-ACSA-2 (astrocyte cell surface antigen-2). We then analyzed their transcriptome by mRNA sequencing of single cells with the C1 Single-Cell Auto Prep System (Fluidigm®). The single-cell transcriptome analyses revealed particular molecular profiles for both neonatal and adult astrocytes. Next, we characterized the cytokine secretion profile of activated adult astrocytes. A distinct profile dominated either by GM-CSF or IL-23 was detected after activating adult astrocytes with TNF-α (or IL-1β) or IFN-γ, respectively. This points to diverse astrocyte subtypes capable of reacting to different stimuli.

PTW11-04

CHOLINERGIC MICRORNA-132 SHEDS NEW LIGHT ON THE LINKS BETWEEN STRESS AND METABOLIC IMPAIRMENTS

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Stressful experiences induce metabolic impairments due to mechanisms that are not fully understood. MicroRNAs (miRNAs) are short non-coding RNAs, 20-25 nucleotides long that simultaneously regulate multiple genes in entire biological pathways. Their roles as stress and metabolic modulators have been independently studied in the brain and in peripheral tissues, while stress-induced changes in brain-to-body miRNA communication as an inducer of metabolic impairments remained unexplored. Here, we report that tetracycline-regulated overexpression in peripheral tissues of the stress-inducible miRNA-132, which regulates numerous neuronal functions via multiple targets, initiates an antisense oligonucleotide-suppressible fatty liver phenotype. Predator scent-induced anxiety induces hippocampal miRNA-132 elevation within 30 minutes, mediating stress-inducible cognitive deficits and anti-inflammatory effects through its acetylcholinesterase target. Strikingly, transgenic mice with peripheral tetracycline-inducible miRNA-132 overexpression presented increased body weight, serum LDL/VLDL and liver-triglyceride levels accompanied by elevated hepatic pro-steatotic transcripts and decreased hepatic miRNA-132 target transcripts. Moreover, non-transgenic diet-induced obese mice with extensive hepatic steatosis, showed similarly increased hepatic miRNA-132, and intravenously injected anti-miRNA-132 oligonucleotides fully reversed this hepatic miRNA-132 excess and hepatic steatosis within one week, reducing liver triglycerides and serum LDL/VLDL by co-elevating multiple lipolysis-associated transcripts. Furthermore, lean LDLR$^{-/-}$ mice, a murine model of familial hyperlipidemia, also presented hepatic miRNA-132 excess, and its antisense-mediated knockdown reduced serum LDL/VLDL levels, indicating global involvement of miRNA-132 in hepatic homeostasis. Our findings suggest that chronic stress-induced elevation of miRNA-132 may exacerbate multiple biological processes in both brain and body tissues, co-expediting cholinergic neurotransmission while blocking inflammatory events and impairing metabolic functioning by enhancing hepatic lipid storage.

PTW12 The Synapse: Signals and Plasticity

PTW12-01

THE BLOCKADE OF NEUROTENSINERGIC NTS2 RECEPTOR INHIBITS NITRIC OXIDE PRODUCTION AND ENHANCES NORADRENALINE UPTAKE AT CNS

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Neurotensin behaves as a neuromodulator or as a neurotransmitter interacting with NTS1 and NTS2 receptors, which bind the peptide with high and low affinity, respectively. Neurotensin modulates Na/K-ATPase activity, an essential enzyme for the maintenance of ionic gradients. Na/K-ATPase properties can be modified by the administration of L-NAME, an inhibitor of nitric oxide (NO) synthesis and by levocabastine, an antagonist for NTS2 receptor. In the search of a potential interaction between neurotensin NTS2 receptor and nitric oxide synthase (NOS), levocabastine was administered to rats and NOS activity was evaluated in cerebral cortex synaptosomal membranes. Taken into account the relationship between catecholaminergic and neurotensinergic systems and the action of catecholamines on the activity of NOS, it was of interest to study the potential relationship between NTS2 receptor and ^{3}H-noradrenaline uptake by hypothalamus slices. Levocabastine (50 µg/kg, i.p., 30 min) or vehicle (saline solution) was administered to male Wistar rats. One group of rats was employed to obtain cerebral cortex synaptosomal membranes by differential and gradient centrifugation for evaluation of NOS activity. In another group of animals, anterior and posterior hypothalamic sections were processed for the assay of ^{3}H-noradrenaline uptake. NOS activity in synaptosomal membranes was decreased roughly by 50% both by levocabastine administration or by in vitro addition of 10^{-5}M levocabastine. Concomitantly, levocabastine administration enhanced ^{3}H-noradrenaline uptake by 35% and 12% in anterior and posterior hypothalamus, respectively. Results suggested an interrelationship between NTS2 receptor, NO synthesis and noradrenaline levels at central nervous system.

