LBA01

Involvement of the Brain Renin-Angiotensin System in the progression of diabetes-induced amnesia

Akash Gautam[1], *Jayashree T.[1], Athar Siddiqui[2]*

[1]Centre for Neural and Cognitive Sciences, University of Hyderabad, Hyderabad, India

[2]School of Medical Sciences, University of Hyderabad, Hyderabad, India

The Renin Angiotensin System (RAS) plays a vital role in glucose metabolism by regulating a complex hormonal cascade and maintaining blood pressure, water intake and electrolyte balance. However, it has been observed that the RAS is dysregulated under diabetic conditions. Brain RAS is a separate component of the RAS within the brain, and it has been implicated through recent studies in cognitive dysfunctions under diabetic conditions. This study was undertaken to determine the involvement of brain RAS components in diabetes-induced amnesic conditions and to design suitable interventions for treating such disorders.

We used adult male SD rats and divided them into Vehicle, diabetic, amnesic, and diabetic+amnesic. Diabetes was induced by a single IP injection of Streptozotocin (STZ) at a dose of 55 mg/kg body weight. Amnesia was induced by an IP injection of Scopolamine at a dose of 1 mg/kg body weight dissolved in normal saline for 15 days. The diabetes-induced amnesia was confirmed through behavioural, biochemical and molecular tests. The expression level of two major brain-RAS components, AT1 and AT2, was analyzed through qPCR and Western blotting in the hippocampus and prefrontal cortex. Finally, we confirmed the involvement of these components using an Angiotensin receptor blocker (telmisartan) in the above animal groups.

Our behavioural, biochemical and molecular assays showed significant memory loss in the diabetic groups compared to the vehicle control group. For the brain-RAS components, the mRNA and protein expression analysis showed that AT1 levels were elevated ($p<0.001$), but AT2 levels were decreased ($p<0.001$) in the diabetic and amnesic groups compared to the control group in both regions of the brain. The telmisartan treatment ameliorates mitochondrial dysfunction, as revealed by the measurement of lactic acid content in the treated groups compared to the untreated groups. Our findings reveal that increased AT1 (with a simultaneous decrease in AT2) expression in the diabetic group might contribute to memory impairment, which can be targeted to ameliorate the diabetes-induced amnesic effects.

LBA02

Targeting fructose metabolism as a modulator of microglial metabolic reprogramming in Alzheimer's disease

Annalise M Sturno[1], James E Hassell[1], Katelyn M Baker[1], Garrett J Potter[1], Dean Oldham[1], Nicholas Cleland[1], Lucas Guerrero[1], Ana Andres-Hernando[1], Miguel A Lanaspa[1], Kimberley D Bruce[1]

[1]*Division of Endocrinology, Metabolism & Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO 80045*

Alzheimer's disease (AD) is a devastating and ultimately fatal age-associated neurodegenerative disease characterized by dysregulated brain metabolism. The precise mechanisms that drive changes in oxidative metabolism and glycolysis in AD remain elusive. Current literature suggests that microglia–the innate immune cells of the brain – play a key role in AD pathology. Whether this role is protective or detrimental depends on microglia phenotype and function, which is intrinsically linked to metabolism. When activated, microglia shift away from oxidative phosphorylation and towards glycolysis. Chronic activation leads to 'metabolic reprogramming', which drives microglial dysfunction and AD pathology. We have recently shown that microglia shift their metabolism towards glycolysis in aging (Cleland et al., 2024). Notably, this metabolic programming is characterized by an increase in GLUT5—a fructosespecific transmembrane transporter encoded by SLC2A5—in the brains of 5xFAD aged mice, and human monocyte-derived microglia, particularly in aged females. In addition, high-fructose diets lead to increased weight and frailty in female mice, highlighting increased vulnerability to fructose metabolism. Taken together, we hypothesize that fructose metabolism may drive the metabolic reprogramming of microglia associated with AD pathology in a sex-specific manner. Here, we show that modulating fructose intake and metabolism has the potential to restore microglia function and slow disease progression. Specifically, depleting Keto Hexokinase (KHK) —downstream of GLUT5 in the fructolysis pathway—improves age-associated changes in microglial morphology in ADvulnerable regions of the brain (e.g., thalamus). Mice lacking Aldose Reductase (AR) — the rate-limiting enzyme in the polyol pathway — also exhibit reduced microglial activation with aging. Additionally, metabolomic data from microglia isolated from AR KO mice confirm decreased fructose processing and increased antioxidant production. Because intracellular KHK and AR are challenging pharmacological targets, we have generated the first conditional GLUT5 knock-out mice to further address our hypothesis. Overall, our findings support the notion that fructose metabolism contributes to the metabolic reprogramming of microglia that precedes AD pathology and may be a novel target for the development of new strategies to improve outcomes for individuals living with or at risk of developing AD, particularly women.

LBA03

Identifying synaptome aberrations in C9orf72 Frontotemporal Dementia patient-derived cortical neurons

Ashton Spillman[1,2], Ritin Sharma[3], Anna Bonfitto[3], Krystine Garcia-Mansfield[3], Brooke Lovell[3], Lauren Gittings[1], Ignazio Piras[3], Patrick Pirrotte[3], Kendall Van-Keuren Jensen[3], Matt Huentelman[3], Rita Sattler[1]

[1]Barrow Neurological Institute (Phoenix, AZ), [2]Arizona State University (Tempe, AZ), [3]Translational Genomics Research Institute (Phoenix, AZ)

Neuronal function and communication rely on the tightly-regulated maintenance of synaptic networks, which go awry in neurodegenerative diseases such as in Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal Dementia (FTD), resulting in synapse loss. A hexanucleotide repeat expansion in the first intron of C9orf72 is the most common genetic cause of ALS and FTD. Mutations in C9orf72 have been shown to impair synaptic transmission in ALS; however, little is known how this mutation may impact the molecular composition of synapses (synaptome), and therefore the maintenance of these synaptic networks in FTD. Therefore, we hypothesize that the synaptome is aberrant in FTD and thus contributes to the aberrant behavior and function of cortical neurons. Here, we utilize patient-derived induced pluripotent stem cells (iPSCs) differentiated into cortical neurons (CNs), and postmortem frontal and occipital cortex tissue from C9-FTD patients and age-matched healthy controls. To study synapse-specific alterations, a series of biochemical fractionations were performed to obtain synaptosomes, isolated synaptic terminals. Here, these synaptosomes were submitted for mass spectrometry proteomics and RNA sequencing transcriptomics, to assess for protein and mRNA alterations in C9-FTD, respectively. Preliminary proteomics analyses from C9-ALS/FTD derived synaptosomes revealed an upregulation of metabotropic glutamate receptor-2, which is consistent with the cortical hyperexcitability hypothesis of ALS. Interestingly, we detected an upregulation of synaptic scaffolding proteins across synapse compartments, as well as downregulations of SNARE-complex members. To assess for synaptic activity, iPSC-CNs were cultured on Microelectrode Array (MEA) plates to assess for metrics such as firing rate of action potentials, and network bursting and synchrony. These activity-dependent phenotypes would provide insight into cortical neuron behavior in C9-FTD, which is currently unknown. Additionally, iPSC-CNs were grown on coverslips to stain for pre- and postsynaptic markers and quantified for their synaptic densities along neuronal dendrites. Our work aims to identify synaptic aberrations across multiple levels of analysis, from molecular composition to functional activity. Subsequent experiments will investigate the relationship between microglia-synapse interactions by manipulating synapse composition and microglial state to determine the directionality of synapse elimination.

LBA04

Differentiation of human iPSC-derived glia in biomimetic platforms mimicking the sti2ness of healthy and demyelinated brain

Carmen Melendez-Vasquez[2], Bowen Luo[1], Ace Alcantara[2], Hongjun Wang[1]

[1]*Stevens Institute of Technology, Hoboken, NJ.*

[2]*Hunter College and Graduate Center, City University of New York, NY, NY.*

Demyelination, characterized by oligodendrocyte loss and myelin sheath destruction, causes impaired neurological function in many diseases and neurodegenerative processes including multiple sclerosis (MS), leukodystrophies, stroke and aging. This process dynamically alters brain sti2ness. Notably, we found that acute demyelination leads to decreased sti2ness, while chronically demyelinated lesions exhibit increased stiffness. Remyelination, the process of regenerating myelin around the demyelinated axons, is essential to restore the nerve function. Studies have shown that rodent oligodendrocytes progenitor cells (OPC) are mechanosensitive, and that their ability to di2erentiate into oligodendrocytes (OL) is influenced by the mechanical properties of their microenvironment.

To investigate the contribution of tissue sti2ness, on the di2erentiation of human iPSCderived glia (hiPSC-glia), we developed hydrogel systems using pure or hybrid methacrylated hyaluronic acid (HAMA) and gelatin (GelMA). These hydrogels were tuned to mimic the mechanical properties of healthy (~2 kPa), acutely demyelinated (~1–1.4 kPa), and chronically demyelinated (~7 kPa) brain tissue. Electrospun microfibers were integrated into these hydrogels to provide axonal biomimicry. We found that conditions mimicking the sti2ness of healthy brain tissue support the viability of hiPSC-glia and their differentiation into mature OL (Olig2+, O4+). By contrast, sti2er environments mimicking the sti2ness of chronic lesions, reduce the viability of hiPSC-glia and inhibit their differentiation into OL, while promoting astrocyte (GFAP+) di2erentiation. This study provides valuable insights on the optimal mechanical properties of the environment that might promote myelin repair by stimulating the di2erentiation of endogenous OPCs or by transplanting OPC derived from autologous iPSCs into lesioned areas.

LBA05

Mapping the Temporal Profile of c-Fos Activation in Zebrafish Larvae Following Pentylenetetrazole-Induced Convulsions.

Amit Kumar[1,2], Damanpreet Singh[1]*

[1]*Pharmacology and Toxicology Laboratory, Dietetics and Nutrition Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India*

[2]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-201002, India*

Zebrafish (Danio rerio) has been used as an alternative to animal models and gained popularity in epilepsy research for the past few years due to several merits over mammalian models. c-fos, an immediate early expressing gene is a well-known marker to assess the neuronal activity during seizures. However, there are several reports that indicates the variations in the transcription and translation of the expression of c-Fos after the chemoconvulsant exposure. This study aimed to investigate the changes in c-Fos expression at various time points following pentylenetetrazole (PTZ)-induced convulsions in a zebrafish larvae model. Larvae at 7 days' post fertilization (dpf) exposed to 8mM PTZ and showed hyperactivity with clonus-like seizures. Gene expression of c-fos was assessed in the cephalic region of zebrafish larvae at multiple time points ranging from 15 to 90 minutes after PTZ stimulation. Concurrently, whole-mount in situ analysis was performed to examine c-Fos protein expression in the brain at the same time intervals. The findings demonstrated a time-dependent modulation of c-Fos expression, with both mRNA and protein levels increasing initially and peaking at 45 minutes post-PTZ exposure, followed by a subsequent decline up to 90 minutes. Pearson correlation analysis confirmed a strong association between transcriptional and translational changes over time. These results highlight the dynamic nature of c-Fos expression in response to PTZ-induced seizures and emphasize the importance of selecting appropriate time points when using c-Fos as a biomarker in zebrafish convulsion models. The modulation of c-Fos expression could contribute to the development of novel strategies for epilepsy treatment.

