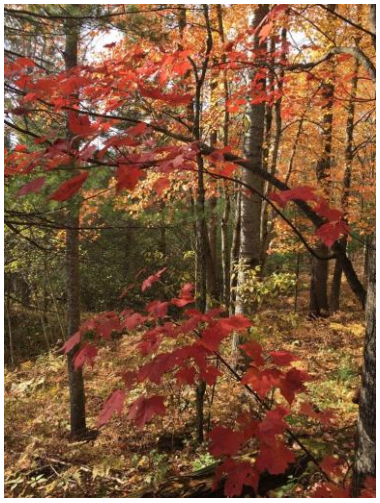
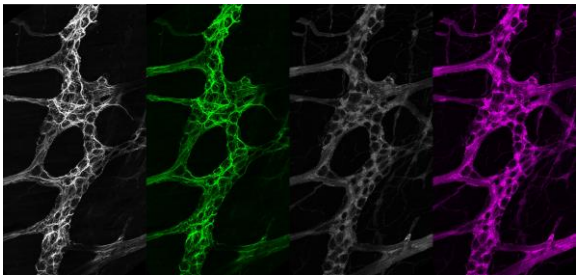
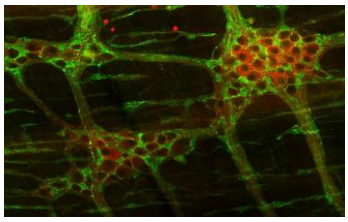
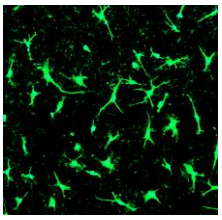
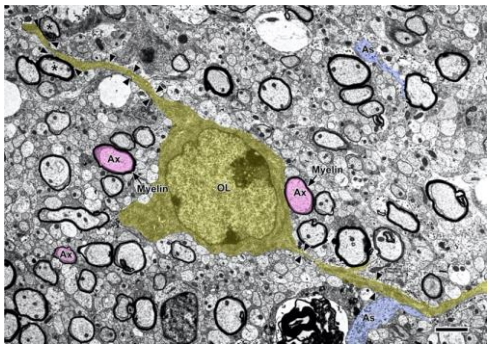
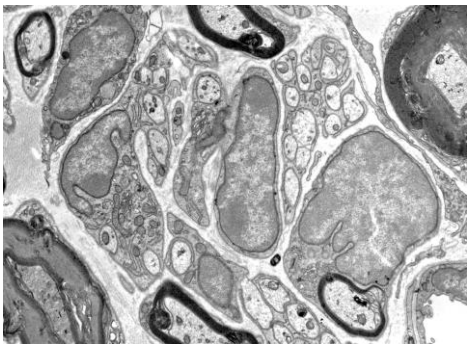
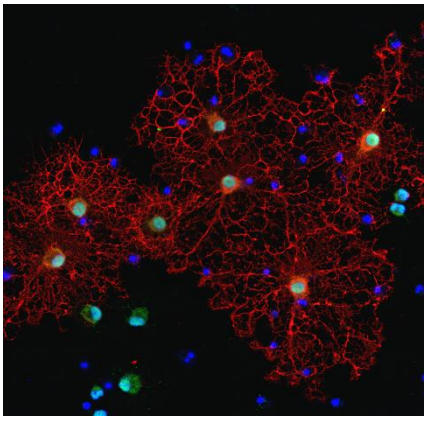
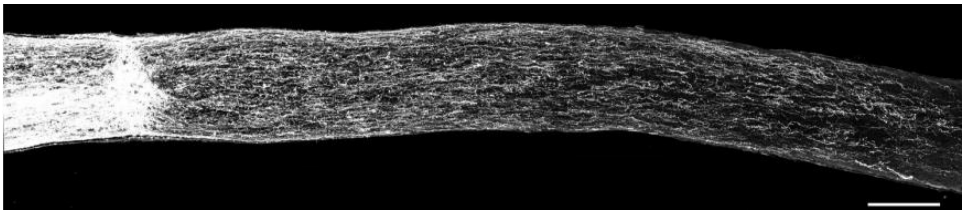
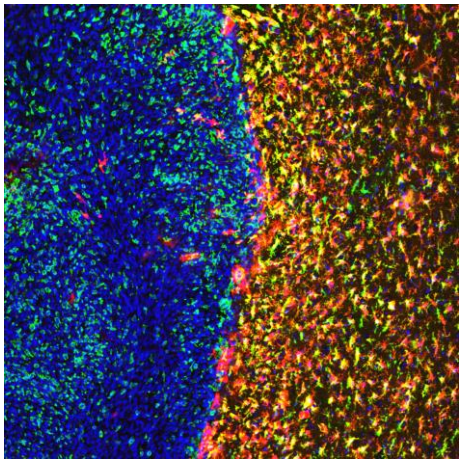
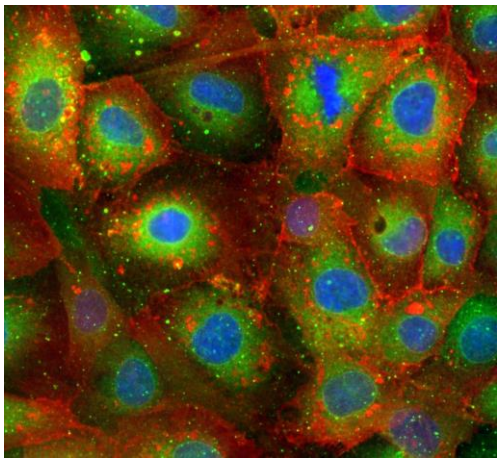
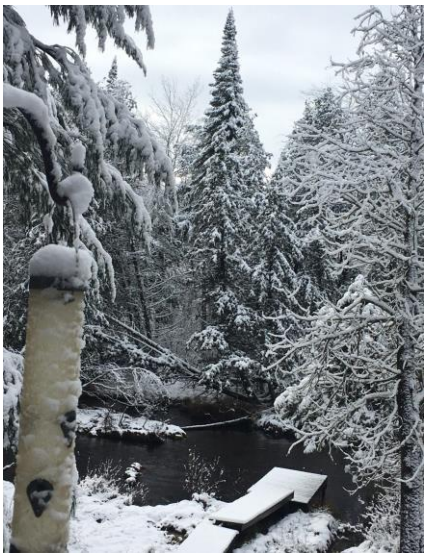
The 14th Great Lakes Glia Meeting

October 6th – 8th, 2024

Park Place Hotel & Conference Center

Traverse City, Michigan

https://greatlakesglia.org

Great Lakes Glia 2024

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Cover Photo Descriptions

Top left and bottom right corners: Images taken by Bob Skoff at his log cabin on the south branch of the Boardman River, South Boardman, MI

Top middle: Submitted by Beatriz Thomasi, Michigan State University. All labels are for enteric glia from the colonic myenteric plexus. The green fluorescence is GFAP and magenta is S100B

Middle 2nd row left: Submitted by Beatriz Thomasi, Michigan State University. Labeling for S100B (glia) and Calcineurin B (mainly neurons) also in the myenteric plexus.

Middle 2nd row right: Submitted by Mark Milner, University of Michigan, Human monocyte-derived microglia-like cells (HMDMis) in culture, differentiated from human peripheral blood mononuclear cells (PBMCs). Green is TMEM119 (homeostatic microglial marker) and light blue is Hoechst (nucleus).

Top right: Submitted by Doug Feinstein, University of Illinois-Chicago. Image taken from the harbor in Traverse City across from the Park Place Hotel. Check it out!

2nd row left: Submitted by Joyce Benjamins, Wayne State University. Low magnification electron micrograph of optic nerve from a young mouse undergoing myelinogenesis. An immature oligodendrocyte (OL, yellow) has extended two processes in opposite directions (arrowheads) into the surrounding neuropil; these processes will eventually myelinate the vast majority of nearby axons (Ax, purple and asterisks). Myelin is the dark ring surrounding these axons. The vast majority of axons are of small diameter and unmyelinated, but they will be myelinated once their diameter increases. The cytoplasm of oligodendrocytes is much darker than other glial cells like astrocytes (As, blue), which are also abundant in white matter tracts and interact extensively with oligodendrocyte cell bodies, myelin sheaths and nodes of Ranvier. Scale bar: 2 micrometers. Original image from Dr. R.P. Skoff with digital (Photoshop, CS6) contrast enhancement and reformatting by Dr. A. Gow.

2nd row middle: Submitted by: Juan Mato, University of Michigan. Image from my studies focusing on peripheral neuropathy in mouse models of Spinocerebellar ataxia Type 3 (SCA3). In the transmission electron microscopy image you can see multiple macrophages (large cells with the dark nuclei) engulfing both myelinating and nonmyelinating Schwann cells along with their associated myelin/axons in an SCA3 mouse sciatic nerve. This image is important to my work to understand the mechanisms/progression of peripheral nerve degeneration in SCA3 as it suggests that pathology in the sciatic nerve of this mouse is sufficient to induce an inflammatory demyelinating response.

2nd row right: Submitted by Alexa Putka, University of Michigan. Immunofluorescence image of primary oligodendrocyte cell culture. Oligodendrocyte progenitor cells were isolated from P5-P7 WT/WT mice and cultured in maturation media for five days. Primary oligodendrocytes were stained with DAPI (nuclei, 405), SREBP2 (master transcriptional regulator of cholesterol biosynthesis, 488), MBP (myelin basic protein, a marker of mature oligodendrocytes, 588), and SOX10 (pan-oligodendrocyte marker, 647).

3rd row left: Submitted by: Roman Giger, University of Michigan Medical School. Immune-mediated axon regeneration in the adult mouse optic nerve. Regenerated axons are traced with cholera toxin beta (CTB) four weeks following injury. Regeneration was induced by intra-ocular injection of beta-glucan.