PTW12-02

THE EFFECTS OF ASTROCYTE-SPECIFIC DELETION OF EPHRIN-B1 ON SYNAPTIC STRUCTURE, FUNCTION, AND MOUSE BEHAVIOR

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Astrocytes play an important role in synapse formation and function making a critical component of a tripartite synapse with astrocytic processes surrounding the presynaptic bouton and postsynaptic dendrites. While several astrocyte-secreted factors have been implicated in synaptogenesis, our knowledge of contact-mediated glial cues that are involved in synapse development is limited. In our studies we investigated the role of astrocytic ephrinB1 in synapse formation in adult mouse hippocampus. Ephrin-B1 is a membrane-bound protein that acts as a ligand for ephrin-B receptor (EphB) activating a series of intracellular signals allowing for cell-cell interactions that mediate either cell adhesion or repulsion during axon guidance, cell migration and proliferation, and synaptogenesis, particularly at excitatory synapses. To study the role of astrocytic ephrinB1 a Cre/LoxP system was used; Cre-inducible loss of ephrinB1 in developing astrocytes was achieved by crossing homozygous floxed ephrinB1 female mice (ephrinB1$^{lx/lx}$) with Cre^{GFAP} male mice and tamoxifen-induced ephrinB1 ablation from adult astrocytes was achieved in ephrinB1$^{lx/lx}$ERT2-Cre^{GFAP} mice. We found a significant increase in the number of excitatory synapses in the SR area of CA1 hippocampus of ephrinB1$^{lx/lx}$Cre^{GFAP} mice identified by co-immunostaining for vGlut1 (presynaptic bouton) and PSD95 (postsynaptic sites). In contrast, we observed impaired maintenance of long-term potentiation in the CA1 hippocampus of ephrinB1$^{lx/lx}$Cre^{GFAP} and ephrinB1$^{lx/lx}$ERT2-Cre^{GFAP} adult mice following stimulation of presynaptic Schaefer collaterals. Along with these changes in synaptic structure and function within the hippocampi, behavioral changes were also observed. Sociability was determined using three-chamber test and showed a decrease in sociability of ephrinB1$^{lx/lx}$Cre^{GFAP} mice as compared to WT mice. Hippocampal learning and anxiety were also tested after astrocyte-specific ablation of ephrinB1 from the adult astrocytes using contextual fear conditioning test. Future studies will determine the molecular basis for ephrinB1 signaling in astrocytes and its role during various developmental stages of synapse development.