LBA06

Evaluation of the Impact of SARS-CoV-2 on the developing brain

Débora Magalhães Portela[1 2]; Emanuelle Vasconcellos de Lima[1]; Bruna Oliveira Andrade[1]; Beatriz Oliveira de Campos[1]; Raissa Rilo Christoff[1 2]; Daniel Messor[1]; Marcos Romário Matos de Souza[3]; Luiz Ricardo Berbert[5]; Luciana Jesus da Costa; Leda Castilho[3]; Leda Castilho[4]; Patrícia Pestana Garcez[2]; Julia Helena Rousauro Clarke[1]

[1]*Research Laboratory in Neuroprogramming (LINP) – Department of Morphological Sciences at the Institute of Biomedical Sciences/UFRJ, Brazil*

[2]*Laboratory of Neuroplasticity (LNP) - Department of Morphological Sciences of the Institute of Biomedical Sciences/UFRJ, Brazil*

[3]*Department of Virology/ Paulo de Góes Institute of Microbiology - Federal University of Rio de Janeiro, Brazil*

[4]*COPPE/UFRJ, Brazil*

[5]*PEMOP3/UFRJ, Brazil*

Data from 2022 showed that more than 196,600 pregnant women had a diagnosis of COVID-19 in the United States. It was observed that SARS-CoV-2 infection during the third trimester of pregnancy triggered cortical changes, with apparent periventricular reactive gliosis. While it is still controversial whether SARS-CoV-2 can cross the placental barrier, the viral Spike (S) protein has been found in the maternal-fetal interface and can persist beyond the acute phase of infection, potentially causing neurological damage on its own. This indicates that not only infection, but also viral particles can affect neurodevelopment, such as gliogenesis and brain connectivity.

Therefore, this study aims to investigate the effects of SARS-CoV-2 on glial reactivity and brain connectivity using a model of S protein exposure during development.

To assess this, 17 µg of Spike (S) protein were subcutaneously injected into postnatal day 1 Swiss mice. Control (Veh) groups received s.c. injection of 10 µl saline. At P10, the animals' brains were collected for immunohistochemistry (IHC) analysis to examine the impact of S protein on the reactivity (GFAP) and number (SOX9) of astrocytes and

microglia/macrophages (Iba1+). Additionally, cortical excitability was assessed by Heat-Induced Seizure at P10 (47-48 °C). The differences between groups were evaluated using the unpaired Student's t-test.

We found astrocytes increase in the cingulate cortex, as evidenced by IHC analysis performed on P10 animals. The Spike group exhibited a higher intensity of GFAP compared to the Veh (n=3; p=0.0313), along with a greater number of SOX9+ cells in the S group compared to the Veh (n=3; p=0.0397) in the same region. However, no significant

differences were found in intensity (p=0,3053; n=3) and number (p=0,3366; n=3) of Iba1+ cells in the cingulate cortex from both Spike and control animals. Behavioral experiments revealed that, at P10, the Spike group exhibited a shorter latency (s) to the onset of seizure (n=5) compared to the Veh (n=7; p=0.0008).

The data suggests that the S protein increases cortical astrocytic reactivity, potentially leading to an inflammatory environment that can influence brain activity, resulting in hyperexcitability.

LBA07

OLX-07010 inhibits tau aggregation and ameliorates motor deficits in mice with mutated human tau and APP

Dhruv R. Patel, Ph.D.[1], Patricia Lopez, M.A[1], Eliot J. Davidowitz, Ph.D., James G. Moe, Ph.D., MBA.

[1]*Oligomerix, Inc., Bronx, New York, United States*

Tau aggregation is a key factor in neurodegenerative diseases like Alzheimer's, PSP, and FTD, leading to synaptic dysfunction, neuronal death, and cognitive decline. The interaction between tau and amyloid pathology exacerbates disease progression and severity. Preventive or therapeutic treatment with OLX-07010, a small molecule inhibitor of tau self-association, inhibited tau aggregation in the htau (PMID: 31771053) and P301L tau JNPL3 mouse models of tauopathy (PMID: 37556474; PMID: 40052227). This study aimed to evaluate the effect of OLX-07010 on tau aggregation in mice co-expressing human P301L-Tau and APPSwe (TAPP), which exhibit tau pathology and gliosis by 3 months of age, leading to motor impairment.

The study was designed with 6 groups including a w.t. age control group of C57BL/6 mice (n=15), a baseline group (n=15), a vehicle group (n=20), and 3 treatment groups receiving 20, 40, or 80 mg/kg/day OLX-07010 formulated in diet (n=20 mice per dose group). The duration of treatment was for six months from 3.5 to 9.5 months of age. The primary endpoint was the reduction of aggregated tau; secondary endpoints included improvements in locomotor and cognitive deficits, and biomarkers for CNS and inflammation.

Biochemical analysis of tau aggregates in cortical biospecimens were conducted using Alphascreen™ and immunoblot assays and CNS and inflammation markers in serum were quantified using the NULISAseq™ CNS Disease Panel. Locomotor behavior was assessed using the Rotarod paradigm and Open Field Test, while cognitive behavior was evaluated using Barnes Maze and Novel Object Recognition Tests.

OLX-07010 treatment significantly reduced insoluble total tau in the cortex at 20 and 40 mg/kg/day doses and significantly inhibited gait initiation failure at all doses. Treatment decreased total tau, ptau 181, 217, and 231, and NEFL in serum suggesting promise for the utility of blood-based biomarkers in clinical evaluation.

LBA08

Defining the role of B-Raf and mTOR Signaling in Spinal Cord Oligodendroglia

Divyangi Krantikumar Kantak[1], Angelina Evangelou[2], Teresa L. Wood[1]

[1]Rutgers-New Jersey Medical School, Newark, New Jersey, 07103

CNS myelination is carefully orchestrated by multiple extracellular and intracellular signal transduction pathways that regulate the formation of myelin sheath during development and adulthood, maintain myelin and axonal integrity throughout life. Recent studies have shown that Raf/Mek/ERK1/2 (MAPK) and PI3K/Akt/mTOR pathway function autonomously and co-operatively to regulate oligodendrocyte differentiation, myelinogenesis and myelin maintenance. Interestingly, deletion of Raf kinases like B-Raf, direct upstream activators of ERKs in neural precursor cells (NPC) of mice results in a striking hypomyelinating and neurodegenerative phenotype and hinders OPC differentiation. Mice lacking mTOR in oligodendrocytes display significantly hypomyelinated spinal cord axons along with impaired oligodendrocyte maturation. However, how B-Raf and mTOR coordinately regulate myelination remains unexplored.

To elucidate the function of B-Raf and/or mTOR in oligodendroglia, we developed a rodent model in which Braf and/or Mtor floxed sequences are conditionally deleted in OPCs by Cre recombinase expression utilizing the CNPase (2',3'-Cyclic nucleotide 3'- phosphodiesterase) promoter. Gene expression analysis revealed significant downregulation of mRNA levels of several myelin proteins crucial for myelin sheath formation and axonal ensheathment in spinal cords of young adult mice lacking Braf or Braf;Mtor. We also observed fewer number of myelinated axons in the spinal cords of 12mo mice. Additionally, mice lacking both Braf and Mtor in oligodendroglia showed more severe motor deficits than mice with individual gene deletions. Our analyses aim to assess the impact of mTOR and/or B-Raf deletions on developmental and adult myelination and the mechanism of interaction between the two signaling pathways.

LBA09

Does remyelination by oligodendrocytes that survive demyelination provide neuroprotection?

Eleonora Scalia[1], Donia Arafa[1], Sarah Neely[1], Marcus Keatinge[1], Julia Meng[1], Anna Williams[1], David Lyons[1]

[1]*The Centre for Discovery Brain Sciences at The University of Edinburgh*

Myelin, the insulating sheath that wraps around axons, is vital for rapid nervous system function. Demyelination, the process wherein myelin is lost, is a hallmark of advancing age and diseases, such as Multiple Sclerosis (MS), wherein neurological disability accumulates over time. That is because demyelination can contribute to neurodegeneration which currently lacks prevention. Following demyelination, the endogenous regenerative process of remyelination can protect neurons from damage, but this fails to prevent neurodegeneration in disease. Therefore, efforts to improve remyelination represent an important strategy to provide neuroprotection. Until recently remyelination was thought only possible via the generation of new oligodendrocytes, but it is now evident that oligodendrocytes that survive demyelination can also contribute to remyelination. However, we do not know if surviving oligodendrocytes provide neuroprotection. The Lyons lab recently developed a zebrafish demyelination model to study remyelination by surviving and newly generated oligodendrocytes, and found that although surviving cells can quickly contribute to remyelination they often mistarget myelin to neuronal cell bodies, true also in MS, implying that remyelination by surviving cells may have both positive and negative consequences. In my PhD project I use zebrafish to determine the consequences of myelination of cell bodies to neurons and to test whether remyelination by surviving and newly generated oligodendrocytes stop or reverse neuronal damage following demyelination. This will determine whether surviving oligodendrocytes could be targeted to promote remyelination and neuroprotection in demyelinating diseases like MS.

LBA10

The beginning of the glymphatic system function represents the end of the susceptibility window for central nervous system ZIKV infection

Eduardo B. Sequerra[1], Jhulimar G. Doerl[1,2], Emanuelly K. C. Chaves[1,2], Lucas G. S. C. Leocádio[1], Vivian A. Becker[1], Diego M. Coelho[3], Josélio M. G. De Araújo[4], Selma M. B. Jerônimo[5,6]

[1]*Brain Institute, Federal University of Rio Grande do Norte*

[2]*Graduate Program in Neurosciences, Federal University of Rio Grande do Norte*

[3]*Graduate Program in Bioinformatics, Federal University of Rio Grande do Norte*

[4]*Microbiology and Parasitology Department, Federal University of Rio Grande do Norte*

[5]*Biochemistry Department, Federal University of Rio Grande do Norte*

[6]*Tropical Medicine Institute, Federal University of Rio Grande do Norte*

Zika virus (ZIKV) infection in postnatal mice leads to a severe disease that can lead to death. However, their susceptibility decreases after the animals reach the second week. During the second week, the glymphatic system (GS) begins to work. Here, we hypothesize that the functional GS reduces new infections by draining the interstitial fluid. We first compared the effect of ZIKV intracerebral injection at an age when the GS is not formed (P1, analysis in P14) and another when the GS is working (P21, analysis in P35). P1-injected animals with 103 PFUs of ZIKV in the lateral ventricle lose weight and display health distress signs, while P21-injected animals do not. To check the GS's function in these animals, we injected 10,000 MW Fluorescein-Dextran in the CSF. We waited for the fluid to be transported to the brain parenchyma for 30 minutes. Our results show that P1-infected animals display a reduction of CSF transport into the brain parenchyma compared to uninfected controls, while P21-injected display no difference. The astrocytes from P1-infected animals display a decrease in their ability to polarize Aquaporin4 expression facing the blood vessels in the thalamus and mesencephalon. Moreover, blood vessels in these regions develop dilations where the CSF flows longer into the parenchyma. P21-infected animals maintain their CSF transport into the parenchyma, Aqp4 polarization by astrocytes, and do not develop blood vessel dilation. We aimed to test if the inhibition of the GS after P14 rescues the susceptibility to ZIKV infection observed in younger animals. We used the drug SR49059, a competitive antagonist of the vasopressin receptor 1a that also reduces Aqp4 expression. The combination of GS inhibition and ZIKV worsens their health, while ZIKV only does not. These animals develop blood vessel dilation in the thalamus and mesencephalon, and the transport of MW Fluorescein-Dextran and 70,000 MW Ovalbumin-Alexa488 into the parenchyma increases. Our data suggest that the development of the glymphatic system represents a decrease in the susceptibility of the CNS to ZIKV infection. Aqp4 brain expression increases during the third trimester in humans, coinciding with reduced cases of fetuses that develop ZIKV-derived malformations.