4th row left: Submitted by Andrew Steelman, University of Illinois. Localization of microglia and macrophages in a syngeneic mouse model of glioblastoma. A Microglia reporter line was generated by crossing Tmem119Cre^{ERT2} mice to B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (Ai14) mice. After tamoxifen injection, these mice were injected with CT-2A glioma cells. At 20 days post injection brain tissue was sectioned and stained for the macrophage/macrophage marker Iba-1 and nuclei stained with Hoechst. Tumor is on the left and brain parenchyma on the right. Microglia and macrophages are spatially distinct in this model.

4th row middle: Submitted by Chuck Sanders, Vanderbilt University School of Medicine. Fluorescence microscopy image of rat Schwann cells expressing human peripheral myelin protein 22 (PMP22). PMP22 is stained red, whereas nuclei are stained blue and the ER marker, calnexin, is stained green. Evident in this image are PMP22-containing inclusions (appearing as punctae) that correlate with type 1A Charcot-Marie-Tooth disease, the most common peripheral neuropathy. (Unpublished image from Katherine Stefanski and Ricardo Capone of the Sanders lab at Vanderbilt University).

Robert Paul Skoff
March 8, 1942 – May 27, 2023

Robert Paul Skoff (1942–2023)
ANS Neuro Volume 16, 2024 Issue 1
Joyce Benjamins, Pamela Knapp & Anne Boullerne

Dr. Robert Skoff, Professor Emeritus, passed away on Saturday, May 27, 2023. He was born on March 8, 1942 in Buffalo, New York. He is internationally recognized for his many contributions to glial biology. Dr. Skoff joined the Department of Anatomy and Cell Biology at Wayne State University in 1978, and was also an associate in the Department of Neurology, as well as a member of the Translational Neuroscience Program and the Center for Molecular Medicine and Genetics. He received his Ph.D. from Boston University and held positions at Washington University and Johns Hopkins University before moving to Wayne State University in Detroit. His research centered on investigations of CNS macroglia, how they were generated, their roles as structural and functional components of the normal brain, and their role in CNS diseases. Bob's research was well-supported throughout his career by grants from the National Institute of Neurological Disorders and Stroke, the National Multiple Sclerosis Society, and the European Leukodystrophy Association. He had a particular fondness for oligodendroglia, and for myelin proteins, especially proteolipid protein (PLP), a CNS myelin protein that is often mutated or over-expressed in humans, causing Pelizaeus-Merzbacher disease.

As a graduate student at Boston University in the late 1960s, Bob studied the function and identification of neuroglial cells that proliferated in the degenerating optic nerve. As a postdoctoral fellow at Washington University in the laboratory of renowned embryologist Dr. Viktor Hamburger, Bob employed electron microscopy to study the organization and development of the spinal cord. The resulting paper described for the first time the fine structure of dendritic and axonal growth cones in the spinal cord and their organization (Skoff & Hamburger, Citation1974). This paper has been referenced hundreds of times.

As an Assistant Professor at Johns Hopkins University in the mid-1970s, Bob began a study of neuroglial cell lineages during normal postnatal development. At that time, little was known about the origins of glia and the morphology of proliferating cells. His research for the first time combined electron microscopy with thymidine autoradiography to identify dividing cells in the brain (Skoff, Citation1975). His findings provided a schema for the lineage of macroglia that became the basis for future studies of glial lineages in vivo. Those studies of glial proliferation continued over several decades, later focusing on proliferation in mouse mutants affecting myelin. In the late 1980s/early 1990s Bob's work reconciled a controversy over the role of bipotential O2A cells (oligodendrocyte-type 2 astrocyte lineage cells) in normal development. While immature glia have considerable plasticity in vitro, and can adopt different fates depending on culture conditions, Bob's work showed that their lineages in vivo are instead very direct. During specific time periods, proliferating immature astrocytes become mature astrocytes, and proliferating immature oligodendrocytes become mature oligodendrocytes (Skoff, Citation1990). He also documented gender differences between male and female oligodendrocytes (Cerghet et al., Citation2006).

At Hopkins, Bob also began studies of a dysmyelinating mouse mutant named jimpy that virtually lacked CNS myelin. His lab was instrumental in showing that massive oligodendroglial death was a major contributor to the paucity of myelin in this mutant, and later in other *Plp1* mutants. The initial studies, published in *Nature* (Skoff, Citation1976), were begun before the mutations in jimpy and in many other dysmyelinating/demyelinating mammals were shown to be due to mutations in the *proteolipid protein1* gene. In the 1990's, Bob's studies

focused on mouse mutants that over-express the *Plp1* gene because in humans most mutations are *PLP1* duplications. Those studies showed that levels of expression of native PLP not only regulate the function of oligodendrocytes but also neurons (Boucher et al., Citation2002; Knapp et al., Citation1986). Bob continued these studies of PLP mutants over the next decades. He demonstrated that many other defects occur in the *Plp1* mutants and showed that the level of PLP expression modulates the amount of cell death. The precise mechanism by which PLP regulates cell survival is unclear, but Bob identified several crucial steps, including the novel and quite provocative finding that native PLP in excess is inserted into mitochondria leading to a massive inflammatory response in both rodents and humans (Tatar et al., Citation2010). Most recently, Bob focused on the transport of PLP into mitochondria, describing the import pathway that it utilizes. He found that insertion of PLP into mitochondria not only occurs in overexpressing *Plp1* mutants but also in humans with Pelizaeus-Merzbacher Disease (Appikatla et al., Citation2014), thereby impacting oxidative phosphorylation.

Bob published over 90 papers, with thousands of citations. He served on numerous editorial boards and study sections. He took special pride in initiating and organizing the Great Lakes Glia meeting, held every other year since 2005 at Crystal Mountain and then Traverse City, MI.

In addition to his reputation as an outstanding scientist, he was always ready and willing to give his advice and time to younger scientists. He trained multiple postdoctoral and graduate students in his laboratory, which was managed for many years by his research assistant, Denise Bessert. His peers always looked forward to stimulating conversations with him at the annual ASN meetings.

Bob's friends remember his sly sense of humor, his collection of orchids, his fondness for a good bourbon or Sazerac, and the epic "man vs. squirrel" battles over the yearly apricot harvest at his yard in West Bloomfield. Bob and his wife Anne traveled extensively, and regaled colleagues with tales of their many misadventures on travels to meetings throughout the world. They both loved northern Michigan, and friends will long remember gourmet meals at their house on Lake Michigan and their rustic cabin on the Boardman River. At the cabin, Bob relished using axe, chain saw and log splitter to cut fallen trees into firewood; he fed both the birds and bears that visited there. In his later years, Bob undertook remodeling the cabin. He is survived by his wife Anne, a daughter, two sons and a grandson.

His wit, scientific insight and friendship will be greatly missed.

https://doi.org/10.1080/17590914.2024.2393559

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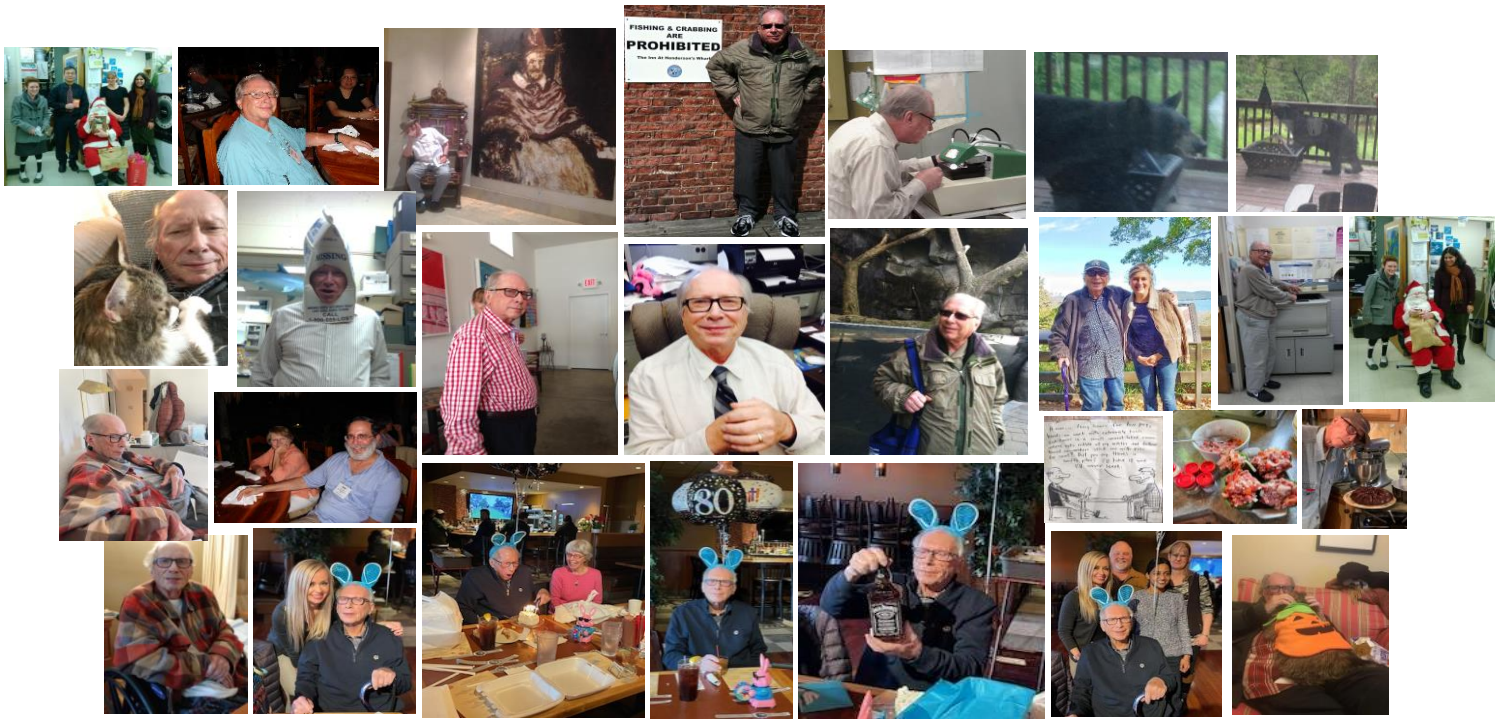