PTW12-03

AGING AND DISEASE ALTER THE RNA INDUCED SILENCING COMPLEX AT THE NEUROMUSCULAR JUNCTION

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The ability of microRNAs to mediate stress-responses in skeletal muscles, including neuromuscular junctions (NMJs), depends on the function of a variety of proteins. Here, we examined levels and distribution of genes involved in microRNA biogenesis, transport and translational repression in skeletal muscles and their synapses. We found that transcripts for DGR8, Drosha, Exportin-2, Dicer and argonaute 2 (Ago2) decrease as muscles mature. Corroborating these findings, we found reduced levels of Ago2 protein in muscles from juvenile and young adult compared to newborn mice. We next visualized the distribution of Ago2 in the extensor digitorum muscles from developing and young adult mice using light microscopy. We found Ago2 dispersed throughout sarcolemma in developing muscle fibers. As mice transition into adulthood, we found that Ago2 gradually concentrates at the NMJ, and primarily abuts acetylcholine receptors within the sarcolemma. However, Ago2 fails to concentrate in ALS-afflicted, injured and aging NMJs. Additionally, we found reduced expression of Ago2 in muscles of aged sedentary mice. To further examine the relationship between Ago2 and changes at the NMJ, we asked if exercise, which has been shown to slow and even reverse aging of NMJs, prevents loss of Ago2 from aged muscles. We found higher levels of Ago2 in aged muscles following a long- and a short-term interval of exercise compared to muscles from age-matched sedentary mice. Together, these findings suggest that restoring Ago2 levels and normal distribution may mitigate degeneration of NMJs during aging and progression of diseases.

PTW12-04

EXPRESSION AND FUNCTION OF FGF BINDING PROTEINS IN THE BRAINS OF DEVELOPING AND ADULT MICE

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Fibroblast growth factor binding proteins (FGFBP) bind and enhance the biological activity of FGF ligands, including those with critical functions in the development of neurons and their synapses. While the function of FGFBPs has been examined in a variety of tissues, little is known regarding their role in the brain. Here, we present data strongly suggesting important roles for FGFBP-1 and FGFBP-3 in brain synapses. We found transcripts for both factors highest in developing cerebral cortex, hippocampus, and cerebellum of mice. Corroborating these findings, FGFBP-1 and FGFBP-3 are more abundant in immature hippocampal neurons in culture. To assess the function of FGFBPs on neurons, we examined cultured hippocampal neurons treated with these factors alone and together with FGF ligands shown to promote synaptogenesis. Compared to controls, hippocampal neurons treated with FGFBPs contained more synaptic-like varicosities. We then examined the impact of deleting FGFBP-1 in the development of hippocampal and cortical circuits in mice. The absence of FGFBP-1 slowed the maturation of these circuits. Deletion of FGFBP-1 also affected the stability of select hippocampal and cortical synapses during normal aging. Together, the findings suggest important functions for FGFBPs at brain synapses.

PTW13 Lipids: Biology and Pathobiology

PTW13-01

LIPID ABNORMALITIES IN MICE DEFICIENT IN PERIPHERAL MYELIN PROTEIN 22

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Altered expression of peripheral myelin protein 22 (PMP22) is associated with hereditary peripheral neuropathies, primarily involving myelin-forming Schwann cells. To study the role of PMP22 in myelin biology we engineered a PMP22-deficient mouse model, which in the heterozygous form models hereditary neuropathy with liability to pressure palsy. Homozygous PMP22-deficient mice display severely hypomyelinated nerves and die around 2-3 months of age. Recently, we reported perturbations in peripheral nerve cholesterol levels in affected mice and found accumulation of apolipoprotein E (apoE) in nerves and Schwann cells. As the liver is the major organ in regulating cholesterol homeostasis, we extended our studies of PMP22-deficient mice from nerve to liver. On gross examination, at 2-3 months of age, PMP22-deficient mice have significantly enlarged livers (20-30% by weight), a phenotype that is more pronounced in males. Histological examination of liver sections indicate hepatosteatosis, with pronounced vacuoles on H&E stained specimen, and accumulation of Oil Red O and Nile red-positive lipid droplets on cryosections. We also detected elevated levels of apoE in the liver of 2-month old PMP22-deficient mice, while plasma apoE levels are decreased by over 30%. In addition, we discovered altered expression of several lipid metabolism-related genes in liver samples, including lipin 1, LRP1 and LDLR. Along with the observed abnormalities in liver, we found irregular, clumped distribution of polar and neutral lipids in the sciatic nerves of affected mice. Double immunolabeling of nerve sections with CD11b and apoE antibodies indicate that while endoneurial macrophages are positive for apoE, they do not account for the significantly elevated levels of apoE. In keeping with this, we found that cultured Schwann cells from PMP22-deficient mice accumulate apoE despite secreting significantly less during a 24h culture period. Together, these findings suggest that PMP22 plays a previously unrecognized role in lipid homeostasis, which may contribute to the pathogenesis of PMP22-linked neuropathies.