LBA11

Simultaneous Expression of Sigma-1 Receptor and Tetraspanins Reveals Mechanisms of Receptor Enrichment in Extracellular Vesicles

Ago Rinken[1], Jolanta Jonane[1], Santa Veiksina[1], Sergei Kopanchuk[1], **Edijs Vavers**[1]

[1]*University of Tartu, Institute of Chemistry, Ravila 14a, 50411 Tartu, Estonia*

Sigma-1 receptor (S1R), an endoplasmic reticulum-localized chaperone protein involved in protein folding, cellular stress response, and apoptosis, has emerged as a promising therapeutic target for neurodegenerative disorders and neuropathic pain, with several ongoing clinical trials. Recent findings suggest that S1R may also play a role in the regulation of cellular secretory pathways and extracellular vesicle (EV) release. This is supported by increasing evidence of S1R's involvement in the modulation of exocytotic processes and its detection within EV populations.

The focus of the present work was the development and application of a robust multigene delivery system for the co-expression of S1R and selected EV markers to study their colocalization in cells and EVs. We employed the MultiBacMam system, a modified baculovirus-based vector optimized for synchronized multigene expression in mammalian cells, by co-expressing fluorescent protein-tagged S1R together with tetraspanins CD9, CD63, or CD81 in human ovarian adenocarcinoma SK-OV-3 cells to further analyse their interaction in cell-derived EVs. The expressed protein constructs were validated through emission spectra profiling and Western blot analysis. Cell conditioned media were processed via tangential flow filtration (TFF) to isolate EVs, which were subsequently subjected to single-particle total internal reflection fluorescence (TIRF) microscopy and quantitative colocalization analysis.

The strongest co-localization of S1R was observed with CD63 demonstrating 60% overlap between S1R-EYFP and CD63-mCherry labelled particles. High colocalization was also found between S1R-mCherry and EGFP-CD9 (40%), while lesser overlap was observed between S1R-YFP and CD81-miRFP670 (17%) labelled EVs. These findings are consistent with intracellular colocalization analyses, which revealed moderate overlap between S1R and CD63 (Pearson = 0.39, SODA = 0.33), supporting their interaction both within the cell and on secreted vesicles, likely through multivesicular endosome–dependent biogenesis and exosomal release pathways. Our findings highlight a novel dimension of S1R biology in the context of EV heterogeneity and suggest potential roles in modulating intercellular communication.

LBA12

Astrocyte gap junctions connect the brain into a gene and protein regulatory fabric

Amanda Charest[1], Lars Udo-Bellner[1], Randy Stout[1]

[1]*New York Institute of Technology*

Gap junctions are protein channels that form direct connections between adjacent cells and have been implicated in intercellular gene regulation (Dermietzel and Spray, 1993). Research from several labs has provided support for gap junctions as pathways for cell-to-cell transfer of RNA (Valiunas et. al. 2005). Gap junction-based intercellular gene regulation can also occur by transfer of secondary messengers. Additionally, gap junctions facilitate intercellular endocytosis (a.k.a. trogocytosis) that induces transfer of proteins and lipids between gap junction-connected cells (Laird, 1996). With collaborators, we demonstrated that intercellular endocytosis can transfer small organelles between astrocytes (Cibelli et. al. 2021). Previous research by collaborators showed evidence of a complex intercellular gene regulatory fabric that was dependent on gap junctions (Iacobas et. al. 2020). Gap junction intercellular gene regulation is poised as a key mechanism in most neurodevelopmental and neurodegenerative brain disorders but have not been well explored.

We sought to apply spatial transcriptomics, super-resolution 3D light microscopy, and new transgene-based tools to determine the roles of intercellular gene regulation in the brain. We examined a potential pathway for intercellular gene regulation: Transfer of nuclear hormone receptor ligands in the control of gene coordination between connected brain cells.

We examined mouse brain tissue and cultured cells with Bruker-Nanostring CosMx. We performed post-spatial transcriptomics immunostaining and imaging with Zeiss 980 and Abberior 3D STED Microscopes for improved cell segmentation and mapped gap junction connectivity with 3D super-resolution imaging. We used custom 3D spatial-transcriptomics and volumetric confocal imaging software to quantify intercellular gene regulation and measure coordination between connected versus non-connected cells to determine genes and pathways of interest. Genetic and other manipulations were used to modify connectivity of cell populations.

Analysis of combined transcriptomics and super-resolution immunofluorescence is ongoing. We will show feasibility for new transcriptomics and protein imaging approaches and will showcase new tools for 3D transcriptomics data analysis. We will report the correlation between gap junction expression and the expression of select genes in first- versus second-order adjacent cells.

LBA13

A role for the p75 neurotrophin receptor in mediating neural progenitor proliferation and oligodendrogenesis in the rat subventricular zone

Anthony A. Williams[1], Steven W. Levison[2], Wilma J. Friedman[1]

[1]*Department of Biological Sciences, Rutgers University, Newark, NJ*

[2]*Department of Pharmacology, Physiology & Neuroscience, New Jersey Medical School, Newark, NJ*

Gap junctions are protein channels that form direct connections between adjacent cells and have been implicated in intercellular gene regulation (Dermietzel and Spray, 1993). Research from several labs has provided support for gap junctions as pathways for cell-to-cell transfer of RNA (Valiunas et. al. 2005). Gap junction-based intercellular gene regulation can also occur by transfer of secondary messengers. Additionally, gap junctions facilitate intercellular endocytosis (a.k.a. trogocytosis) that induces transfer of proteins and lipids between gap junction-connected cells (Laird, 1996). With collaborators, we demonstrated that intercellular endocytosis can transfer small organelles between astrocytes (Cibelli et. al. 2021). Previous research by collaborators showed evidence of a complex intercellular gene regulatory fabric that was dependent on gap junctions (Iacobas et. al. 2020). Gap junction intercellular gene regulation is poised as a key mechanism in most neurodevelopmental and neurodegenerative brain disorders but have not been well explored.

We sought to apply spatial transcriptomics, super-resolution 3D light microscopy, and new transgene-based tools to determine the roles of intercellular gene regulation in the brain. We examined a potential pathway for intercellular gene regulation: Transfer of nuclear hormone receptor ligands in the control of gene coordination between connected brain cells.

We examined mouse brain tissue and cultured cells with Bruker-Nanostring CosMx. We performed post-spatial transcriptomics immunostaining and imaging with Zeiss 980 and Abberior 3D STED Microscopes for improved cell segmentation and mapped gap junction connectivity with 3D super-resolution imaging. We used custom 3D spatial-transcriptomics and volumetric confocal imaging software to quantify intercellular gene regulation and measure coordination between connected versus non-connected cells to determine genes and pathways of interest. Genetic and other manipulations were used to modify connectivity of cell populations.

Analysis of combined transcriptomics and super-resolution immunofluorescence is ongoing. We will show feasibility for new transcriptomics and protein imaging approaches and will showcase new tools for 3D transcriptomics data analysis. We will report the correlation between gap junction expression and the expression of select genes in first- versus second-order adjacent cells.

LBA14

Characterization of a murine repeat dose LPS-Induced inflammation model for early drug discovery research

Asuni A[1], Domondon L[1], Hadi M[2], Tresse-Gommeaux E[3], Andreassen T[4], Christensen R[1], Thygesen C[5], Benmamar-Badel A[1], Ratner C[1], Pedersen J[2], Noe F[1], Nalepa I[1],

[1]*Department of Preclinical Fluid Biomarkers & Occupancy, H. Lundbeck A/S, Copenhagen, Denmark*

[2]*Department of Molecular & Single Cell Pharmacology, H. Lundbeck A/S, Copenhagen, Denmark*

[3]*Department of Histology & Pathology Models, H. Lundbeck A/S, Copenhagen, Denmark*

[4]*Project Management, H. Lundbeck A/S, Copenhagen, Denmark*

[5]*Department of Antibody Discovery & Assay Technology, H. Lundbeck A/S, Copenhagen, Denmark*

Inflammation is implicated in the development of many neurodegenerative diseases. Systemic injection of lipopolysaccharide (LPS) in mice is one of the most widely used in vivo mechanistic model of neuroinflammation. However, there is discrepancy regarding protocol and model characterization throughout the literature. The divergent protocols call for a refinement of the model to ensure reproducibility of prior data. Therefore, we characterised a repeat dose LPS model in mouse for our routine discovery research on neuroinflammation.

The 3xLPS model is induced by repeated intraperitoneal injections of LPS (0.85mg/kg.) for 3 consecutive days in C57BL/6 mice which are weighed daily.

We performed a broad profiling of the induced inflammatory response in biofluids and brain using established techniques like immunohistochemistry (IHC), western blotting (WB), flow cytometry (FC), and Fluidigm qPCR.

We observed activation of the systemic immune response, accompanied by activation and proliferation of microglia, as shown by increased expression of reactivity and proliferation markers (e.g., Iba1, Ki67, CD68). This was substantiated by qPCR analysis, showing significant increase in the transcription of multiple neuroinflammatory markers, and supported by WB data.

We have validated a mechanistic neuroinflammation model, characterised by activation of the immune response accompanied by microglial proliferation and reactivity, enabling early drug research.

LBA15

Regulation of brain pH, CO_2 and O_2 homeostasis is predicted to be activity-dependent and influenced by blood physiology

Botond Antal[1], Douglas Rothman[2], Silvia Mangia[1]

[1]*Center for Magnetic Resonance Research, University of Minnesota*

[2]*Yale School of Medicine*

Maintaining brain homeostasis is essential for normal function. Achieving this balance requires not only ensuring adequate metabolic supply across varying levels of neuronal activity but also removing byproducts, such as protons and CO_2. Recent computational work suggests that key coupling mechanisms, namely neurovascular and neurometabolic coupling, may have evolved to support both the supply and clearance demands involved in sustaining this physiological stability. In this study, we build on this framework by first examining how arterial blood parameters pH, CO_2, and O_2 influence the corresponding steady-state levels in the brain. We find that these arterial properties have a strong impact on brain pH, CO_2, and O_2 even in resting-state, and that altering any one of them can affect all three within the brain. These findings highlight the critical role of systemic physiological states in maintaining brain homeostasis and suggest potential mechanistic links between systemic disorders and brain dysfunction. Secondly, by extending our analysis to states of neuronal activation, we show that the optimal tuning of neurovascular and neurometabolic couplings depend on the level of neuronal activity, suggesting that these mechanisms dynamically adapt to changing metabolic demand. Our results underscore the need for future studies to assess the relative influence of pH, CO_2, and O_2 on this tuning, as well as to explore the potential compensatory role of these dynamic responses under systemic disturbances in blood physiology.

LBA16

Small RNA chaperone promotes TDP43 functionality and motor neuron survival in an in vivo mouse model

Brigid Jensen [3,4,5], Katie Copley [1,2], Matt Davis [3,4,5], Piera Pasinelli [3,4,5], Jim Shorter [1,2]

[1]*Department of Biochemistry and Biophysics,*

[2]*Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania; Philadelphia, PA. U.S.A.*

[3]*Jefferson Weinberg ALS Center,*

[4]*Vickie and Jack Farber Institute for Neuroscience*

[5]*Department of Neuroscience, Thomas Jefferson University; Philadelphia, PA. U.S.A.*

TDP43 cytoplasmic mislocalization is a hallmark feature of ALS, found in 97% of patient post-mortem tissue. Abundant experimental evidence has demonstrated that loss of nuclear TDP43 and aggregation within the cytoplasm is a key driver of motor neuron death. As TDP43 is a DNA/RNA binding protein, essential roles in transcription, translation, RNA processing, and trafficking are all disrupted in this pathological state. A promising area of therapeutic development therefore, has focused on restoration of functional cellular TDP43 through proteins and molecules aimed at preventing or dissolving TDP43 cytoplasmic aggregates.