We miss you Bob

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Schedule

Sunday, October 6th

4:30pm **Opening Reception** **Top of the Park**

6:00pm **Dinner** **Top of the Park**

7:30pm **Session 1: Demyelination and Remyelination in Peripheral Nerves** **Grandview I**
Chair: Jun Li, MD, Houston Methodist Hospital
Weill Cornell Medical College

Chuck Sanders, PhD, Vanderbilt University, TN
Causes and consequence of misfolded peripheral myelin protein PMP22 in peripheral neuropathies

Chelsey J. LeBlang, PhD, Harvard University, MA
Satellite glial contact advances maturation of human induced sensory neurons: Generating a model of peripheral neuropathy

Jun Li, MD, Houston Methodist Hospital and Weill Cornell Medical College, TX
Kinases in the peripheral nervous system

Sophie Belin, PhD, Albany Medical College, NY
The new role of the PMP2 fatty acid chaperone in peripheral myelination

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Monday, October 7th

8:00am **Breakfast** **Top of the Park**

8:45am **Keynote Presentation** **Grandview I**

R Douglas Fields, PhD

University Maryland, Baltimore, MD

Plasticity of myelin structure for experience-dependent optimization of conduction velocity

9:45am **Break**

10:00am **Poster Blitz** **2 minute Student/Postdoc presentations** **Grandview I**

10:30am **Session 2: Mechanisms of Neurodegeneration in Multiple Sclerosis** **Grandview I**

Chair: Tara de Silva, PhD, Cleveland Clinic, OH

Kedar Mahajan, MD, Cleveland Clinic, OH

Thalamic pathology: implications for neurodegeneration in MS

Tara M. DeSilva PhD, Cleveland Clinic, OH

Synaptic loss during inflammatory demyelination

Roman Giger, PhD, University of Michigan, MI

Axonal regeneration and the role of inflammation

Noon **Free time**

3:00pm **Poster Session** **Grandview II**

4:30pm **Session 3: Lipids in the CNS: Activation and Toxicity** **Grandview I**

Chair: Jeffrey Dupree, PhD, Virginia Commonwealth University, Richmond, VA

Andrew Lieberman, MD, PhD, Michigan Neuroscience Institute Affiliate, MI

Apolipoprotein mimetics for treatment of acid sphingomyelinase deficiency

Andrew (Drew) Steelman, PhD, University Illinois Urbana-Champaign, IL

The myelin lipidome and activation of neuroinflammation

Jeff Dupree, PhD, Virginia Commonwealth University, VA

The role of sulfatide in maintaining CNS structure and function

Sijia He, PhD, UT Health San Antonio, TX

Sulfatide loss drives Alzheimer's disease-like CNS and peripheral phenotypes

7:00pm **Dinner** **Top of the Park**

8:30pm **Posters and Refreshments** **Grandview II**

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Tuesday, October 8th

7:30am **Breakfast** **Top of the Park**

8:15am **Keynote Presentation** **Grandview I**

David Spray, PhD

Albert Einstein College of Medicine, NY

Coupled glial networks in health and disease

9:15am **Break**

9:30am **Session 4: Astrocytes: Supportive and Disruptive Roles** **Grandview I**

Chair: Chris Proschel, PhD, University of Rochester, NY

Margot Mayer-Proschel, PhD, University of Rochester, NY

The role of astrocytes in Iron deficiency and Pb exposure

Thomas Delgado, MS, University of Rochester, NY

Transglutaminase 2 attenuates astrocytic neurosupportive function by dampening upregulation of fatty acid transport & utilization

Kurt Hauser, PhD, Virginia Commonwealth University, VA

Astrocyte: microglial cross talk: dealing with drug abuse and HIV

Chris Proschel, PhD, University of Rochester, NY

Disruption of astrocytic protein translation in vanishing white matter disease

11:30am **Lunch Break Out** **Grandview I**

12:00pm **Session 5: Exosomes in Neurodegenerative Diseases** **Grandview I**

Chair: Mike Nichols, PhD, University of Missouri-St. Louis, MO

Simon Kaja, PhD, Loyola University, Chicago, IL

Trophic actions of astrocyte derived exosomes in glaucoma

Mike Nichols, PhD, University of Missouri-St. Louis, MO

Interplay between extracellular vesicles and amyloid-β protein

Alan Dombkowski, PhD, Wayne State University, MI

Exosomes of astrocytic origin are associated with seizures in patients with tuberous sclerosis complex

Joyce Benjamins, PhD, Wayne State University, MI

Apoptosis-related differences in molecular profiles of exosome-enriched extracellular vesicles from multiple sclerosis B cells compared to controls

2:00pm **End of Meeting**

Abstracts

#1

The Oral Biome of the Multiple Sclerosis patient, what can the OMS do to help?

Raza Hussain[1,2], Demetrios Skias[3,4], Carolyn Bevan[4], Michael Carrithers[3,4], Douglas L Feinstein[5], Anne Boullerne[5]

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2. Oral & Maxillofacial Surgery Dpt. University of Illinois, Chicago 60612
3. Neurology & Rehabilitation Dpt. University of Illinois, Chicago 60612
4. Neurology Section, Jesse Brown Veterans Affairs Medical Center, Chicago 60612
5. Anesthesiology Dpt. University of Illinois, Chicago 60612

Statement of the problem: Changes in the microbiome can provide information about disease progression, severity, and drug efficacy; and can contribute to disease by changing levels of active metabolites. Most studies look at the gut biome by examining fecal samples; however differences also occur in the oral biome for example between Alzheimer's Disease and non-neurological controls (Bouftas et al. 2021). Using saliva samples, we previously showed differences in the oral biome of identical twins who had divergent Multiple Sclerosis (MS) severity (Boullerne et al. 2020). The goal of this study is to confirm differences in a larger cohort of subjects and determine how salivary metabolites are affected.

Materials and methods: In this study we collect saliva samples from a total of 80 subjects (20 MS and 20 Control; both White and AA). After 1 year we collect a follow-up sample. After consent, subjects undergo a dental exam to record signs of disease, count cavities, missing teeth, and any other damage. Exclusion criteria are mouthwash the same day; antibiotics in past 30 days; daily antivirals; and eating or drinking 1-hour preceding collection. Inclusion criteria are a definitive diagnosis of MS; age 18-74 y/o; and either White or AA. After collection, saliva is sequenced for 16S ribosomal RNA, and screened for metabolites by mass spectroscopy.

Data analysis: Statistical Methods: Principal Component Analysis (PC) to compare groups, unpaired T-tests to compare means, and Pathway Analysis to identify altered metabolic pathways.

Results: To date 16 MS subjects and 9 controls were analyzed. Comparison of bacterial taxa (phylum to species) between the MS cohort versus the control cohort showed several differences without reaching significance. However, comparison of White males (MS to control) (Figure 1) shows significantly increased phylum Firmicutes in MS patients, and almost significant reduced Proteobacteria. Further comparisons are in progress. Untargeted metabolite screening on all samples and PC analysis shows that the 2 groups are distinct, aside from 3 outliers in the MS and control groups (Figure 1). There are about 450 metabolites significantly different between the 2 groups, and pathway analysis points to Folate (Vitamin B12) metabolism and One-Carbon pool for methylation processes. A targeted analysis for differences in Small Chain Fatty Acids (SCFAs), which can affect Tcell functions, identified an almost significant increase in isoValeric Acid in the MS cohort. Comparisons to compare White males (MS, n=3 to control, n=6 including Hispanic white) found about 250 significantly different metabolites, and pathway analysis again points to Folate metabolism.

Conclusions: Initial results indicate significant differences in the oral microbiome between MS and controls independent of gender or race. Differences in sub-cohorts require larger groups to confirm significance. Similarly, we identified many metabolites significantly altered in MS versus controls, with changes in Folate, which can be metabolized by bacteria and can regulate systemic inflammatory processes. Together, these findings confirm that the oral microbiome differs between MS and controls along active metabolites.

References: Bouftas M. A Systematic Review on the Feasibility of Salivary Biomarkers for Alzheimer's Disease. J Prev Alzheimers Dis 2021, 8:84-91.
Boullerne AI et al. Deep DNA metagenomic sequencing reveals oral microbiome divergence between monozygotic twins discordant for multiple sclerosis severity. J Neuroimmunol. 2020, 343:577237.

#2

Cerebellar lipid dysregulation is conserved across Spinocerebellar ataxia type 3 patients and mouse models

Alexandra F. Putka[1,2], Varshasnata Mohanty[3], Vikram O. Sundararajan[1], Stephanie M. Cologna[3,4], Hayley S. McLoughlin[1,5]

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Background and Objective: While RNA-sequencing and proteomic studies abound in the neurodegenerative disease literature, one class of macromolecules remains understudied: lipids. Our lab studies Spinocerebellar ataxia type 3 (SCA3), the most common dominantly inherited ataxia. We recently identified cholesterol/sterol biosynthetic processes as the top gene ontology biological pathways dysregulated in SCA3 transgenic mice compared to WT controls. However, whether transcriptional dysregulation translated to altered lipid levels remained unknown. Here, we investigated dysregulated lipid profiles in SCA3 disease.

Methods: Cerebella from post-mortem SCA3 patients and healthy controls were obtained from the Michigan Brain Bank. Cerebella from YACQ84 transgenic and *Atxn3*-Knockout mice were collected at 16 weeks of age. Lipids were isolated from all samples using the Folch extraction method and quantified by positive and negative ion mode liquid chromatography-mass spectrometry. Lipid identifications were obtained via Lipid Annotator Software and differential analysis was carried out. Lipids with a fold-change < -1.5 or > 1.5 and a p-value < 0.05 (unpaired Student's two-tailed t-test) were considered differentially expressed.