PTW13-02

ETHANOL, PHOSPHOLIPASE A2 SIGNALING, AND NEURODAMAGE IN ADULT RAT BRAIN

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Repeated ethanol binges in adult rats elicit hippocampal (HI) and entorhinal cortical (EC) neurodegeneration accompanied by significantly increased intraregional levels of cytosolic phospholipase A_2 (cPLA$_2$ GIVA), its phosphorylated (activated) form, and secreted PLA$_2$ (sPLA$_2$ GIIA)—whereas regions lacking neurodamage displayed no increases. Similar PLA2 elevations occur in rat adult-age (~60 d) organotypic HI-EC slice cultures binged for 4 days with ethanol (100 mM), concomitant with oxidative stress-dependent neurodegeneration as measured using propidium iodide (Tajuddin et al., PLoS 2014). With the slice cultures and PLA$_2$ inhibitors we examined whether PLA$_2$-mediated release of neuroinflammatory lipid mediators--notably, arachidonic acid-- is involved in the ethanol-provoked neurodamage. Indeed, addition of cPLA$_2$ inhibitor, arachidonyl trifluoromethyl ketone (5-25 μM), significantly suppressed 63-87% of neurodegeneration in ethanol-binged HI-EC slices. Likewise, treatment of HI-EC slices with the sPLA$_2$ inhibitor, aristolochic acid (50-500 μM), prevented nearly all of the binge ethanol-induced neurodamage. Exploring possible upstream regulators of the PLA$_2$ enzymes, we examined with an aquaporin-4 (AQP4) inhibitor, tetraethyl ammonium chloride (TEAC), the possible role of this water channel, since it was also elevated by ethanol binges (Tajuddin et al., 2014), is linked by others to neuroinflammation, and could underlie our previously documented brain edema. Throughout a range of 0.01-1 mM, TEAC prevented ~80% of ethanol-related neuronal death; however, abrogation of cPLA$_2$ and sPLA$_2$ potentiation ensued only at the highest TEAC concentration, suggesting that neurodegenerative pathways in addition to the above PLA$_2$ enzymes were being triggered by AQP4. In summary, the emerging scheme for neuroinflammatory lipid signaling encompasses elevation and activation of cPLA$_2$ and sPLA$_2$, promoted only in part by AQP4 elevations, in binge ethanol-induced brain neuronal demise. Supported by NIH U01 AA018279 (MAC).

PTW14 Late Breaking Abstracts

PTW14-01

BRAIN FATTY ACID SYNTHESIS INHIBITION WORSENS STROKE OUTCOMES IN THE RODENT MODEL

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Previously, our laboratory demonstrated a neuron-specific increase of fatty acid (FA) synthesis from glutamate under hypoxia conditions in vitro. Based on our in vitro data, we hypothesized that FA synthesis increase from glutamate may have a protective role under hypoxia. In the present study, we tested this hypothesis in vivo in the rodent stroke model. A transient stroke in mice was modeled using a middle cerebral artery occlusion with microfilament. To decrease brain FA synthesis, a pharmacological inhibition was induced by i.c.v. drug injection. Inhibition of brain FA synthesis significantly decreased fatty acid synthesis from glutamate, and increased brain glutamate levels under basal conditions. Under stroke, FA inhibition significantly increased neurological deficiency score, edema, and infarct volume. These data support our hypothesis that increased FA synthesis under hypoxia/ischemia conditions may have a protective role for neuron survival in vivo under stroke. Previously, our laboratory demonstrated a neuron-specific increase of fatty acid (FA) synthesis from glutamate under hypoxia conditions in vitro. Based on our in vitro data, we hypothesized that FA synthesis increase from glutamate may have a protective role. In the present study, we tested this hypothesis in vivo in the rodent stroke model. A transient stroke in mice was modeled using a middle cerebral artery occlusion with microfilament. To decrease brain FA synthesis, a pharmacological inhibition was induced by i.c.v. drug injection. Inhibition of brain FA synthesis significantly decreased fatty acid synthesis from glutamate, and increased brain glutamate levels under basal conditions. Under stroke, FA inhibition significantly increased neurological deficiency score, edema, and infarct volume. These data support our hypothesis that increased FA synthesis under hypoxia/ischemia conditions may have a protective role for neuron survival in vivo under stroke.