In this study, we have used a small RNA molecule which effectively binds wildtype TDP43 as well as various TDP43 mutations with high affinity, and which disrupts the ability TDP43 to aggregate. We have utilized our acute in vivo modeling system, where a TDP43 protein variant confined to the cytoplasm (TDP43□NLS1) was expressed across the cervical region of adult mouse spinal cord. Following 7 days of TDP43□NLS1 expression, animals received saline or small RNA treatment. We show that while untreated animals have a progressive loss of motor neurons and associated behavioral grip strength, animals treated with this small RNA maintain motor neuron number and have partial grip strength retention. Assessment of TDP43 revealed that puncta number increased in untreated animals, but this increase was significantly attenuated in treated animals. Furthermore, the puncta which did remain in treated animals were significantly smaller in size. Finally, TDP43 cellular functionality was restored specifically in treated animals, as was indicated in a splicing assay of a known mouse mRNA target which is differentially spliced through TDP43 activity. For all experiments, there were 10 animals per group for Day 0, Day 3 +/- treatment, and Day 5 +/- treatment cohorts.

Overall, these results indicate that this small RNA chaperone molecule, which showed promise in in vitro assays, is effective at restoring function and promoting motor neuron survival in an in vivo model of TDP43 pathology. Further development of this molecule and optimization of pharmacokinetics and pharmacodynamics in a longitudinal mouse model of ALS with TDP43 pathology will establish the potential for clinical translation.

LBA17

The orphan receptor GPR27 regulates the dynamics of second messengers and the mechanosensitivity of 3T3 cells and astrocytes

D. Kuhanec [1,2], E. Sanjković [1,2], M. Kreft [1,2,3], H. H. Chowdhury [1,2], R. Zorec [1,2]

[1]*Laboratory of Neuroendocrinology – Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

[2]*Laboratory of Cell Engineering, Celica Biomedical, Ljubljana, Slovenia*

[3]*Department of Biology, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia*

GPR27 is an orphan receptor and belongs to a family of super-conserved receptors expressed in the brain (SREB)1. GPR27 appears to play a role in insulin production2, lipid metabolism3 and is also involved in L-lactate homeostasis (LL). It has been shown that GPR27 stimulation enhances aerobic glycolysis (AG) and LL production in NIH-3T3 MEF cells and primary rat cortical astrocytes4. Here, we investigated whether activation of the GPR27 receptor by GPR27 surrogate agonist (1 μM) involves signalling via the second messengers Ca2+ and cAMP. We used a Förster resonance energy transfer (FRET)-based cAMP nanosensor to monitor cytosolic cAMP with high temporal resolution in single cells. Intracellular Ca2+ was monitored with Ca2+ indicator Calbryte 520AM in real time. Our preliminary results show that stimulation of GPR27 with a surrogate agonist increases [Ca2+]i but not [cAMP]i in WT 3T3 cells. In astrocytes, the GPR27-surrogate agonist also caused an increase in [Ca2+]i. Interestingly, in control experiments, we also observed an increase in [cAMP]i in both 3T3 cells and astrocytes in response to the addition of vehicle (extracellular solution). In 3T3 cells with CRISPR-Cas9 GPR27 knockout, the vehicle-induced increase in [cAMP]i was greater than WT controls. Transfection of GPR27KO 3T3 cells with a plasmid encoding GPR27 attenuated the vehicle-induced increase in [cAMP]i. Additionally, we observed that the addition of vehicle also induced an increase in [Ca2+]i in GPR27KO 3T3 cells. Previous studies of mechanosensitive signalling in mouse and rat astrocytes have suggested the possible involvement of mechanically activated channels, including PIEZO channels5 and most notably TRPV4 channels6. We observed that both TRPV4 and Piezo1 channels are more abundantly expressed in GPR27KO 3T3 cells, which could explain the observed responses after vehicle stimulation. It appears that GPR27 not only plays a role in AG and LL production but is also likely involved in the mechanosensitivity of cells.

LBA18

Iron Accumulation in the Inflamed Brain: The Role of Blood-Brain Barrier Endothelial Cells

James. R. Connor[1], Kondaiah Palsa[1], Timothy Helmuth[1], Elizabeth B. Neely[1]

[1]*Department of Neurosurgery, Penn State College of Medicine, Hershey, PA, USA, 17033*

The endothelial cells (ECs) of the blood-brain barrier (BBB) mediate the major iron transport to the brain, but it is not clear whether inflammation increases or decreases iron flux across the intact BBB. Brain inflammation leads to an increase in the amount of iron in brain tissue, however, existing studies indicate that astrocytes release a protein, hepcidin, that should limit iron release into the brain during inflammation. Herein, we investigated the role of inflammation on iron release and iron uptake transporters in vivo. Our recent in vitro study discovered a novel alternate mechanism that iron transport across the BBB is mediated via the extracellular vesicles (EVs). We induced brain inflammation in three-month-old C57BL/6 mice by intracerebroventricular injection of lipopolysaccharide (LPS). The LPS-induced inflammation did not increase the permeability of the BBB for larger molecules as evidenced by the similar levels of albumin in the LPS and control groups. The LPS injection activated microglia and astrocytes compared to the control mice. Furthermore, brain inflammation increased the iron levels in brain parenchyma and decreased iron levels in brain microvasculature (BMV). Brain inflammation degraded BMV FPN1, and increased CD63 indicating the increased iron levels in the brain occurred via EVs release from the BMV to the brain. Moreover, brain inflammation dysregulated the BMV iron homeostasis and induced iron deficiency in BMV by increasing the Transferrin receptor expression, which increased iron uptake. Lastly, we injected the mice intraperitoneally with GW4869, an inhibitor of sphingomyelinase 2 (nSMase2), a key regulatory enzyme generating ceramide that is necessary for EV formation. GW4869 was found to reduce iron accumulation in the inflamed brain parenchyma compared to control (LPS alone) mice. This is the first study to demonstrate that EVs are the source of brain iron accumulation during inflammation.

LBA19

Descending serotonergic modulation of primary sensory neurons through 5-HT3A receptors

Elizabeth S. Silagi [1,2], Jing Peng [2,3], Kenza Ashworth [1], David D. Ginty [2,3]*, Rosalind A. Segal [1,2]*

[1]*Cancer Biology Department, Dana-Farber Cancer Institute*

[2]*Department of Neurobiology, 3Howard Hughes Medical Institute, Harvard Medical School*

Nociceptive information processing in the spinal cord dorsal horn is modulated by descending pathways from the brain. Descending serotonergic (5-HT) projections from the brainstem to the dorsal horn modulate nociceptive signaling by altering activity of dorsal root ganglion (DRG) neurons and spinal cord interneurons. Among serotonin receptors implicated in pain modulation, the role of the ionotropic 5-HT3A receptor (5-HTR3A) remains unclear—specifically, whether it facilitates or inhibits nociception. Previous scRNA-seq studies revealed Htr3a enrichment in vGAT+ inhibitory interneurons of the spinal cord deep dorsal horn. We found that 5-HTR3A is also present and locally translated in the central axons of DRG neuron subtypes terminating in the dorsal horn. Studies using 5-HTR3A global knockout mice suggest a facilitatory role for 5-HTR3A in persistent pain, with selective reductions in the second phase of formalin-evoked pain behavior. However, this model does not delineate between the contributions of 5-HTR3A in DRG neurons and interneurons. Clinically, intrathecal 5-HTR3A antagonists alleviate symptoms in certain chronic pain conditions, including fibromyalgia and cancer pain, but have limited success in others, including low back pain, and their cellular site of action remains unclear.

To investigate how 5-HTR3A modulates nociceptive information processing, we generated a conditional knockout (cKO) mouse lacking Htr3a in Nav1.8+ sensory neurons to ask whether conditional loss of 5-HTR3A alters pain behaviors under normal and paclitaxel-induced persistent pain conditions (16mg/kg). Behavioral measurements—including open field, von Frey, dynamic brush, and thermal preference—revealed hypersensitivity of cKO mice to static and dynamic mechanical stimuli (n=9 mice/condition). We also assessed whether loss of 5-HTR3A affects DRG neuronal responses to 5-HT and pain-related stimuli using in vitro calcium imaging. Our data revealed that 5-HT pretreatment suppressed capsaicin-evoked calcium responses in control DRGs but not cKOs, implicating 5-HTR3A in dampening excitability of C-fiber DRG neurons (n=3 mice/condition). However, 5-HT-evoked responses persisted in some axons from cKO DRG neurons, suggesting 5-HT modulation is sensory neuron subtype-specific. These findings support a role for DRG-expressed 5-HTR3A in the descending modulation of persistent pain, potentially through non-canonical primary afferent depolarization and presynaptic inhibition. Further characterization of 5-HTR3A function may inform precision targeting of serotonergic analgesics.

LBA20

Citrate Production is Modulated by L-lactate and GPR27 agonists in 3T3 cells and astrocytes

E. Sanjković [1,2], D. Kuhanec [1,2], M. Kreft [1,2,3], H. H. Chowdhury [1,2], R. Zorec [1,2]

[1]*Laboratory of Neuroendocrinology – Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

[2]*Laboratory of Cell Engineering, Celica Biomedical, Ljubljana, Slovenia*

[3]*Department of Biology, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia*

Astrocytes are neuroglial cells with many homeostatic functions, including the regulation of brain energy metabolism. Astrocytes can convert D-glucose to L-lactate in a process known as aerobic glycolysis 1. In culture, and likely in vivo, astrocytes represent the main source of mitochondrial citrate production and its secretion into the intercellular space. Citrate, which is produced in the Krebs cycle, is involved in the regulation of glycolysis and gluconeogenesis. Although the concentration of citrate in the cerebrospinal fluid is relatively high, ranging from several tens to several hundred μmol/L, its precise role remains unclear 2,3. It has been shown that aerobic glycolysis in astrocytes can potentially be activated with certain G-protein coupled receptor (GPCR) agonists, including those activated by noradrenaline and L-lactate 4,5. GPR27 is an orphan GPCR and a member of the super-conserved receptors expressed in the brain (SREB). It has been shown that stimulation of GPR27 enhances aerobic glycolysis and L-lactate production in 3T3 murine embryonic fibroblasts (MEF) and astrocytes 5. Here, we investigate the impact of the stimulation with extracellular L-lactate on the production of citrate and further involvement of the intracellular citrate concentration ([citrate]i) in aerobic glycolysis through GPCR agonist stimulation in astrocytes and 3T3 cells. We used a genetically encoded fluorescent biosensor to monitor cytosolic citrate with high temporal resolution in single cells. Cells were stimulated with L-lactate (2 mM) and GPR27 surrogate agonists (1 μM). Our preliminary results show that extracellular L-lactate significantly increases [citrate]i in 3T3 cells and rat astrocytes. Similarly, stimulation of GPR27 with surrogate agonists also increases [citrate]i in 3T3 cells and rat astrocytes. These results indicate that L-lactate and GPR27 receptor activation modulate mitochondrial citrate production, which may affect energy metabolism in the cell itself and, under in vivo conditions, more broadly in the brain.