Results: SCA3 patients and YACQ84 transgenic mice share reduced cerebellar lipid content, with a majority of differentially expressed lipids downregulated. We identified seven lipids reduced in both SCA3 patients and YACQ84 mice, representing potential lipid biomarkers of disease. In contrast, *Atxn3*-Knockout mice displayed increased cerebellar lipid content, with most differentially expressed lipids upregulated. No lipid changes were uniformly observed across SCA3 patients, YACQ84 mice, and *Atxn3*-Knockouts, suggesting ATXN3 loss-of-function is likely not the primary driver of lipidomic alterations in SCA3.
Conclusion: We uncovered broad lipid dysregulation in SCA3 patients and mice likely due to mutant ATXN3 toxic gain-of-function. This work establishes lipid perturbations as an uncharacterized feature of SCA3. Future work will elucidate the progression of lipid signatures in SCA3 Knock-in mice, which may help uncover when therapeutic intervention is most valuable. Additionally, we plan to explore the connection between lipid dysregulation and impaired oligodendrocyte maturation and myelination, which are established features of SCA3 disease.

#3

Neuronal and glial pathology in the spinal cord of Spinocerebellar ataxia type 3 patients and mouse models

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While spinal cord involvement is implied in the name of the debilitating neurodegenerative disorder spinocerebellar ataxia type 3 (SCA3), very little is known about how this disease affects the spinal cord. SCA3 is a polyglutamine repeat expansion disease that causes a heterogeneous range of symptoms including coordination, sensory, and motor impairments, all of which could at least partially be caused by spinal cord dysfunction. Cervical spinal cord atrophy, (volumetric loss determined by MRI), is a well-established biomarker of SCA3, present before symptom onset and progressing with disease severity. It remains unknown if this atrophy is present throughout the spinal cord and what cell types are responsible. Here, we begin to address these gaps by characterizing neuropathology in the spinal cord of a Knock-in SCA3 mouse model, as well as in patient postmortem tissue. Histological spinal cord analysis within these models reveals disease hallmark ATXN3 nuclear accumulation in neuronal and glial cell populations as well as a decrease in mature oligodendrocytes. Bulk RNAseq of cervical spinal cord from end-stage SCA3 KI mice provides evidence of neuronal, microglial, and oligodendrocyte dysfunction in SCA3. This work begins to characterize spinal cord neurodegeneration in SCA3, providing the foundation for future studies elucidating cell-type specific contributions to disease and potential therapeutic targets.

#4

Peripheral nerve Schwann Cell abnormalities are tied to neuropat hic features in mouse models of Spinocerebellar ataxia type 3

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Background and Objective: Spinocerebellar ataxia type 3 (SCA3) currently has no effective treatment despite being the most common dominantly inherited ataxia. While research into this disease has focused on neurodegeneration of vulnerable brain regions such as the cerebellum and brainstem, work elucidating neuropathology in the peripheral nervous system in SCA3 is lacking. More than half of SCA3 patients present with peripheral neuropathy, and sensory abnormalities and chronic pain in the periphery are often reported. Schwann cell-mediated myelination abnormalities are a common pathological underpinning to nerve degeneration in several neuropathic diseases. Thus, my work aims to characterize the progression of myelin-related peripheral neuropathy features in two SCA3 mouse models to provide a foundation for future therapeutic studies.

Methods: YAC Q84 transgenic and Knock-In (KI) Q300 mouse models of SCA3 were assessed for nerve conduction abnormalities, sensory behaviors, and histological markers of peripheral neuropathy across the disease timeline in comparison to wildtype littermate controls and *Atxn3* Knock-out (KO) mice.

Results: The YAC Q84 and KI Q300 mice reflect SCA3 patient sensorimotor nerve conduction velocities, while the *Atxn3* KO mouse did not display any nerve conduction abnormalities. Behaviorally, the KI Q300 mice display loss of thermal sensation and abnormal response to mechanical stimuli. The YAC Q84 display relatively milder abnormal sensory responses while the *Atxn3* KO mice behave comparably to wildtype littermates. Histological analysis of YAC Q84 nerves show pathological ATXN3 nuclear inclusions in myelinating Schwann Cells. Further ultrastructural assessments of YAC Q84 and KI Q300 sural nerves show demyelination and large axon losses as well as reductions in myelinated fiber densities, respectively relative to wildtype mice. In contrast, *Atxn3* KO mice sciatic nerve fibers show *hyper*myelination relative to wildtype mice.

Conclusion: Our results confirm neuropathology in SCA3 peripheral nerves that are likely functionally tied to behavioral and electrophysiological manifestations of peripheral neuropathy. This neuropathology implies a deleterious effect of mutant ATXN3 toxic gain of function in Schwann cells. Understanding the effects of Schwann cells on SCA3 peripheral neuropathy is critical for pursuing new therapeutic avenues for patients and those suffering from similar hereditary ataxias. In the future, Cre-driven conditional deletions of mutant ATXN3 in Schwann cells may be beneficial in assessing the necessity of Schwann cell pathology for onset and progression of neuropathic features in the KI Q300 mice.

#5

Understanding The Myelin g ratio From First Principles, Its Derivation, Uses And Artifacts

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In light of the increasing importance for measuring myelin g ratios – the ratio of axon-to-fiber (axon + myelin) diameters in myelin internodes – to understand normal physiology, disease states, repair mechanisms and myelin plasticity, there is urgent need to minimize processing and statistical artifacts in current methodologies. Unfortunately, many contemporary studies fall prey to a variety of artifacts, which reduce study outcome robustness and slow development of novel therapeutics. Underlying causes stem from a lack of understanding of the myelin g ratio, which has persisted more than a century. An extended exploratory data analysis from first principles (the axon-fiber diameter relation) is presented herein and has major consequences for interpreting published g ratio studies. Indeed, a model of the myelin internode naturally emerges because of (1) the strong positive correlation between axon and fiber diameters and (2) the demonstration that the relation between these variables is one of direct proportionality. From this model, a robust framework for data analysis, interpretation and understanding allows specific predictions about myelin internode structure under normal physiological conditions. Further, the model establishes that a regression fit to g ratio plots has zero slope, and it identifies the underlying causes of several data processing artifacts that can be mitigated by plotting g ratios against fiber diameter (not axon diameter). Hypothesis testing can then be used for extending the model and evaluating myelin internodal properties under pathophysiological conditions (accompanying article). For without a statistical model as anchor, hypothesis testing is aimless like a rudderless ship on the ocean.

#6

A Statistically-Robust Model Of The Axomyelin Unit Under Normal Physiologic Conditions With Application To Disease States

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Despite tremendous progress in characterizing the myriad cellular structures in the nervous system, a full appreciation of the interdependent and intricate interactions between these structures is as yet unfulfilled. Indeed, few more so than the interaction between the myelin internode and its ensheathed axon. More than a half-century after the ultrastructural characterization of this axomyelin unit, we lack a reliable understanding of the physiological properties, the significance and consequence of pathobiological processes, and the means to gauge success or failure of interventions designed to mitigate disease. Herein, we highlight shortcomings in the most common statistical procedures used to characterize the axomyelin unit, with particular emphasis on the underlying principles of simple linear regression. These shortcomings lead to insensitive detection and/or ambiguous interpretation of normal physiology, disease mechanisms and remedial methodologies. To address these problems, we syndicate insights from early seminal myelin studies and use a statistical model of the axomyelin unit that is established in the accompanying article. Herein, we develop and demonstrate a statistically robust analysis pipeline with which to examine and interpret axomyelin physiology and pathobiology in two disease states, experimental autoimmune encephalomyelitis and the rumpshaker mouse model of leukodystrophy. On a cautionary note, our pipeline is a relatively simple and streamlined approach that is not necessarily a panacea for all g ratio analyses. Rather, it approximates a minimum effort needed to elucidate departures from normal physiology and to determine if more comprehensive studies may lead to deeper insights.

#7

Myelinating models : we are not there yet

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Myelinating models began with Betty Geren's "jelly roll" after she discovered in 1954 that peripheral nervous system (PNS) myelin sheath originates from Schwann cell spiraling around the axon a linear membrane extension over many layers. The spiral revolution immediately launched a frenetic search for its counterpart in the central nervous system (CNS). Protractedly, owing to tortuous long cellular processes connecting internodes to the oligodendrocyte perikaryon, Richard and Mary Bunge in 1961 partly extrapolated the first 3D spatial representation of a CNS myelin internode. A year later they provided the electron microscopy proof that myelin sheath is a spiraling oligodendrocyte membrane extension. A spiral however proved mentally difficult to grasp, prompting Asao Hirano and Herbert Dembitzer in 1967 to devise the first flat unrolled myelin model, now ubiquitous. Soon, a complete oddity was discovered first in PNS (Webster, 1971) then in CNS (Stempak and Knobler, 1972): spiral direction alternates several times within the same internode. That proved too challenging and is still not resolved. But the search for the spiral drive was on. Easier to study in PNS, Richard Wiggins showed in 1980, in a virtually forgotten study, that Schwan cell generates new myelin at the inner tongue. In 1989, the Bunges again published a landmark paper showing just that. Their model was dubbed "carpet crawler" by others, and the creative imagination of investigators minted other names for their models in PNS or CNS, depending whether a coiling process or lateral wrapping would occur first: "yo-yo" and "ribbon" (Pedraza et al, 2009 from David Colman), "liquid croissant" (Sobottka et al, 2011 from Norbert Goebels), "corkscrew" (Ioannidou et al, 2012 from Susan Barnett), and "Sarape" (Kerman et al, 2015 from Fred Gage). Other models chose concurrent spiraling and wrapping (Bauer et al, 2009 from Charles ffrench-Constant) and an outlier theory of "myelinophore organelles" by Sara Szuchet and coll. (2015). Some 25 years after the PNS "carpet crawler" model, Mikael Simons finally provided the proof with sophisticated electron microscopy and genetic engineering that CNS myelin grows at the inner tongue (Snaidero et al, 2014). The quest to uncover the drive molecular mechanism was solved a year later powered by actin, independently by Simons and Ben Barres in 2015. The flat unrolled model has seldom been updated twice by Robert Lazzarini (Gow et al, 1999) and Julia Edgar and coll. (2021). To this day, there is no myelinating model integrating all myelin ultra-structures, especially the cytoplasmic channels traversing the mature compact myelin sheath besides established outer, paranodal, and inner loops, all revealed in 1921 and 1928 by Pio del Rio-Hortega.