NIH Grant 5R01AG042819-04 and NIH funded COBRE Mass Spec Core Facility Grant 5P30GM103329-04

PTW14-02

REACTIVE ASTROCYTES CONTRIBUTE TO TUMOR-ASSOCIATED EPILEPSY

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Up to 70% of patients with primary brain tumors suffer from seizures during the course of the disease. High levels of extracellular glutamate have been reported in glioma patients and animal models of glioma, which are likely the cause for seizures in these patients. In the healthy brain, astrocytes are responsible for ion- and neurotransmitter homeostasis and are capable of clearing even millimolar levels of glutamate. We therefore hypothesized that the homeostatic functions of astrocytes surrounding brain tumors are impaired perpetuating seizure generation.

In order to address a potential role of astrogliosis in the generation of tumor-associated epilepsy, we took advantage of a xenograft mouse model of glioma that presents with an increasing frequency of seizures during the course of the disease. Strong astrocyte activation characterized by increased expression of intermediate filaments in all astrocytes, changes in morphology and proliferation of a subset of astrocytes was found in regions surrounding the tumor.

In mature quiescent astrocytes, high expression of the potassium transporter Kir4.1 is required for K^+ clearance from the extracellular space and generates the negative resting membrane potential of astrocytes at ca. -85mV. Whole cell patch clamp recordings of astrocytes in acute brain slices of tumor-bearing or sham operated animals showed a reduction of K^+ currents and corresponding depolarization of astrocytes at rest and impaired potassium uptake.

Glt-1 and GLAST transporters take up glutamate more efficiently at negative resting potentials. Since Kir4.1 establishes a significant portion of the resting membrane potential, impairment of this channel should also compromise glutamate uptake. Additionally, expression levels of Glt-1 were reduced, whereas GLAST expression was increased in astrocytes surrounding the tumor.

In summary, astrocytes were severely reactive in close proximity of the tumor. This affects their ability to equilibrate extracellular K^+ levels and might also affect glutamate homeostasis. Thus, reactive astrogliosis likely contributes to seizure generation in glioma patients.

PTW14-03

TRIACETIN ALTERS BRAIN AND WHITE MATTER LIPID COMPOSITION IN MICE SUBJECTED TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Acetate supplementation, induced with glycerol triacetate (GTA), promotes histone acetylation, increases anti-inflammatory cytokine levels, and stimulates fatty acid synthesis. Previously, we found that acetate supplementation prevented the loss of spinal cord ethanolamine and choline glycerophospholipid, phosphatidylserine, and cholesterol in a mouse experimental autoimmune encephalomyelitis (EAE) model, an autoimmune model for multiple sclerosis. To determine if EAE and treatment also alters whole brain lipid synthesis, we quantified the effect treatment had on whole brain and whole brain white matter lipid content. These experiments were performed in control mice and mice subjected to EAE that were treated daily with either water or GTA (4.0g/kg body weight) for a total of 40 days. We found that acetate supplementation significantly increased the content of white matter phosphatidylinositol (29%) in EAE mice compared to controls. Further EAE resulted in a significant decrease in white matter ethanolamine plasmalogen (29%) and phosphatidylcholine (35%) compared to controls. Treatment with GTA did not alter the white matter content of ethanolamine plasmalogen or phosphatidylcholine. However, acetate

supplementation significantly increased by 12% whole brain phosphatidylcholine and phosphatidylserine levels in mice subjected to EAE compared to EAE mice treated with water. These data suggest that EAE can significantly alter whole brain phospholipid levels but not to the extent that is found in the spinal cord and that triacetin therapy can increase brain lipid synthesis in EAE. It remains to be determined if the changes in the brain lipid content are a result of an increase in deposition or a decrease in breakdown.