LBA21

Shining a Light on Dopamine Dynamics with Novel dLight 3.0 Sensors

Erin Scott [1,3], Jacob Roshgadol [1,3], Julie Chouinard [1], Rochelin Dalangin, Shouvik Majumber [1], Katharine Borges [2], Tanner Steveson [3], Kenta M. Hagihara [4], Tarun Kamath [5], Bart Lodder [5], Bryan J. MacLennan [6], Carina Soares-Cunha [7], Patrick Melugin [8], Nikki Tjahjono [1,3], Karan Mahe [9], Kiyoto Kurima [10], Cody Siciliano [8], Viviana Gradinaru [9,] Kaspar Podgorski [6], Tim Hanks [3], Bernardo Sabatini [5], Salil Bidaye [1], Na Ji [2], JeI Wicken [10], Hidehiko Inagaki [1], Lin Tian[1]

[1] *Max Planck Florida Institute for Neuroscience*

[2] *University of California Berkeley*

[3] *University of California Davis*

[4] *Allen Institute*

[5] *Harvard University*

[6] *Howard Hughes Medical Institute*

[7] *ICVS - Life and Health Sciences Research Institute*

[8] *Vanderbilt University*

[9] *California Institute of Technology*

[10] *Okinawa Institute of Science and Technology*

Neuromodulators such as dopamine are conserved across species to regulate behavior and allow for the brain to quickly adjust its functional state in response to environmental demands. The timing and magnitude of dopaminergic release in targeted brain regions are critical for shaping appropriate behavioral responses including voluntary movement, reinforcement learning, and decision making. However, current dopamine sensors lack the dynamic range and aIinity to detect real-time fluctuations of the broad spectrum of dopamine release that underlie these behaviors. To overcome this, we have developed the dLight 3.0 series - a set of fluorescence intensity and lifetime sensors that outperform existing sensors in dynamic range, aIinity, and kinetics in areas of both dense and sparse dopamine innervation. Herein we exemplify dLight 3.6 and 3.8s increased sensitivity in vitro and ex vivo recordings in superior colliculus, hippocampus, prefrontal cortex, and striatal regions. We also demonstrate the sensor's ability to simultaneously record fluorescence intensity and lifetime changes in reward collection and motor timing tasks. These experiments demonstrate the utility of the dLight 3.0 series for in vivo measurements in previously undetectable circuits and emphasize their potential for advancing our understanding of dopamine's role in diverse brain functions.

LBA22

Evaluation Of The Role Of 6-Phosphofrutokinase-2/Fructose 2,6 Biphosphatase In The Glioblastoma Progression

E. Lima, Flavia[1], Janeiro, José Marcos[1]; Magarão, Rebecca Luisa[1]; Magalhães, Eduardo[1]; Leser, Felipe[2]; Geraldo, Luiz Henrique[3]; Garcia, Celina[4]; Fonseca, Anna[5]; Dubois, Luiz Gustavo[1,2]

[1] *Institute of Biomedical Sciences, Federal University of Rio de Janeiro, UFRJ, Rio de Janeiro, Brazil;*

[2] *Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, USA;*

[3] *Neurology Department, New York University, New York, USA;*

[4] *Institute of Medical Sciences, UFRJ, Rio de Janeiro, Brazil;*

[5] *Paulo Niemeyer State Brain Institute, Rio de Janeiro, Brazil.*

Glioblastoma (GBM) is one of the most common and aggressive tumors that affects the Central Nervous System. The median survival rate of patients is approximately 15 months after the diagnosis. Some features of this deadly tumor, like metabolism, have become widely studied, mainly its cellular mechanisms and new approaches for the treatment. During the progression of GBM, the metabolism remains dysregulated, and the expression of genes and proteins associated with a high glycolytic flux are upregulated. One of these proteins is the 6-phosphofructo-2-kinase/fructose-2,6-bifosfatase 3 (PFKFB3), a crucial enzyme that participates in the third step of the glycolysis pathway, acting as an allosteric activator of phosphofructokinase 1. Besides its function in metabolism, PFKFB3 has been well characterized in the context of cancer. The increase of its expression is related to various events in the tumor cells contributing to the enhance of malignant features. However the role of PFKFB3 is not fully understand yet in GBM. In this study we evaluated the function of PFKFB3 during the progression of GBM in both in vivo and in vitro approaches. We have used PFK15, a potent and selective PFKFB3 inhibitor and verified that, when the protein is inhibited, cell viability (MTT) and proliferation (BrdU) decrease significantly after 24h and 48h of treatment in cultured GBM cells. In addition, scratch assay data indicated decreased migration in cells treated with the inhibitor for 24 hours. Cell survival analysis, utilizing TUNEL assay and Annexin V/Pi flow cytometry, suggested an increased apoptosis in PFK15-treated cells. Understanding the effects of PFKFB3 in the GBM context can be helpful as it reveals new targets for future therapies.

LBA23

Hippocampal cellular aberrations and decrease in arborization and synaptic density induced by prenatal kolanut consumption in Sprague Dawley rat offspring

Foluso A. Atiba [1,2], Pillani Nkomopezi [3], Felix E. Mbajiorgu [2] Amadi O. Ihunwo [2]

[1]*Missouri School of Dentistry and Oral Health, A. T. Still University, Kirksville, MO 63501, USA.*

[2]*School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.*

[3]*Department of Anatomy and Physiology, University of Johannesburg, South Africa.*

Kolanut is one of the snacks taken by pregnant women to prevent morning sickness symptoms. However, it is uncertain if prenatal exposure to kolanut consumption will alter the hippocampal physiology and then induces to oxidative stress, alteration in cholinergic activities and changes in morphology. The aim of this study was to evaluate the consequences of prenatal exposure to kolanut consumption on arborisation of the dendrites and morphological changes in the hippocampus of the Sprague Dawley rats.

Sprague Dawley pregnant rats were exposed to 400 mg/kg kolanut consumption from first day until parturition. Offspring were grouped as postnatal day (PND) 0, 7, 21, 56 and 70 in both control and experimental groups. Brain sections were processed for malonaldehyde (MDA), acetylcholine (Ach) and Brain-derived Neurotrophic Factor (BDNF) measurement and Golgi-cox impregnation staining for morphology assessment.

Prenatal kolanut consumption exacerbated oxidative stress, increased the level of BDNF expression and down-regulated acetylcholine release in rat hippocampus at the different PNDs. The total number of synaptic densities, soma (cell body) density and diameter, dendritic spine density, length, and branches at CA1, CA2, CA3 and DG were significantly reduced. Additionally, prenatal kolanut consumption reduced arborization of the dendrites and dendritic constriction and fragmentation, cause loss of spines, alteration in spine morphology across PNDs.

Therefore, prenatal kolanut consumption during pregnancy has anti-neuroprotective effect by inducing oxidative stress, altering cholinergic system activity, stimulating over-expression of BDNF protein, and concomitantly causing changes in morphology of the hippocampal neurons.

LBA24

Oligodendrocyte dysfunction drives human cognitive decline

Georgina A Craig [1,2,3], Emeric Merrour [4], Luise Seeker [5], Keon Arbabi [6], Jessica Thapar [1,2], Jamie Rose [3], Steven Mitchell [7], Josef Priller [8], Colin Smith [7], Tara Spires-Jones [3], Lu Sun [9], Anna Williams [3,5], Simon Cox [10], Shreejoy Tripathy [6], Brian Popko [4], Veronique E Miron [1,2,3]

[1] *Keenan Research Centre, St. Michael's Hospital, Unity Health Toronto, Canada.*

[2] *Department of Immunology The University of Toronto, Canada.*

[3] *UK Dementia Research Institute, The University of Edinburgh, UK.*

[4] *Department of Neurology, University Northwestern Feinberg School of Medicine, Chicago, USA*

[5] *Institute for Regeneration and Repair, The University of Edinburgh, UK.*

[6] *Centre for Addiction and Mental Health, Toronto, Canada.*

[7] *Centre for Clinical Brain Sciences, The University of Edinburgh, UK.*

[8] *Neuropsychiatry division, Universitatsmedizin, Berlin, Germany.*

[9] *Department of Molecular Biology, University of Texas Southwestern, Texas, USA*

[10] *Lothian Birth Cohorts, Department of Psychology, The University of Edinburgh, UK.*

Decreases in cognitive function are a hallmark of aging. However, why some individuals experience more severe cognitive decline while others remain cognitively resilient is unclear. We sought to determine neuropathological correlates of cognitive decline using rare human brain samples from the world's longest-running study on cognition, the Lothian Birth Cohort. Participants were born in 1936 in Scotland and had cognitive testing from age 11 until death, allowing unparalleled assessment of cognitive trajectories across life for >1,000 individuals.

Here, we investigated neuropathological and transcriptomic change in white matter, given its important role in cognition and vulnerability to pathology with age. We found severe cognitive decline correlated with loss of large-diameter axons, axonal atrophy, and bnormally thick myelin. Single nuclei RNA-sequencing associated cognitive decline with a surprising increase in oligodendrocytes (OLs), contrasting their roles as positive odulators of cognition in youth, a result validated by immunofluorescence. Bioinformatic pathway analyses of OLs enriched in cognitive decline pointed to reduced activity of NRF2 antioxidant signaling, supported by a reduction in OLs expressing NRF2 protein. To test the functional contribution of reduced NRF2 activity in OLs, we generated an OL-specific conditional knockout of NRF2 (PlpCreERT2Nfe2l2fl.fl), where inducing recombination at 6 months led to reduced large-diameter axons and thicker myelin in aged mice (14-20 months), similar to that observed in humans with more severe cognitive decline. Together, these data suggest pathological oligodendrocytes in aging contribute to axonal damage and cognitive decline.

LBA25

TFEB coordinates mitochondrial metabolism to promote Schwann cell-mediated nerve regeneration.

Hana Choi [1], Akash Patel [1], and Haesun Kim [1]

[1] *Rutgers University, Department of Biological Sciences, Newark, NJ, USA*

Peripheral nerve injuries affect over 20 million people worldwide and often result in incomplete recovery. Regeneration depends on Schwann cells, which adopt a repair phenotype to clear myelin debris, support axon regrowth, and remyelinate regenerated fibers. These energy-intensive processes require substantial metabolic adaptation, yet how Schwann cells reprogram their bioenergetics during repair remains poorly understood. Previous studies suggest that Schwann cells provide metabolic support to axons, relying on mitochondrial respiration and glycolytic intermediates such as lactate (Viader et al., 2011; Beirowski et al., 2014). Mitochondrial dysfunction in Schwann cells leads to axonal degeneration even with intact myelin, underscoring the importance of mitochondrial health. In other systems, the transcription factor EB (TFEB) orchestrates mitochondrial biogenesis and oxidative metabolism in response to stress (Mansueto et al., 2017; Pastore et al., 2017). Recent work from our lab (Patel et al., 2024) demonstrated that TFEB and its homolog TFE3 are critical for the injury-induced transcriptional reprogramming required for Schwann cells to transition into a repair state. Loss of TFEB/TFE3 impairs the formation and proliferation of repair Schwann cells, leading to delayed axonal regeneration and functional recovery. However, the role of TFEB in regulating mitochondrial metabolism in Schwann cells following nerve injury remains unknown. Bulk RNA sequencing of Schwann cells expressing constitutively active TFEB (TFEBS211A) in doxycycline-dependent manner revealed upregulation of genes involved in core metabolic pathways, including glycolysis, TCA cycle and oxidative phosphorylation, suggesting a TFEB-driven activation of metabolic programming. Consistently, the expression levels of mitochondrial proteins Tomm20 and Cox8A, were also elevated in Schwann cells expressing TFEBS211A, further supporting enhanced mitochondrial function. We now aim to test whether TFEB promotes mitochondrial biogenesis and oxidative phosphorylation in Schwann cells, and whether this bioenergetic reprogramming accelerates axonal regrowth in injured sciatic nerves using Schwan cell specific TFEB overexpression and TFEB/TFE3 dKO mouse models. By defining how TFEB-driven metabolic reprogramming contributes to axonal regeneration, it will provide new insight into the mechanisms of axon-glia metabolic coupling.