#8

Lanthionine Ketimine Ethyl Ester Increases Oligodendrocyte Proliferation Both In-Vitro and In-Vivo

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Lanthionine ketimine ethyl ester (LKE) is a synthetic bioavailable cell penetrating derivative of the naturally occurring metabolite, lanthionine ketimine. Several studies have shown neuroprotective effects of LKE in attenuating pathology in animal models of Multiple Sclerosis (MS), Alzheimer's Disease, spinal cord injury; as well as providing neuroprotection and induction of oligodendrocyte (OLG) maturation in-vitro. The basic objective of this work is to study the effects of LKE on OLGs using mouse primary OLGs in-vitro, and in the cuprizone (CPZ)-induced demyelination mouse model of MS. Briefly, primary OLG progenitor cells (OPCs) were cultured in-vitro from post-natal day 0-2 mouse pups. The brains were dissected, trypsinized, then plated in OPC specific medium containing platelet-derived growth factor (PDGF-AA) and basic fibroblast growth factor (bFGF). The OPCs were seeded in poly-D-lysine coated chamber slides and treated with vehicle or LKE (10 μM) for 72h, fixed, then used for immunocytochemical analysis for PDGFRα to label immature OLGs, and proliferating cell nuclear antigen (PCNA) to measure cell proliferation. Similarly, primary neural stem cells (NSCs) were cultured and treated with LKE for 72h via the same protocol, plated in NSC medium containing bFGF and epidermal growth factor (EGF), then analyzed for Sox2 and Ki67 immunoreactivity. Immunocytochemical analysis revealed an increase in the number of PDGFRα+/PCNA+ OPCs indicating increased OPC proliferation in the LKE treated group as compared to control group. Similar analysis was done for Sox2+/Ki67+ NSCs and comparable differences were observed between the two groups. To examine effects of LKE on OLG proliferation in-vivo, female C57BL/6 mice were placed on 0.2% CPZ chow for either 3 or 6 weeks, and then remyelination allowed to occur for 2 weeks on control chow or chow containing 100 ppm LKE. Brains were collected, then dissected into specific regions Corpus Callosum (CC), hippocampus (HC), and cerebellum (CB) to be used for immunostaining and RNA analysis. Initial RNA-seq analysis of CC RNA indicated that LKE treatment increased the expression of proliferation promoting (e.g., BDNF) and differentiation inhibiting (e.g., Lingo1, NOGO receptor) mRNAs. These preliminary results demonstrate the proliferative action of LKE on OPCs as well as NSCs, which may contribute to its known neuro-protective and OPC maturation roles. On going qPCR and immunostaining studies are being carried out to confirm RNAseq results and to determine the cellular localization of mRNAs that are upregulated by LKE during remyelination. This work was funded in part by grants from the National MS Society and the Department of Veterans Affairs

#9

Investigating Lanthionine Ketimine Ethyl Ester's Role in Remyelination and Neuroprotection in the Cuprizone Model of De-remyelination

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Cuprizone-induced de-remyelination (CPZ) serves as a chemically induced model of reversible oligodendrocyte pathology in animal subjects, exhibiting certain pathological parallels with human Multiple Sclerosis (MS). Post-cessation of CPZ intoxication reveals spontaneous remyelination histologically, however, it does not always correlate with functional recovery. This phenomenon may partially explain why various agents that enhance remyelination in animal studies have not met expected clinical outcomes in human trials. Remyelination also serves as an axonal protection strategy and may enable functional recovery, but the mechanisms of myelin repair, especially after chronic insults remains poorly understood.
Lanthionine Ketimine Ethyl Ester (LKE), a synthetic derivative of lanthionine ketimine with improved bioavailability, has shown neuroprotective effects in various animal models of neuropathology. In the current study, our goal is to elucidate the specific neuroprotective mechanisms of LKE in the CPZ model that are distinct from its effects on spontaneous remyelination, potentially leading to enhanced neuroprotection and functional recovery. In the CPZ model, the corpus callosum (CC) is the principal site of demyelination and subsequent remyelination. To identify potential neuroprotective actions, we carried out RNAseq analysis of CC RNA isolated from control mice, mice administered 0.2% CPZ in chow for 6 weeks, and CPZ-treated mice with 2 weeks recovery on control chow or chow containing 100 ppm LKE. As expected, CPZ significantly decreased close to 500, and increased close to 900 mRNAs. Recovery on control chow led to restoration of about 400 of those mRNAs. However, compared to CPZ, recovery in the presence of LKE led to significant changes in 1,854 identified mRNA, including upregulation of 554 mRNA that were unaffected by CPZ, and 108 mRNAs that were downregulated by CPZ.

Pathway analysis was then carried out utilizing KEGG 2021, an expert-curated knowledge base, and SynGO 2022, a synapse-centric, evidence-based database. KEGG analysis of the set of 108 mRNAs identified pathways including phosphatidylinositol signaling, serotonergic synapse, arachidonic acid metabolism, steroid biosynthesis, inositol phosphate metabolism, retrograde endocannabinoid signaling, Ras signaling pathway and enhanced synaptic signaling. SynGO analysis identified several pathways involved in pre- and post-synaptic membrane structure and function, including regulation of voltage-gated ion channel and membrane potential. In contrast, analysis of the 554 mRNAs were not reduced by CPZ but increased by LKE, identified pathways encompassing circadian entrainment, calcium signaling, axon guidance, cGMP-PKG Signaling, oxytocin signaling, glutamatergic synapse, apelin signaling, actin cytoskeleton regulation, and enhanced synaptic signaling pathways, particularly synaptic vesicle priming and postsynaptic actin cytoskeleton organization, along with elevated calcium level regulation.

Together, these data suggest that recovery after CPZ in the presence of LKE not only accelerates processes of remyelination but also increases the expression of mRNAs involved in pre- and post-synaptic functionality. Current studies include carrying out comparative analyses with other notable databases, as well as confirmation of changes in mRNAs using qPCR and immunostaining methods.

This work was funded in part by grants from the National MS Society and the Department of Veterans Affairs.

#10

Effects of lanthionine ketimine on acutely isolated mouse oligodendrocyte progenitor cells.

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Lanthionine ketimine ethyl-ester (LKE) is a synthetic derivative of lanthionine ketimine (LK) which is naturally occurring metabolite of nonproteinogenic amino acid lanthionine. LKE is involved in neuronal and oligodendrocyte maturation. Previously we have shown that LKE can increase remyelination in the cuprizone induced model of demyelination, reduce clinical scores in the EAE mouse model of MS, as well as increase differentiation of oligodendrocyte precursor cells (OPCs) in vitro. LKE regulates the activity of collapsin response mediator protein-2 (CRMP2) which is thought to mediate its actions. In the current study we are evaluating effects of CRMP2 conditional knockout (cKO) as well as LKE treatment on OPCs as well as on mature oligodendrocytes. To generate oligodendrocyte specific CRMP2 cKO we crossed CRMP2 floxed mice with a PDGFRa-CreER mouse. To confirm cellular specificity, we crossed PDGFRa-CreER mice to TdTomato expressing reporter mice. The resulting PDGFRa-Cre-ER : TdTomato mice, after treatment with tamoxifen showed Tomato reporter expressing in OPCs, confirmed by colocalization staining with PDGFRa but not with GFAP or Iba1. In order to confirm OPC specific CRMP2 cKO in vivo, and to evaluate LKE effects in the cKO mice, we are optimizing a method for isolating and culturing OPCs from brains of 1 week old and 1 month old mice. This method makes use of enzymatic digestion and mechanical dissociation of brain tissue into single cell suspension followed by selective isolation of OPCs by PDGFRa antibody conjugated magnetic beads, then culture in OPC proliferation media. While we obtain comparable yields of total cells from mice of either age, cells obtained from older mice were more heterogenous population. The cells obtained from younger mice exhibit enrichment in OPCs and confirmed by IHC staining with Olig2, PDGFRa, GFAP, Iba1. Following treatment with thyroid hormone T3, the majority of OPCs differentiated into PLP and MBP expressing cells. Additional staining shows that both OPCs and mature oligodendrocytes express CRMP2. Current studies are testing the ability of LKE and LKE derivatives to induce maturation of the acutely isolated OPCs, and isolating OPCs from PDGFRa-CreER: CRMP2 f/f mice to generate CRMP2 deficient cells. This work was funded in part by grants from the National MS Society and the Department of Veterans Affairs.

#11

PMP2 Regulates Myelin Thickening and ATP Production During Remyelination

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It is well established that axonal Neuregulin 1 type 3 (NRG1t3) regulates developmental myelin formation as well as EGR2-dependent gene activation and lipid synthesis. However, in peripheral neuropathy disease context, elevated axonal NRG1t3 improves remyelination and myelin sheath thickness without increasing Egr2 expression or activity, and without affecting the transcriptional activity of canonical myelination genes. Surprisingly, Pmp2, encoding for a myelin fatty acid binding protein, is the only gene whose expression increases in Schwann cells following overexpression of axonal NRG1t3. The purpose of this study is to clarify the function of PMP2 upregulation downstream of NRG1t3-mediated hypermyelination during development and remyelination. We generated mice overexpressing NRG1t3 and knocked out for Pmp2 (Pmp2-/-;NRG1t3OE). We utilized nerve crush surgery as a remyelination model, and measured mouse sciatic nerve morphology, electrophysiology, and molecular pathways during development and remyelination. We also used a fluorescent fatty acid analogue to quantify fatty acid uptake, and a Seahorse analyzer to measure ATP production in the Pmp2-/-;NRG1t3OE mouse sciatic nerves during development and remyelination. Here, we demonstrate PMP2 expression is directly regulated by NRG1t3 active form, following proteolytic cleavage. Then, using the Pmp2-/-;NRG1t3OE mouse model, we demonstrate that PMP2 is required for NRG1t3-mediated remyelination. We demonstrate that the sustained expression of Pmp2 in NRG1t3OE mice enhances the fatty acid uptake in sciatic nerve fibers and the mitochondrial ATP production in Schwann cells. In sum, our findings demonstrate that PMP2 is a direct downstream mediator of NRG1t3 and that the modulation of PMP2 downstream NRG1t3 activation has distinct effects on Schwann cell function during developmental myelination and remyelination.