PTW14-04

IMMUNOREGULATORY AND MYELIN REPAIR THERAPIES IN T CELL-MEDIATED MOUSE MODELS OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system characterized by demyelination and neurodegeneration in response to perivascular T-cell and mononuclear cell infiltration. Currently available disease modifying therapies, for reduction of frequency and severity of relapses, are global immunosuppressants acting through non-specific inhibition of T-cell activation/function and/or trafficking. These FDA-approved drugs have limited efficiency and are often associated with serious side effects. We have recently demonstrated an effective means of ameliorating disease in a mouse model of MS through tolerance induction in autoreactive T cells using i.v. infusion of 500nM poly-(lactic-*co*-glycolic acid) nanoparticles coupled with or encapsulating myelin peptides (Ag-PLG NP) that effectively reduces disease burden in relapsing-remitting (R-EAE) and in chronic-progressive (C-EAE) mouse models of experimental autoimmune encephalomyelitis (EAE) by reducing inflammatory cell infiltration. Today, there are no FDA-approved therapies for enhancing myelination despite successful *in vitro* and *in vivo* preclinical testing of several molecules/compounds reported to promote oligodendrocyte differentiation/maturation. As autoimmunity and neurodegeneration underlie MS, effective disease modifying therapies need to both regulate the immune system and promote restoration of neuronal function, including remyelination. This research tests the hypothesis that remyelination can be more efficiently induced in mice in which the underlying autoimmune response is specifically regulated. We examined the effects of therapies employing drugs which promote myelin repair by stimulating oligodendrocyte progenitor cell expansion, homing and/or differentiation combined with nanoparticle tolerance-based immunoregulatory therapies on T cell-mediated EAE mouse models of multiple sclerosis. We examined clinical disease progression, behavioral outcomes, CNS immune and inflammatory responses, and flow cytometry-based enumeration of cells of the oligodendrocyte lineage. These pre-clinical trials may provide a novel and safe targeted approach that can be translated into effective disease modifying therapies for MS patients.

PTW14-05

TEMPORAL REGULATION OF TNF PATHWAY DURING SEIZURE DEVELOPMENT IN A MOUSE MODEL OF EPILEPSY

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Inflammation is a key component of many acquired forms of epilepsy; however, we do not understand how triggering events lead to potentially irreversible anatomical changes and seizures (Vezzani 2011). Tumor necrosis factor-α (TNF) coordinates the immune response against viral and bacterial invaders in the CNS and is elevated during acute epileptogenesis in some models (Weinberg 2013). We hypothesize that the TNF pathway is involved in viral-induced seizure onset, and regulation points in this inflammatory pathway are potential pharmacologic targets for drug-resistant patients.

Mice were intra-cortically injected with Daniel's strain of Theiler's murine encephalomyelitis virus (TMEV n=4-8), or PBS (n=4-5), monitored for handling induced seizures, and inflammatory pathway changes were assessed with RT-PCR and western blot for TNFα cytokine, TNF receptors (TNFR1 & TNFR2), and β-actin.