LBA26

Mouse models of Duchenne muscular dystrophy reveal novel roles for dystrophin isoforms in oligodendrocyte development and myelination

Holly Colognato[1], Zijian Shao[1], Andrea Arreguin[1]

[1] *Stony Brook University*

Duchenne muscular dystrophy (DMD) is caused by mutations in the gene that encodes dystrophin proteins (Dp). The largest Dp, Dp427m, has a critical role in muscle health and stability. DMD also leads to cognitive deficits and neurodevelopmental abnormalities, of which the underlying dysfunction remains unknown. Intriguingly, the dystrophin gene contains a complex set of internal promoters, leading to a unique pattern of dystrophin protein expression in the brain (Dp427c, Dp140, Dp71). The loss of Dp140 and Dp71 in particular, which only occurs in a subset of DMD causing mutations, is linked to substantially higher incidence of intellectual disability and neuropsychiatric conditions. Diffusion tensor imaging has uncovered white matter abnormalities in DMD patients, including lower fractional anisotropy, a potential indicator of impaired myelin maturation. To test the hypothesis that dystrophin loss impairs myelination, we examined oligodendroglial development and myelin in DMD mouse models with distinct dystrophin gene mutations, each with a differential loss of dystrophin proteins. In *Dmd-mdx* mice (Dp427-), we observed reduced OPC (oligodendrocyte progenitor cell) proliferation, fewer mature oligodendrocytes, thinner myelin, and delayed myelin compaction. In contrast, at postnatal day 14 in the corpus callosum of *Dmd-mdx3cv* mice (Dp427- /Dp140-/Dp71-), we found increased OPC density and myelin protein levels, despite highly abnormal myelin ultrastructure. However, later in postnatal development, *Dmd-mdx3cv* mice had less myelin compared to control littermates. To address whether these changes in myelin were due to an oligodendrocyte-intrinsic defect, pan-dystrophin siRNA designed to mimic *Dmd-mdx3cv* was used in primary oligodendrocyte cultures. Pan-dystrophin siRNA in OPCs led to increased proliferation, which following a switch to differentiation-promoting medium, accelerated oligodendrocyte differentiation and myelin gene expression. RNA-seq in oligodendrocytes treated with pandystrophin siRNA revealed significantly altered lipid metabolism. These findings demonstrate that brain dystrophin isoforms regulate developmental myelination, providing a mechanistic link to neurodevelopmental dysfunction in DMD.

LBA27

Alterations in RBM47 Expression and Nrf2 Activity in the Hippocampus Following Time-Dependent Melatonin Administration

Hye-Sun Lim, Gunhyuk Park

[1] *Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine, 111 Geonjae-ro, Naju-si, Jeollanam-do, 58245, Republic of Korea*

The regulation of physiological processes such as the sleep-wake cycle, immune function, anxiety, and circadian rhythms is intricately coordinated by various endogenous molecules. Among them, melatonin—an indoleamine produced primarily by the pineal gland—plays a significant modulatory role. Although closely linked to circadian biology, melatonin does not directly control circadian rhythms. Instead, these rhythms are principally entrained by environmental light, which influences the suprachiasmatic nucleus (SCN) and, in turn, modulates melatonin synthesis. This light-dependent mechanism ensures alignment between internal biological clocks and the external light-dark cycle. In this study, we explored the neuroprotective effects of melatonin administration at distinct times of day and night, aiming to identify optimal timing for therapeutic application. We applied these findings to rodent models of aging, dementia, and neuroinflammation to evaluate potential translational relevance. Our data revealed that RNA-binding motif protein 47 (RBM47) and the antioxidant regulator Nrf2 are involved in the signaling cascade influenced by the timing of melatonin administration. To investigate the regulatory role of RBM47 on Nrf2 activity, we employed siRNA and CRISPR-mediated knockdown in hippocampal neuronal cells, along with lentiviral vector injections in mice. In 5xFAD, aging, and neuroinflammatory models, daytime administration of melatonin produced greater antioxidant effects than nighttime treatment. Furthermore, molecular analysis indicated that RBM47 expression varied depending on the timing of melatonin exposure, with lower levels correlating with enhanced Nrf2 activation. These findings suggest that downregulation of RBM47 may enhance antioxidant defense mechanisms in the hippocampus. As a result, daytime melatonin administration could serve as a promising strategy for antioxidant-based therapeutic interventions in neurodegenerative conditions.

LBA28

GABA transport in astrocytes is implicated in the regulation of cortical EEG henotypes and behaviors in mouse model of Fragile X Syndrome.

Iryna M. Ethell [1], Victoria A. Wagner [1], Alexandra Varallo [1], Anna O. Norman [1], Maham Rais [1], Jordan Wimberly [1], Walker Woodard [1], Xinghao S. Shuai [1], Timo P. Piepponen [3], Maija Castren [3], Khaleel A. Razak [2],

[1]*Division of Biomedical Sciences, School of Medicine, University of California, Riverside, CA, USA;*

[2]*Psychology Department and Psychology Graduate Program, University of California, Riverside, CA, USA;*

[3]*Department of Physiology, University of Helsinki, Helsinki, Finland*

Fragile X syndrome (FXS) is a leading genetic cause of autism-like symptoms, including sensory hypersensitivity and cortical hyperexcitability, resulting from epigenetic silencing of the Fragile X messenger ribonucleoprotein (Fmr1) gene. Recent observations in humans and Fmr1 knockout (KO) animal models of FXS suggest abnormal GABAergic signaling. As most studies have focused on neuronal mechanisms, the role of astrocytes in mediating altered inhibition in FXS is largely unknown. Therefore, the main goal of the present study was to determine how astrocytes may contribute to abnormal GABAergic signaling and PV cell development, leading to cortical hyperexcitability and behavioral alterations in FXS. First, we found that human FXS astrocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs) show ~7-fold increase in GABA levels compared to their control counterparts using high-performance liquid chromatography (HPLC). Similar to FXS human astrocytes Fmr1 KO mouse astrocytes showed increased levels of GABA, potentially due to an up-regulation of GABA-synthesizing enzymes GAD65/67 assessed with western blotting and immunostaining. Second, we observed that astrocyte-specific Fmr1 deletion during P14-P28 period reduced inhibitory connections in the cortex. Third, astrocyte-specific Fmr1 conditional KO mice also manifested electroencephalogram (EEG) phenotypes that are remarkably similar between the mouse model of FXS and the human condition, such as abnormal resting delta-gamma and alpha-gamma power coupling and enhanced power of onset response to sound trains, which were normalized following acute inhibition of GABA transport in astrocytes with SNAP. Finally, increased locomotor activity and exploratory behaviors in open field test, but not impaired social novelty preference, were normalized in SNAP-treated KO mice. Our findings suggest astrocytes play a key role in the development and function of inhibitory circuits, and GABA transport in astrocytes is potential therapeutic target for FXS-associated phenotypes.

LBA29

Cortical layer-specific density changes of interneuron subtypes in dementia progression

Isabel Paine [1], Lisa Lowery [2], Elizabeth McDonough [2], Dan Meyer [2], Jennifer Leubke [3], Patrick Hof [1], Merina Varghese [4]

[1] *The Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai*

[2] *GE HealthCare Technology & Innovation Center*

[3] *Anatomy and Neurobiology, Boston University*

[4] *George & Anne Ryan Institute for Neuroscience, University of Rhode Island*

Neurodegeneration and the development of extracellular amyloid-beta plaques and intracellular neurofibrillary tau tangles show regional and cortical layer specificity in the progression of Alzheimer's disease (AD); however, the involvement of distinct cell types, characterized by protein expression, is insufficiently understood. We used multiplexed immunofluorescence to visualize interneuron subpopulations in human postmortem tissue. We compared the dorsolateral prefrontal cortex (DLPFC), a region vulnerable to degeneration, to the primary visual cortex (V1), a comparatively less affected region, in patients across clinical dementia rating (CDR). Patients had a CDR score of 0 (healthy), 0.5 (mild cognitive impairment), or 3 (severe dementia). Using scikit-learn and a custom annotation and training workflow plugin in FIJI, Support Vector Machine models were trained to segment cells (DAPI+) and classify all neurons (HuD+), as well as neurons expressing parvalbumin (PV+), calbindin (CB+), and/or calretinin (CR+). Cell density was calculated for each interneuron type by brain region, cortical layer, and CDR, and analyzed using one-way ANOVA with Tukey HSD. Overall neuron density showed no significant changes with CDR score in either DLPFC or V1. CB+ neuron density did not display change significantly in DLPFC. In V1, CB+ density changes with CDR were inconsistent. Notably, in layer 6 of V1, CB+ density was significantly higher in CDR 0.5 compared to CDR 0 ($p = 0.04$) and CDR 3 ($p = 0.02$). CR+ and PV+ neuron density did not decrease with CDR in either DLPFC or V1. These results suggest CB+ interneurons may be more responsive early in disease, such as in the context of oxidative stress, contributing to resistance of V1 to degeneration. Although unexpected that overall neuron density did not show significant decreases, the present study was a pilot experiment with relatively small groups using thin tissue sections. In our previous stereological studies, pyramidal neuron subpopulations positive for non-phosphorylated neurofilament protein (NPNFP) decline in the DLPFC but not V1; NPNFP+ neuron density is currently being evaluated for these cases. These findings contribute to a larger understanding of selectively vulnerable neocortical neuronal populations in AD and overall progression of the disease in identified neuron types.

LBA30

Development of next generation probes for correlative light and electron microscopy

Isabelle Cuthbert [1,2], Matthew Wilkinson [2], James Flanaghan [2], Steve Roome [2], Paul Verkade [3], Elek Molnar [1]

[1]*School of Physiology, Pharmacology and Neuroscience, University of Bristol, UK*

[2] *Hello Bio Ltd, Unit 3, IO Centre, Cabot Park, Avonmouth, Bristol, UK*

[3] *School of Biochemistry, University of Bristol, UK*

Correlative light and electron microscopy (CLEM) is a powerful tool that combines the ability to undertake live cell imaging of fluorescence microscopy followed by fixation and imaging at sub-nanometer resolution using electron microscopy. Currently, probes used for CLEM consist of antibodies conjugated with fluorophore and gold nanoparticles to enable detection of target proteins by fluorescence and electron microscopy respectively. However, the close proximity of the gold nanoparticle to the fluorophore quenches up to 95% of fluorescent signal, limiting the effectiveness of this technique.

This project has developed novel probes consisting of antibodies conjugated to both gold nanoparticles and fluorophore, but with a structural design that prevents fluorophore quenching. Through use of spectrophotometry, fluorescence and electron microscopy we have optimised probes to prevent quenching of the fluorophore and therefore enable use of these probes in CLEM.

These novel probes will enable study of protein localisation in the nanometer scale, for example linking cellular activity to the ultrastructure of the neuron. These novel probes will improve the accuracy and reliability of CLEM, enabling it to become a widespread technique in neuroscience and neurodegenerative disease research.