#12

Effect of IFN-γ and GM-CSF on glial activation and oligodendrocyte viability in primary mouse mixed glial cultures.

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Multiple sclerosis (MS) is an autoimmune, inflammatory demyelinating and neurodegenerative disease of the central nervous system. The T cell derived cytokines IFN-γ and GM-CSF have both been implicated in disease progression of both MS as well as experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS. In the current study, we aimed to characterize the effects of IFN-γ and/or GM-CSF stimulation on mixed glial cultures containing microglia, oligodendrocytes and astrocytes. Here, primary glial cells were cultured from brains of male and female neonatal mouse pups, then challenged with media, recombinant mouse IFN-γ (10ng/ml), GM-CSF (10ng/ml) or both cytokines for 72h. Following treatment, the number of microglia and oligodendrocytes were determined by immunocytochemistry. Changes to inflammatory factors were screened by proteome profiler array and select cytokines and chemokines confirmed by ELISA. Stimulation with IFN-γ increased microglia cell number and altered their morphology. Moreover, IFN-γ, but not GM-CSF, reduced the total number of O4+ oligodendrocytes, an effect that was more pronounced in less mature cells. Consistent with glial activation, thirty-three cytokines were upregulated at least two-fold in supernatants from cultures treated with both IFN-γ and GM-CSF. Bioinformatics analysis revealed many of the proteins that were upregulated by treatment were under the transcriptional control of STAT1 and STAT5, but also NFkB. Treatment with IFN-γ specifically increased levels of chemokines (i.e. CCL22, CCL2, CCL3/4, CCL5, CXCL10 and CXCL1) involved in attraction of monocytes, dendritic cells, T cells and neutrophils, but decreased levels of proteins (i.e. PDGF, FGF and LIF) that promote oligodendrocyte progenitor cell proliferation and maturation. Notably, no sex differences were observed in any measure. These findings provide insight into how cytokine modulation can influence inflammatory responses in EAE, offering a deeper understanding of the immunological mechanisms that may contribute to MS pathology. This research contributes to the broader effort to develop targeted therapeutic strategies for managing MS relapses and highlights the importance of cytokine interactions in the progression of autoimmune diseases.

#13

Myrf conditional knockout inhibits myelin gene expression without altering glucose dependence or glycolytic capacity.

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Myelin regulatory factor (*Myrf*) is a transcription factor within oligodendrocytes (OLs) that controls expression of genes involved in myelination, including *Plp1* and *Mag*. Conditional deletion of *Myrf* in adult OLs leads to cognitive impairment, demyelination, and ataxia. Here, we asked whether changes to myelin gene expression co-occur with changes to OL metabolic capacity. Sox10cre-ER^{T2}:Myrf$^{fl/fl}$ mice (Myrf^{CKO}) exhibited no behavioral abnormalities at 1 week post-gavage (p.g.) up until 6 weeks p.g., at which time they exhibited signs of ataxia, burrowed less, and took longer to complete an irregularly spaced ladder test compared to their cre-negative littermates (Myrf^{WT}). Myrf^{CKO} exhibited decreased *Myrf* expression within a day of initial tamoxifen exposure, followed by decreased *Plp1* and *Mag* expression that reached peak suppression by 1 week p.g. In contrast, gene expression of key glycolytic enzymes *Pdha1*, *Pfkp*, *Pkm*, and *Prkaa2* was unchanged in Myrf^{CKO} compared to Myrf^{WT}. To assess OL metabolic capacity, we performed SCENITH flow cytometry analysis on brain tissues from Myrf^{CKO} and Myrf^{WT} mice. Despite dramatic decreases in OL viability and MAG protein expression following *Myrf* deletion, we observed no changes to glucose dependence or glycolytic capacity of viable OLs in Myrf^{CKO} compared to Myrf^{WT} at 1 week p.g. Interestingly, MAG levels in the myelin debris fraction were not altered at 1 week p.g. but were decreased in Myrf^{CKO} compared to Myrf^{WT} at 6 weeks p.g., indicating that 6 weeks was sufficient time for significant turnover of MAG protein in the myelin sheath. Taken together, these data indicate that changes to myelin gene transcription are not inherently linked to changes to OL metabolic capacities.

Our findings show that S100B is a glial mediator that controls cellular activity in gut neurocircuits that underlie motor behaviors of the intestine. Disrupting this gliotransmitter system impairs gut motor function and neuron activity and may contribute to digestive diseases.

#14

Enteric glial S100B controls enteric motor neurocircuit spontaneous activity

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Enteric glia regulate gut motor function through interactions with enteric neurons but specific glial mechanisms remain poorly understood. Patterns of gut motility such as colonic motor complexes (CMC) are driven by central pattern generators in the enteric nervous system (ENS). These enteric circuits share similarities with central pattern generators in the brain in which astrocytes play key roles through the S100B protein. We hypothesized that enteric glial S100B controls gut motor function by affecting ENS excitability and coordinated activity.

Glial S100B release was studied by ELISA in whole-mount preparations while CMC motor activity was studied in organ bath experiments. Cell activity of ENS was studied using Ca^{2+} imaging in whole-mount preparations of myenteric plexus from mice expressing genetically encoded calcium indicators (GCaMP5g) in Wnt1+ cells (glia and neurons) or ChAT+ neurons. S100B was manipulated using arundic acid (AA, 50 μM) pentamidine (300 μM), and S100B antibodies (1:1000).

Myenteric plexus whole-mounts spontaneously released S100B and AA reduced enteric S100B release by 51%. Reducing S100B release or blocking its extracellular buffering activity diminished CMC occurrences ($p<0,0001$). Cell live imaging studies showed that changes to enteric neurons and glial excitability may drive these effects. S100B production inhibition with AA did not eliminate spontaneous cellular activity but reduced glial and neuronal spontaneous peaks ($p<0.0001$ for both). Pentamidine also reduced the amplitude of spontaneous enteric neuron responses ($p<0.01$) and this effect was most prominent in males.

Opposite effects were observed in females where inhibiting S100B with pentamidine increased spontaneous neuron peak amplitudes ($p<0.0001$). Rhythmic activity among cholinergic neurons in the proximal colon is thought to underlie CMC generation. Ca^{2+} imaging experiments in samples from *Chat^{Cre}::GCaMP5g* mice showed that inhibiting S100B release impaired spontaneous cholinergic neuron activity in this region ($p<0.001$).

#15

Enteric pannexin-1 regulates intestinal motility

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Objective: Integration between neural, immune, and microbial systems underlies complex gastrointestinal functions, and abnormal crosstalk contributes to disease. Pannexin-1 (Panx1) is a membrane channel expressed by enteric neurons that contributes to intercellular signaling mechanisms that drive enteric neuron death during inflammation. Despite having a clear role in pathophysiology, the physiological functions of neuronal Panx1 in the enteric nervous system remain unknown. We hypothesized that neuronal Panx1 signaling regulates enteric neurocircuits controlling motility through effects on neuron-neuron and neuro-glia communication in the gut.

Methods: Panx1 expression in the murine enteric nervous system was assessed by immunolabeling. Gastrointestinal transit time, small intestinal transit, and colonic motility were measured by red carmine gavage and colonic bead assay in wild-type and knock-out mice for neuronal Panx1 (*Wnt1^{Cre};Panx1$^{f/f}$*). Neuronal and glial activity were measured by calcium imaging following local application of 1 mM ADP or 1 mM ATP in the distal colon from *Wnt1^{Cre};Panx$^{f/f}$;GCaMP-tdT$^{f/f}$* and *Wnt1^{Cre};GCaMPTdt$^{f/f}$* animals.

Results: Panx1 is expressed by neuronal subtypes in the myenteric and submucosal plexuses, but not by enteric glia. Available single-cell RNA-seq data suggest that intrinsic primary afferent neurons are the main cell type that expresses Panx1. Deleting neuronal Panx1 did not affect total gastrointestinal transit time ($p=0.22$) or small intestinal transit ($p=0.95$) in either sex; however, it doubled colonic transit time in males ($p=0.009$). The absence of Panx1 in Wnt1$^+$ cells increased neuronal and glial responses to ADP ($p=0.008$ and $p<0.0001$, respectively) and ATP ($p=0.0005$ and $p=0.0021$, respectively), which may indicate a cellular mechanism underlying the change observed in colonic motility. Interestingly, deleting Panx1 only induced an increase in glial responses to ADP in males, whereas both neuronal and glial activity increased in females.

Conclusion: These findings show that neuronal Panx1 is essential for the physiologic regulation of intestinal motility through mechanisms that include intercellular signaling between enteric neurons and glia in gut motor neurocircuits. Panx1 could, therefore, be an important therapeutic target for intestinal motility and inflammatory disorders.

#16

Burrowing behavior as an assessment for disease progression in the CT-2A mouse model of glioblastoma.