Expression of TNFα levels in TMEV-infected mice increased 150 to 200 fold at 5 days post-infection (dpi) for both mRNA and protein, before decreasing at 14 dpi. This demonstrates strong temporal correlation between seizure activity and inflammatory cytokine expression. However, protein expression for TNF receptors (TNFR1 & TNFR2) decreased and did not correlate with the significantly increased gene expression, nor with the general seizure phase. This could be due to translational or post-translational regulation which may highlight additional pharmacologic targets in future studies. It is also possible that cross-regulation from other cytokine pathways may influence TNFRs expression. The ratio of TNFR1 to TNFR2 affects the balance between proapoptotic and immunomodulatory pathways. The protein ratio significantly increased through 5 dpi, however the ratio of mRNA significantly decreases by 14 dpi. Taken together, this data as well as seizure resistance in TNFR1 KO mice (Kirkman 2009) suggests TMEV acute seizure development may be due to TNFα signaling via TNFR1. Selective inhibition of TNFR1 at the protein level could be beneficial in suppressing epilepsy seizure development.

PTW14-06

ALTERATIONS IN BRAIN METABOLISM IN THE POSTNATAL DAY 10 RAT MODEL OF NEONATAL HYPOXIA-ISCHEMIA AT TERM

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It is well established that energy metabolism is impaired after hypoxia-ischemia (H/I) in the postnatal day (PND) 7 rat pup model of near-term neonatal H/I. However, much less is known about the brain alterations in the PND 10 rat model of H/I at term equivalent

birth. Ten day old rat pups underwent carotid artery ligation followed by 60 minutes of hypoxia at 8% O_2. We administered intraperitoneal injections of [1,2-^{13}C]glucose to rat pups 30 min prior to euthanasia and determined metabolism in both ipsilateral (right) and contralateral (left) sides of brain using ^{13}C-NMR spectroscopy. Our preliminary studies demonstrate that metabolism is substantially higher in the brains of 10 day old rats compared to PND 7, due to considerable maturation of the brain. Our preliminary studies suggest that there is more profound impairment in both neuronal and astrocytic specific pathways of metabolism after H/I at PND 10 compared to H/I injury in the less mature PND 7 rat pup. These studies underscore the importance of determining changes in brain in the PND 10 rat model of term H/I injury since the alterations in brain, and thus neuroprotective strategies needed, may be different in the term and near term brain.

Supported by NIH grant 5P01-HD016596

PTW14-07

VARIABLE THERMOLYTIC RESPONSE TO A1 ADENOSINE RECEPTOR AGONIST IS MANAGED WITH SURFACE WARMING

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Targeted temperature management of core body temperature (T_b) between 32-36°C improves neurological outcome after cardiac arrest and is in clinical trials for stroke. Cooling, however, is compromised by shivering. ^{6}N cyclohexyl adenosine (CHA) is an A_1 adenosine receptor (A_1AR) agonist that induces hibernation and torpor in ground squirrels, hamsters and fasted mice and induces torpor-like effects in rats. While CHA is highly effective at inhibiting thermogenesis and produces a decrease in core body temperature, efficacy varies between individual animals. Here we tested the hypothesis that a sufficient thermolytic response could be induced by increasing the dose of CHA to 1.0mg/kg and that core body temperature could be modulated with a Peltier controlled, radiant heated and cooled surface. We also performed ^{1}H-NMR metabolomic analysis on forebrain 24h after CHA to assess metabolic consequence of overcooling. Male Sprague-Dawley rats 3-4 months old were instrumented with iButton dataloggers (Maxim Integrated, Sunnyvale, CA) or CTA-F40 transmitters (DSI Inc.) to monitor core body temperature (T_b) and IPTT transponders to monitor subcutaneous temperature. CHA (1.0 mg/kg) was delivered IP at an ambient temperature of 17°C. In 10 rats, minimal T_b ranged between 21.1 and 36.6°C within 24h after injection with a minimum median of 28.6 (Q1, 28.6; Q3, 32.2) at 6h. Metabolomic analysis did not show evidence of cerebral ischemia, but did reveal an association between minimal T_b and brain tissue levels of trimethyl glycine (betaine). A subgroup of animals treated with 1.0mg/kg CHA was placed on the Peltier controlled surface set to an initial temperature of 17°C. Modulation of surface temperature prevented overcooling and improved control of target temperature.