LBA31

Therapeutic Potential of Cb1 Receptor Inhibition in Glioblastoma with Emphasis on Mitochondrial Metabolism

Medeiros, Isabelle [1]; Diniz, Luan[1]; Romão, Luciana [1]

[1]*Neurobiology Applied to Biomedicine Laboratory, Institute of Biomedical Sciences, ICB/UFRJ, RJ, Brazil.*

Glioblastoma (GBM) is the most common primary tumor of the central nervous system, originating from glial cells. It presents notable cellular, genetic, and metabolic heterogeneity, including necrosis, high mitotic activity, and microvascular proliferation, classifying it as a high-grade glioma. The uncontrolled growth and proliferation of GBM cells can be partially explained by metabolic reprogramming involving both mitochondrial energy metabolism and intracellular calcium signaling. GBM is highly resistant to conventional oncological treatments, which have limited effects on patient survival. In this context, the search for new therapeutic strategies becomes essential, and the endocannabinoid system has emerged as a promising target. This system consists mainly of cannabinoid receptors 1 and 2 (CB1r and CB2r), their endogenous ligands anandamide and 2-arachidonoylglycerol (2-AG), and the enzymes responsible for their synthesis and degradation. Previous studies have demonstrated CB1r overexpression in GBM, suggesting its potential as a therapeutic target. Therefore, this study aimed to evaluate the antitumor effects of CB1r inhibition in GBM cell lines.

We treated GBM02 cells with different concentrations of WIN (a CB1r agonist), AM251 (a selective CB1r antagonist), and a vehicle control. To assess cell viability, we performed MTT assays, intracellular ATP quantification, and extracellular LDH release measurements. Time-lapse microscopy was used to monitor cell migration and proliferation (cytokinesis). To evaluate the mechanism of cell death induced by treatments, we performed Annexin V/Propidium Iodide staining followed by flow cytometry. Mitochondrial morphology was assessed using TOMM20 labeling, and mitochondrial membrane potential was measured with the JC-1 probe.

AM251 treatment significantly reduced the viability of GBM02 cells compared to controls, without affecting human astrocytes. It also decreased proliferation, altered migration speed, and induced morphological changes consistent with apoptosis. Furthermore, AM251 increased GBM cell sensitivity to temozolomide (TMZ). Additional findings revealed treatment and time-dependent changes in mitochondrial morphology and membrane potential.

In summary**,** our findings suggest that CB1 receptor inhibition may represent a promising therapeutic strategy for glioblastoma treatment.

LBA32

TET3-mediated regulation of hippocampal neuronal morphology

James M. Kina[1], Wilma J. Friedman[1].

[1] *Department of Biological Sciences, Rutgers University-Newark, NJ.*

The regulation of neuronal morphology during development is critical for proper hippocampal circuit formation. Several factors are involved in regulating neuronal morphology, including neurotrophins such as brain-derived neurotrophic factor (BDNF). However, the spectrum of genes that are targeted by neurotrophin signaling to regulate neuronal morphology is likely different than those involved in other processes, such as maintaining survival. Epigenetic processes alter the conformation of chromatin to change how accessible genes are to transcription factors. Ten-eleven translocation (TET) enzymes play a key role in DNA demethylation and changing the expression status of methylated genes from being silenced to actively expressed. Of the three distinct TET enzymes, TET3 is the most abundant in the brain and hippocampus. Therefore, we hypothesized that TET3 activity is important in regulating hippocampal neuronal morphology. Here, we demonstrate that TET3 knockdown in E18 hippocampal neurons leads to extensive dendritic growth and complexity. Interestingly, inhibition of Trk signaling greatly reduces the TET3 knockdown-induced growth, with a similar effect also observed by inhibiting the Akt or Erk1/2 pathways. Further, we are investigating the spectrum of genes that are influenced after knocking down TET3.

LBA33

Chemogenetic control of Schwann cell development and myelination

Jazmin G. Corral[1], Veronica T. Cheli[1], Pablo M. Paez[1].

[1]*Institute for Myelin and Glia Exploration, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, The State University of New York, University at Buffalo, Buffalo, New York*

Schwann cells (**SCs**), found in the peripheral nervous system (**PNS**), primarily function to form the myelin sheath around axons, providing insulation and facilitating rapid signal transmission, as well as supporting nerve regeneration and providing trophic support to neurons. In the CNS, the participation of G-protein coupled receptors (**GPCRs**) in oligodendrocyte Ca2+ dynamics and maturation has previously been shown. However, how Ca2+ signaling induced by GPCRs affects the development and function of myelinating glia in the PNS is unknown. Via *Cre*-mediated recombination, we expressed spcifically in SCs an excitatory GPCR based on the human muscarinic M3 receptor (**hM3Dq**). The hM3Dq **DREADD** (***D**esigner **R**eceptors **E**xclusively **A**ctivated by **D**esigner **D**rugs*) is commonly used to enhance neuronal excitability, and it is specifically activated by clozapine-N-oxide (**CNO**), an inert metabolite of the antipsychotic drug clozapine. We have found that the activation of hM3Dq by CNO in immature SCs induces the release of Ca++ from internal stores, decreases Ca++ influx mediated by ATP and acetylcholine receptors, and completely inhibits SC maturation and myelin protein synthesis in vitro. In the same line, hM3Dq activation in Sox10-positive SCs during early postnatal development significantly delayed the myelination of the sciatic nerve and reduced the density of mature SCs in vivo. In contrast, hM3Dq activation in mature myelinating SCs induced myelin loss, neurodegeneration of adult peripheral nerves, and disrupted the motor coordination of adult mice. These results indicate that SC Ca++ signaling can be effectively modulated by excitatory DREADDs such as hM3Dq and that these receptors can be used to control SC development and myelination.

LBA34

Cortical Circuit Alterations and Activity-Dependent Molecular Changes in a Mouse Model of Autism

Jeronimo Lukin [1,2,3,4,5], Rodrigo Muñoz-Castañeda [6], Marta Garcia-Forn [1,2,3,4,5], Lauren Dierdorff [1,2,3,4,5], Khaled Althobaiti [1,2,3,4,5], Wei Wang6, Zhuhao Wu [6], Silvia De Rubeis [1,2,3,4,5]

[1] *Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, USA.*

[2] *Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, USA.*

[3] *The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, USA.*

[4] *Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, USA.*

[5] *The Alper Center for Neural Development and Regeneration, Icahn School of Medicine at Mount Sinai, New York, USA.*

[6] *Appel Alzheimer's Disease Research Institute, Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York.*

Altered cortical circuits in the developing brain have been strongly linked to autism spectrum disorders (ASD) and described in ASD-relevant mouse models. Mutations in DDX3X cause DDX3X syndrome, a neurodevelopmental condition often co-morbid with ASD. Our lab generated the first mouse model with construct and face validity for DDX3X haploinsufficient mutations. Ddx3x+/- female mice have abnormal neocortical development and present abnormal behavior in open field exploration (OF). The present study aims to bridge the gap between these behavioral phenotypes and circuit abnormalities. Understanding how mutations in DDX3X regulate brain circuits might offer a new key to decipher the complexity of circuit alterations in ASD. Using Ddx3x+/- female mice, we 3D-mapped brain neural activity after a 10min Open Field (OF) exploration task, combining iDISCO technique, Fos immunostaining and light-sheet microscopy to identify affected regions influencing abnormal behaviors. Ddx3x+/- mice exhibit a distinct pattern of neuronal activation upon OF experience compared to a Ddx3x+/+ control mice. Notably, enhanced neural activation is observed in different regions of the cortex including the retrosplenial cortex (RSP), a structure involved in spatial information processing. To elucidate the role of this region and to explore the potential benefits of manipulating this circuits in the Ddx3x+/- mice, I employed chemogenetics using stereotaxic injections of excitatory and inhibitory DREADDs in this region and testing their effect on OF behavior in Ddx3x+/- and control females. In addition, I am approaching dendritic spines changes after behavior in both genotypes and I am conducting transcriptomics analysis to identify the molecular signature altered in Ddx3x+/- mice in response to experience, with the goal of identifying potential molecular targets that can help to elucidate the cellular mechanisms underlying this behavior. Our research reveals critical cortical regions involved in the aberrant OF spatial exploration in Ddx3x+/- female mice, shedding light on the complex interplay between genetics, neuronal activity and brain function. Notably, these findings. identified promising targets for malleable interventions, offering avenues for therapeutic development.

LBA35

Regulation of local translation: a novel role for sarm1 in axon degeneration

Jesus Zuniga Paiz [1,2], Maria F. Pazyra-Murphy [1,2], Rachael Davis [2], Guillaume Adelmant [2], Rosalind A. Segal [1,2]

[1] *Department of Neurobiology, Harvard medical school*

[2]*Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA.*

Axon degeneration is a common physiological event in most neurodegenerative pathologies. The conserved enzyme, Sarm1, executes axonal degeneration by catalyzing vital steps within the degenerative cascade. Canonically, activated Sarm1 functions as an NADase, altering cellular redox state and controlling calcium flux. However, the Sarm1-mediated mechanism of axon degeneration remains elusive in the dorsal root ganglion (DRG) neurons of the peripheral nervous system. To investigate the Sarm1 functional interactions ediating progressive axonal degeneration, we performed co-immunoprecipitation mass pectrometry (IP-MS) in DRGs treated with paclitaxel (600nM), a chemotherapeutic agent that induces a "dying back" axonal phenotype typical of chemotherapy-induced peripheral neuropathy (CIPN). RNA-binding proteins (RBPs) and translational machinery were enriched within the Sarm1-binding cohort in both control and paclitaxel conditions. These RBPs are components of axonal ribonucleoprotein complexes responsible for mRNA transport and translation. We further validated that Sarm1 associates with the RBP, Srrm2, using IP Western blot and co-localization in distal DRG axons using immunocytochemistry in microfluidic chambers. These results demonstrate a novel role for Sarm1 in regulating axonal integrity through local translation. However, it is still not known how protein ynthesis is locally regulated in axons after injury and if translational regulation is a key mechanistic step in the degenerative cascade. To address this, we cultured primary DRG neurons in compartmentalized Xona microfluidic devices and investigated the effects of paclitaxel (120 nM) using immunofluorescence imaging techniques. First, we confirmed that Sarm1 percent area increased in DRG axons following treatment, implying an increased distribution of Sarm1 in injured axons. Next, we treated DRGs with puromycin (2 μM) and quantified nascent peptides. We observed increased puromycin signal intensity and percent area in axons following paclitaxel treatment (n=3), with localization to branch points, growth cones, and swellings. Additionally, we assessed ribosome distribution via ribosomal RNA signal and found both increased abundance and localization in axons post-treatment, mirroring the distribution of nascent peptides and linking axonal ribosome organization to changes in translational status. Together, these findings suggest that Sarm1 may influence axonal degeneration in DRG neurons through modulation of local protein synthesis and highlight axonal translation in the degenerative cascade triggered by chemotherapeutic injury.