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Glioblastoma (GBM) represents the most common type of primary brain tumor. Despite aggressive treatment, less than 5% of patients survive to 5 years after diagnosis. Thus, there is a critical need to develop effective therapeutics. Mouse models represent an important preclinical tool with which to investigate GBM pathogenesis. However, progression analysis in these models is reliant on imaging and survival studies, which can be cumbersome, and at times, subjective. Burrowing is an instinctive mouse behavior that is disrupted during conditions that cause neuroinflammation and/or neurodegeneration. In the current study we questioned whether burrowing behavior could be used to predict tumor progression as well as determine if changes to behavior correlate with numbers of microglia and/or tumor infiltrating macrophages (TAM) in a syngeneic model of GBM. In order to distinguish between microglia and infiltrating macrophages, a microglia-specific reporter line was generated by crossing homozygous Tmem119Cre^{ERT2} mice to homozygous B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (Ai14) mice. Transmembrane 119 is a marker in microglia that can be used to distinguish it from other immune cells in the tumor microenvironment (TME) to specifically visualize localization throughout tumor progression. At 6 weeks of age, recombination was induced in male (n=9) and female (n=9) mice by tamoxifen injection (5µg/day for 5 days) administered *per os*. At 8-12 weeks of age, mice were anesthetized and stereotaxically injected with 5x10^4 CT-2A GBM cells in a total volume of 0.5 µL. After surgery, burrowing behavior was monitored after 2 and 24 hr each day for up to twenty days post injection (p.i.). Body weights were recorded daily and mice were scored for clinical signs of disease. Equal numbers of sex matched mice were euthanized on days 4, 8 and 20 post injection (p.i.) and brains processed for tumor infiltrating microglia and macrophage analysis. We observed a continual reduction in burrowing behavior that initiated at day 5 p.i. Reductions in burrowing behavior preceded weight loss and clinical signs of disease. Regression analysis indicated a significant negative correlation between TAMs and burrowing behavior measured at 24 hrs. These data indicate that burrowing behavior may provide a helpful tool with which to assess disease progression in tumor-bearing mice and also that the number of TAMs may be indicative of poor prognosis.

#17

Microglia increase morbidity and mortality in a synergetic mouse model of glioblastoma

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A hallmark of glioblastoma (GBM), the most aggressive and common brain cancer, is the tumor mass often consisting of ~30% microglia and macrophages (TAMs), yet their precise contributions to tumor development and progression remain unknown. Microglia possess the ability to interact with glioma cells, further progressing the cancer's distinct ability to proliferate uncontrollably and invade surrounding tissue. Despite these findings, the issue of distinguishing between microglia and other TAMs precluded attributing findings to microglia alone. Recent findings show transmembrane protein 119 (TMEM119) is exclusively expressed in microglia, enabling their distinction from other myeloid populations such as macrophages. We have leveraged the fact that *Tmem119* is expressed in microglia, but not macrophages, to create a transgenic mouse line that enables the conditional deletion of *Csf1r* in microglia, causing their depletion. To determine the involvement of microglia in disease progression, control ($Csf1r^{fl/fl}$; MG^{WT}) and microglia conditional knockout mice ($Tmem119Cre^{ERT2}$:$Csf1r^{fl/fl}$; MG^{CKO}) received a stereotaxic injection of syngeneic CT-2A glioma cells. Body weights, burrowing behavior and clinical signs were measured throughout the experiment. The effects of microglia depletion on immune cells within the tumor microenvironment was determined by immunofluorescence and flow cytometry. Immunofluorescent staining revealed an approximate 70% reduction in $Iba1^{+}$ microglia within the cortex, hippocampus, and striatum of MG^{CKO} mice when compared to MG^{WT} control mice. Moreover, MG^{CKO} mice exhibited prolonged survival, increased burrowing behavior and decreased weight loss compared to MG^{WT} mice. Flow cytometry analysis performed on cells isolated from the brains of tumor-bearing mice indicated that MG^{CKO} had reduced monocyte, macrophage, and CD4 T cell populations, but increased cytotoxic CD8 T cell populations compared to MG^{WT} control mice. Collectively, we developed a mouse model that provides depletion of microglia alone, enabling greater understanding of this cell type's role in GBM pathogenesis. Furthermore, Cre-mediated depletion of microglia significantly impacted survival, weight change, and burrowing efficiency, showing a strong correlation with the volume of TAM trafficking into the TME.

#18

Inhibition of neuroinflammation by a bacterial polysaccharide

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Neuroinflammation is an important factor contributing to the development of neurodegenerative diseases. Blocking inflammatory signaling pathways in CNS is a promising strategy for treatment of neurodegenerative diseases. Inflammation is triggered by innate immune cells, and we have identified a probiotic molecule, exopolysaccharide (EPS), secreted by a commensal bacterium, *Bacillus subtilis,* that induces immunological tolerance of innate cells. We showed that EPS generates inhibitory macrophages and dendritic cells, which inhibit a variety of inflammatory diseases, including sepsis by infection with *Staphylococcus aureus, C. Rodentium* -induced colitis*,* allergic eosinophilia, autoimmunity, and graft vs host disease. Here, we used the “inflammamouse”, a mouse model in which tissues express a luciferase biosensor for inflammation, to study if EPS can prevent neuroinflammation induced by injection of LPS. We showed that mice pre-treated with EPS 1 day prior to the lethal challenge with LPS do not display either brain or systemic inflammation. Similarly, pro-inflammatory cytokine levels were significantly lower in brains and serum of EPS pre-treated mice compared to PBS controls after LPS challenge. To test if EPS affects microglia, we pretreated the microglial cell line, SIM-A9, with EPS and then challenged with LPS, and measured the pro-inflammatory mediator, NO, using the Greiss assay. We found that pre-treatment with EPS significantly decreased (by 30%) nproduction of NO by microglial cells treated with LPS. Our data suggest that EPS inhibits neuroinflammation induced by LPS and could be used for treatment or prevention of neurodegenerative diseases.

#19

Long-acting anti-coagulants (LAARs) induce glial cell activation in adult rabbit cerebellum

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Long-acting anti-coagulant rodenticides (LAARs) are used to eradicate rodent infestations. Often called superwarfarins, LAARs were introduced after several rodent strains developed resistance to the anticoagulant Warfarin. LAARs prevent blood clotting by reducing Vitamin K1 (VK1) levels, a necessary co-factor for activation of clotting factors. Increased usage of LAARs has led to accidental and intentional exposures to humans. One of the most potent and commonly used LAARs is brodifacoum (BDF), a strongly hydrophobic, metabolically stable derivative of warfarin. BDF undergoes enterohepatic recirculation which extends its half-life, and it was shown that treatment with the bile sequestrant Cholestyramine (CSA) which blocks enterohepatic recirculation increased clearance of BDF from the body. We previously showed that BDF increased neuropathology in adult rats; and caused cell death when applied directly to astrocytes or neurons. In the current study we examined the effects of BDF on neuropathology in adult New Zealand White (NZW) rabbits, which we established to be a better model to examine LAAR poisoning than rodents. BDF (200 ug/kg) was administered once (by gavage) to adult male NZW rabbits, together with VK1 (5 mg/kg i.p., given daily) to prevent anticoagulation and death. Some animals were also treated with CSA (0.67 gm/kg by gavage, daily for 6 days). After 22 days, animals were euthanized, and brains collected for immunohistochemistry and lipid analysis. To analyze glial cell activation, immunohistochemistry was carried out by staining cerebellar sections for GFAP and Iba1. Initial results indicate a significant increase in both GFAP and Iba1 in animals administered BDF; and reduced staining in animals that received treatment with CSA. Lipid analysis shows that BDF decreased sulfatides C12:0 and C24:0; decreased sphingomyelin C18:0; increased Sphingomyelin C24:1; and increased ceramide C18:0. These changes were all prevented by treatment with CSA. These results suggest that BDF induces glial cell activation and modifies lipid synthesis and/or metabolism; effects which could contribute to subsequent neuropathology in poisoned subjects. Treatment with a bile sequestrant such as CSA represents a novel approach to minimize consequences of LAAR poisoning.

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#20

Downregulation of Pantothenate Kinase and Upregulation of Pantothenate in Liver Kinase B1 Deficient Mouse Astrocytes

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Liver Kinase B1 (LKB1) is a master kinase involved in regulating activation of multiple downstream kinases, and is associated with cell polarity, metabolism, proliferation, and inflammatory activation. Astrocyte activation and metabolic function have important roles in regulating neuroinflammation and neuropathology, and we previously showed that knockdown of LKB1 from astrocytes exacerbated pathology in the EAE model of MS. To better understand how LKB1 influences astrocyte physiology, we carried out studies using primary mouse astrocytes treated with siRNA to knockdown (KD) LKB1. In brief, primary astrocytes were cultured from mouse pups from postnatal day 0 – 2. The brains were dissected, trypsinized and plated in DMEM + 10% FBS growth medium. The astrocytes were seeded into poly-L-lysine coated coverslips or culture plates and treated with scramble or LKB1 siRNA for 24 hours. Untargeted metabolomics showed an increase in Pantothenate (Vitamin B5) in conditioned media from KD cells. Pantothenate is phosphorylated by Pantothenate Kinase (PanK) to generate 4-phosphopantothenate, and mutations in PanK have been shown to cause neurodegeneration. Immunocytochemical analysis for GFAP, PanK2, and LKB1 revealed a decrease of LKB1, as well as GFAP expression in siRNA treated astrocytes compared to control cells. Interestingly, staining for PanK2 appeared to be slightly increased in the KD cells. Similarly, qPCR analysis for GFAP, PanK2, and LKB1 showed altered expression of these mRNAs as compared to control. RNAseq analysis showed that LKB1 KD increased 167 and decreased 179 mRNAs (QValue < 0.05 and greater than 1.5-fold change) versus controls. KEGG analysis showed that several metabolic pathways were reduced, including Pantothenate and CoA biosynthesis. These findings indicate that Pank2 gene expression and protein levels are altered in LKB1 KD astrocytes, thereby suggesting a crucial role of the Pantothenate pathway in maintaining metabolic homeostasis in mouse brain astrocytes. Further work is being performed to confirm these preliminary findings.

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#21

Disease-Associated Microglia Impact Synaptic Circuits in Multiple Sclerosis

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Microglia, the macrophages of the central nervous system, constantly surveil their microenvironment under homeostatic conditions. Our previous work on Multiple Sclerosis (MS), an inflammatory, demyelinating disease, identified that most homeostatic microglia transition into reactive phenotypes and these reactive microglia eliminate retinal ganglion cell synapses in the lateral geniculate nucleus early in disease, causing visual dysfunction in multiple MS-relevant preclinical animal models. However, several reactive microglial phenotypes exist, and it remains elusive which reactive phenotype these synapse-engulfing microglia adopt and if this transition is essential for synaptic circuit disruption. Here, we investigate the so-called disease-associated microglial phenotype (DAM), as it is the predominant reactive microglial phenotype in neurodegenerative diseases including MS. We have intriguing new preliminary data showing decreased synaptic densities exclusively surrounding reactive DAM but not homeostatic microglia in the visual system, suggesting that microglial adaption of a DAM phenotype is essential for synaptic circuit disruption in demyelinating disease. To further test this, I will knockout established DAM regulatory factors specifically in microglia, previously shown to block or promote a shift towards the DAM phenotype, respectively and determine whether modulating the homeostatic-to-DAM transition affects synaptic connectivity in demyelinating disease. I will analyze microglia reactivity, as well as structural and functional synaptic circuits. Further, it is unexplored whether DAM transition of human microglia is governed by the same regulatory proteins, therefore, in a highly translational approach, I am currently also evaluating the role of these regulators in the homeostatic-to-DAM transition and microglia-synapse interactions in human monocyte-derived microglia-like cells (HMDMi). Together, this work will advance our fundamental, functional knowledge of the DAM phenotype and reveal if DAM manipulation comprises a therapeutic target to protect against synaptic loss and the associated functional vision decline that is relevant to MS and other related neurodegenerative diseases.

#22

Müller Glia Cells – A Target for Steroid Therapy to Control Diabetic Macular Edema

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Purpose: Diabetic macular edema (DME) is characterized by fluid buildup within the macula, an area that is defined by only cones and Müller cells and the point of sharpest vision. Newest research suggests that DME is driven by underlying chronic inflammatory events and Müller cells have been identified to produce and release pro-inflammatory mediators under hyperglycemic conditions such interleukin-1beta, a product of active caspase-1. Standard protocol of care to control DME in patients are intravitreally injected anti-VEGF (vascular endothelial cell growth factor) drugs. However, in 30% of patients with DEM those drugs have no effects. The only alternative therapy are steroids, although very little is known about their mechanism of action in the diabetic retina. In general, steroids are considered to alleviate inflammation and swelling. Thus, this study was focused on understanding the effects of fluocinolone acetonide, a steroid clinically used to treat DME, on retinal Müller cell function when exposed to hyperglycemic conditions.

Materials and Methods**:** Non-diabetic and STZ-diabetic wild-type (WT) and caspase-1-/- knockout (Cas-1-/-), mice were used to determine the effect of caspase-1 on Müller cell viability and the progression of diabetic retinopathy in vivo. Müller cells (rat cell line rMC-1; isolated human cells hMC) were treated with either 5mM or 25mM glucose in the presence or absence of FAc at different concentrations for up to 96 hours. Outcome measurements to assess Müller cell functions included measurements of caspase activities using different fluorescence peptide probes, assessment of cell swelling and cell death using DeNovix CellDrop FL.

Results: Our studies confirmed that active caspase-1 is causing Müller cell loss and subsequent diabetic retinopathy in the STZ animal model. Furthermore, our in vitro studies identified that treatment of Müller cells with FAc (0.05ng/ml) reduced hyperglycemia-induced cell death by 100±6% ($n>6$, $p<0.0001$) in rMC-1 and by 94.5±5.5% ($n>6$, $p=$ $p<0.0001$) in hMC, respectively. 0.05ng/ml of FAc given 48 hours after the hyperglycemic insult significantly inhibits hyperglycemia-induced cell death (25.6±7.4%) compared to cells treated in 25mM glucose without FAc (44.8±6.27%) ($n>6$, $p<0.0001$) indicating strong interventional capabilities of the drug. In addition, FAc reduced hyperglycemia-induced activation of the pro-inflammatory caspase-1 signaling pathway by 96±4% at 96 hours ($n>6$, $p=0.0003$). Interestingly, FAc (0.05ng/ml) also reduced hyperglycemia-induced cell swelling by 90±10% ($n>6$; $p=0.037$).

Conclusions: Fluocinolone acetonide targeted and improved Müller cell function and viability in a hyperglycemic environment by inhibiting pro-inflammatory events. Considering that diabetic macular edema has strong pro-inflammatory components expanding the use of steroid therapy, either as a therapy on its own or in combination with anti-VEGF therapy, should benefit patients with DME.

#23

GABAergic Modulation of Gut Motor and Sensory Neurocircuits Differentially Impacts Gut Function Between Weaning and Adulthood.

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Functions of the gastrointestinal tract such as motility are controlled by the enteric nervous system (ENS), a neuroglial network embedded in the wall of the intestine, while gut sensory information is conveyed by extrinsic nerve fibers that innervate the gut from dorsal root ganglia. Gut motility and pain are modified by Gamma-Aminobutyric acid (GABA), but the cellular targets and underlying mechanisms remain poorly understood. GABAA and GABAB receptors are expressed by enteric neurons and glia and GABAergic signaling in the brain undergoes a well-known switch during maturation. Therefore, we hypothesized that GABA modifies gut motor and sensory functions through effects on enteric neurons, glia and sensory afferent nerve terminals and that the effects of GABA change during the maturation of the ENS in weaning.

The effects of GABA on enteric neuronal and glial activity, as well as on colonic nociceptive fibers, were assessed using Ca2+ imaging in Wnt1GCaMP and Trpv1GCaMP mice before weaning and at adulthood. Gut motor function was studied in organ bath experiments by recording colonic migrating motor complexes (CMCs).

GABA (1 mM) drove Ca2+ responses among myenteric neurons and glia in the distal colon, which were larger in both glia ($p<0.001$, $\Delta F/F0=0.10$ before weaning vs 0.06 in adults) and neurons ($p=0.0021$, $\Delta F/F0=0.18$ before weaning vs 0.12 in adults) prior to weaning. However, 10 μM GABA induced stronger activation of Neurons in adult mice ($p=0.005$, $\Delta F/F0=0.14$ before weaning vs 0.18 at adult age). In the proximal colon, only 1 mM GABA produced robust activation of neurons both in terms of response amplitude and the number of responsive cells before the weaning. TRPV1+ sensory nerve terminals did not exhibit acute responses to GABA, but a 30-minute incubation with 10 μM GABA reduced basal activity in TRPV1+ fibers and decreased responses evoked by capsaicin. Incubation with 1 mM GABA completely blocked TRPV1+ fiber activation ($\Delta F/F0$ of peak response = 0.053 in CT, 0.034 with GABA 10 μM and -0.028 with GABA 1 mM). CMC recordings revealed specific region effects of GABA of motor function whereby GABA increased the amplitude of contractions in aboral regions in adult mice and oral regions before weaning.

These findings indicate that GABA differentially regulates gut motor and sensory functions before and after weaning through effects on enteric neuronal and glial activity and TRPV1+ fiber sensitivity. Ongoing studies explore cellular signaling mechanisms underlying these effects and how they may be altered following inflammation.

#24

Pre-clinical Model of Pelizaeus-Merzbacher Disease: An In Vivo Study

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Pelizaeus-Merzbacher Disease (PMD) is a rare neurodegenerative disorder affecting the brain's white matter, a leukodystrophy primarily affecting myelinogenesis. The disease is principally caused by mutations of the PROTEOLIPID PROTEIN 1 (PLP1) gene located on the X-chromosome, which encodes the major protein component of CNS myelin. The most common mutations in PMD patients are duplications and triplications of the PLP1 gene, leading to protein overexpression and causing cellular toxicity and death of oligodendrocytes. A transgenic mouse model of Plp1 gene duplications (Nave-66) has been studied by several groups including our own. These mice have a lifespan between 150 - 200 days. To mitigate disease symptoms in these transgenic mice, we tested if a single intraventricular injection of an AAV-Olig001vector - an adeno-associated virus (AAV) with tropism for oligodendrocytes, encoding EGFP and an inhibitory short-hairpin sequence targeting Plp1 mRNA (shRNA, miR-1333) - into 4-6 days old mice could suppress PLP1 overexpression and reduce cytotoxicity. A scrambled shRNA (miR-1307) was used as a control. This exploratory study aimed to regulate PLP1 levels in mutant mice as a potential treatment strategy for PMD patients. Effectiveness of the AAV treatment was evaluated through various behavioral tests including forearm strength test, rotarod test, and lifespan measures. Further, qPCR was used to examine postmortem Plp1 expression levels intreated mice. Because PMD is X-linked recessive, only male mice between 1 to 6 months of age were included in the study. In all behavioral tests, we did not find evidence of disease mitigation in the Olig001-miR-1333 group compared to the Olig001-miR-1307 controls. Further, Kaplan-Meier analysis indicated that lifespan was not extended. However, we observed an approximate two-fold reduction in levels of Plp1 mRNA in brain homogenates of active versus scrambled miRNAs (whole tissue between Bregma and Lambda analyzed). The mRNA encoding EGFP was also abundant in this tissue, indicating that AAV expression persisted for at least 7 months post-injection. Finally, we observed strong and widespread EGFP fluorescence staining in midbrain/brainstem cryostat sections. Our findings underscore the challenges ahead for improving the efficacy of the Olig001 therapy and alleviating PMD symptoms. Nevertheless, we conclude that our approach in Plp1 overexpressing mice is a technical success, and this preliminary research serves as a critical foundation for future experiments and studies aimed at refining therapeutic strategies for PMD.

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