LBA36

Elevated expression of PMP2 promotes myelin formation

Jiayue Hong [1], Simar Arora [1], Yannick Poitelon [1], Sophie Belin [1]

[1] *Myelin Lab, Dpt. Neuroscience and Experimental Therapeutics, Albany Medical College*

Peripheral demyelinating neuropathies affect 3 million people worldwide, causing a progressive decline in neuromuscular function, numbness, muscle weakness, fatigue, and pain. With treatment options remaining palliative, peripheral demyelinating neuropathies remain a major public health problem. Inducing Schwann cell remyelination is the essential therapeutic step towards restoring the rapid conduction of action potentials along axonal fibers. Previously, we found that overexpressing peripheral myelin protein 2 (PMP2), a fatty acid (FA) chaperone can increase Schwann cell myelination *in vitro*. Now we are looking to determine if Schwann cell PMP2 overexpression can safely and effectively increase myelination *in vivo*. We hypothesize that **high and sustained expression of PMP2 can safely increase Schwann cell myelination through modulating Schwann cell FA metabolism**. To address this hypothesis, we generated a novel transgenic mouse model (PMP2OE) that overexpresses PMP2 in all Schwann cells using the Myelin Protein Zero-Cre recombinase. We show that PMP2 overexpression can increase myelin thickness *in vivo* without upregulating other major myelin proteins and promyelinating pathways. Using *ex vivo* FA uptake assay, we show that PMP2OE nerves can obtain more FAs from extracellular sources. Furthermore, we notice an enrichment of PMP2 in the endoplasmic reticulum and the nuclear regions, indicating that overexpressed PMP2 may be involved in lipid membrane synthesis and gene transcription regulations. We also demonstrate that high and sustained PMP2 expression in Schwann cells does not have detrimental effect on motor and sensory nerve functions or cause ER stress. We conclude that PMP2 overexpression can safely increase Schwann cell myelination, possibly in part by increasing FA uptake. The long-term goal of this study is to understand the molecular regulation of Schwann cell fatty acid metabolism that are beneficial to myelination in demyelinating peripheral neuropathies, and to facilitate the development of treatments targeting those pathways to improve myelination, nerve repair and function.

LBA37

Levels of circulating apolipoprotein E are altered in COVID-19 patients

Jorge Sáez-Leyva [1,2,] María-Salud García-Ayllón [1,2,3], Javier Sáez-Valero [1,2,4]

[1]*Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, San Juan de Alicante; Spain;*

[2] *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), San Juan de Alicante, Spain;*

[3] *Unidad de Investigación, Hospital General Universitario de Elche, FISABIO, Elche, Spain; 4Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain.*

Apolipoprotein E (apoE) is a 299-amino acid glycoprotein that is abundantly present in plasma, cerebrospinal fluid, and other biological fluids. ApoE is a multifunctional protein involved in lipid metabolism and cholesterol homeostasis. In addition to its lipid-related functions, unlipidated apoE can modulate key cellular signaling pathways through receptor interactions. In humans, three major allelic variants of the APOE gene exist: ε2, ε3, and ε4. The ε4 allele (APOE4) is a well-established genetic risk factor for Alzheimer's disease, and emerging evidence suggests a possible association between APOE4 and both susceptibility to and severity of COVID-19. Furthermore, apoE has been reported to interact with and potentially modulate the levels of angiotensin-converting enzyme 2 (ACE2), the primary receptor used by SARS-CoV-2 to enter host cells. Despite these associations, little is known about how apoE protein levels are altered in the context of COVID-19. In this study, we measured circulating apoE levels in patients during the acute phase of COVID-19 infection and following recovery. Given the coexistence of immature glycoforms, fragments and apoE-containing complexes alongside the mature glycoform, we employed electrophoresis and western blotting techniques to separate and quantify these distinct apoE species. Our findings reveal that patients in the acute phase of COVID-19 exhibit significantly decreased levels of mature apoE species. However, approximately two months post-recovery, apoE levels returned to baseline ranges in the same individuals. Correlations between apoE and ACE2 levels were also assessed. In addition, apoE was examined in individuals experiencing long COVID. In summary, we report a marked decrease in circulating apoE during active COVID-19 infection, which may reflect compromise in its diverse biological functions and potentially contribute to disease pathophysiology.

LBA38

HIV-1 Transgenic Oligodendrocytes Exhibit Impaired Differentiation and Altered Lipid Metabolism

Jun Shin[1], Anushka Singhal[2], Kelly L. Jordan-Sciutto [1], Judith B. Grinspan [2], Marisa A. Jeffries[2]

[1]*Department of Oral Medicine, University of Pennsylvania, Philadelphia, PA,*

[2]*Division of Neurology,Children's Hospital of Philadelphia, Philadelphia, PA*

Approximately 30-50% of people with HIV (PWH) are affected by HIV-associated neurocognitive disorders (HAND) that are strongly linked to white matter abnormalities. Unpublished data from our lab indicate myelin lipid dysregulation in an HIV-1 transgenic (Tg) rat model. Based on this and our previous studies on the effect of select antiretroviral therapy (ART) drugs on oligodendrocyte (OL) maturation, our hypothesis is that HIV and/or ART alters oligodendroglia differentiation and lipid metabolism for myelination. In this study, primary mixed glial cultures from neonatal HIV-1 Tg rats yielded fewer oligodendrocyte precursor cells (OPCs) and these OPCs exhibited reduced proliferation compared to WT cultures. These HIV-1 Tg OPCs also exhibited impaired differentiation into mature OLs compared to controls. Interestingly, Western blots revealed unchanged expression of MBP and PLP, two major myelin proteins, but a significant reduction in CNP, an enzyme abundant in the myelin sheath. Additionally, while expression of lipoprotein receptor protein 1 (LRP1) and fatty acid synthase (FASN) were unchanged, we observed a significant reduction in low-density lipoprotein receptor (LDLR), which takes up extracellular lipid for myelin production. *In vitro*, when OPC cultures from WT rats was treated with ART combination containing tenofovir disoproxil fumarate and emtricitabine, OLs exhibited significantly reduced overall LDLR fluorescent intensity as well. Our data suggest that the HIV and/or ART treatment trigger complex responses in OL maturation and myelin lipid metabolism, which may contribute to white matter abnormalities in HAND. As astrocytes are a major external source of cholesterol for OLs, ongoing studies are investigating how HIV and ART impact the critical process of lipid transport from astrocytes to OLs. Additionally, immunohistochemistry is underway to assess OL populations and myelination *in vivo*. Understanding these mechanisms could lead to targeted therapeutic strategies for improving cognitive outcomes in PWH, particularly in adolescents undergoing critical periods of brain development.

LBA39

Changes in Tau Expression Affect Neuroligin-1 in Hippocampal Neurons

Kamelija Horvatović [1,2,4], Satabdee Mohapatra[2], Lisa Diez [2], Barbara Vuic [3,4], Dubravka Švob Štrac [3,4], Susanne Wegmann [2]

[1]*Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia*

[2]*German Center for Neurodegenerative disorders - DZNE, Berlin, Germany*

[3]*Ruđer Bošković Institute, Zagreb, Croatia*

[4]*Faculty of Science, University of Zagreb, Zagreb, Croatia*

Recent evidence suggests a potential role for the post-synaptic cell adhesion molecule neuroligin-1 (NLGN1), a key player in synaptic plasticity, during the initial stages of Alzheimer's disease (AD) in the hippocampus. Despite overlapping regulatory mechanisms, including the phosphorylation by Ca2+/Calmodulin-dependent protein kinase 2 (CAMK2), the pathological influence of the microtubule-associated protein Tau on NLGN1 has not yet been investigated. The aim of this work is to explore whether there is a direct relationship between the change in Tau and NLGN1 expression.

Primary mouse hippocampal neurons (P0-2) overexpressing human Tau (AAV GFP-2N4R Tau) were harvested on DIV13 for evaluation of NLGN1, total Tau (tTau), phosphorylated Tau (pSer262, relevant for microtubule detachment and regulated by CAMK2; pSer396, often associated with Tau pathology) and CAMK2 levels by western blot and immunocytochemistry. In addition, human neuronal SH-SY5Y cells were transfected with NLGN1 and Tau to test the interaction of the two proteins by live-cell imaging. Kruskal-Wallis was used for multiple comparisons followed by Dunn's post-hoc analysis.

Human Tau overexpressing neurons showed reduced levels of NLGN1 (-63%, $p<0.001$) compared to control (GFP overexpressing) neurons. In parallel, we detected an increase in p-Tau pSer262 (+92%, $p<0.01$) but not p-Tau pSer396.

This pilot study implies that accumulation of Tau and its phosphorylation at Ser262 may be related to a disruption of NLGN1 levels in hippocampal neurons, suggesting a potential role of somatodendritic, phosphorylated Tau on postsynaptic integrity early in AD pathogenesis.

LBA40

Rapid Activation of Neurogenesis and Mitotic Gene Networks During Interbout Arousal in Hibernating Arctic Ground Squirrels

Drew KL[1], Nguyen V[1], Yoldi Bergua I[1], Frare CA[2], Rice SA[1], Fedorov VB[1], Goropashnaya AV[1]

[1]*Center for Transformative Research in Metabolism, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775*

[2] *School of Physiology, Pharmacology, & Neuroscience, University of Bristol, Bristol, BS8 1TD, United Kingdom*

Hibernation exemplifies extreme neural plasticity; whether rewarming from hibernation triggers neurogenesis is unknown. We hypothesized that interbout arousal (IBA) in hibernating arctic ground squirrels (AGS) activates neurogenic programs. We performed RNAseq and immunohistochemistry (IHC) to identify and quantify adult hippocampal neurogenesis (AHN) collected from AGS at three states; summer active, torpor and IBA. Total RNA was used for cDNA library construction and sequencing and analyzed using CLC Genomics Workbench. Genes were mapped to the AGS genome. GSEA was performed using curated gene set collections from MSigDB. Significance was set at FDR<0.05. In a separate group of animals, we used IHC to quantify AHN by the number of doublecortin-positive (DCX+) and Ki67-positive (Ki67+) neuronal precursor cells in the dentate gyrus. Cell cycle related genesets involving G2/M transition were overexpressed in torpor and IBA, versus summer. Notably, GTSE1, which is critical for G2/M transition, showed upregulation of partners MKI67 and PLK1 in string analysis. The inflammatory response geneset was also upregulated in the hibernation season (FDR<0.05). Despite similar geneset expression during torpor and IBA, IHC showed significant reduction of AHN during torpor that rebounded during IBA. The presence of immature neurons (DCX+) increased 18-fold in the IBA phase and 13-fold during the summer active phase compared to torpor ($p<0.0001$). We saw a similar trend in the number of actively dividing cells (Ki67+). RNAseq showed RBM3, a cold-shock RNA binding protein, was also upregulated in both torpor and IBA. Gene expression suggests increased capacity for mitosis during hibernation and IHC suggests a coordinated "regenerative burst" during rewarming, potentially clearing cellular debris (immune response). Moreover, results point to RBM3 as a potential driver of neurogenesis during the hibernation season with mitosis suppressed by cold tissue temperature during torpor. Findings reveal a neurogenic window during interbout arousal, with implications for understanding brain repair.

LBA41

Altering Extracelluar Matrix Catabolism To Prevent White Matter Vascular Brain Injury And Cognitive Decline In Patients With Alzheimer's Disease

Larry S. Sherman [1*,2], Fatima Banine [1], Weiping Su [1], C. D irk Keene [3], Stephen A. Back [4]

[1]*Division of Neuroscience, Oregon National Primate Research Center, Beaverton, Oregon USA.*

[2]*Department of Cell, Developmental and Cancer Biology, Oregon Health & Science University, Portland, Oregon USA.*

[3]*Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington USA.*

[4]*Departments of Pediatrics and Neurology, Oregon Health & Science University, Portland, Oregon USA*

Vascular contributions to cognitive impairment and dementia (VCID) affects millions of people world-wide, the majority of whom have co-morbid Alzheimer's disease (AD). VCID is linked to vascular brain injury (VBI) and leads to white matter injury (WMI) associated with cognitive decline. WMI is linked to arteriolosclerosis, which causes microvascular ischemia that promotes degeneration of brain vascular endothelial cells (BVECs) and brain capillary leakages. One way that BVECs degenerate is via ferroptosis, a form of programmed cell death that involves iron overload and reactive oxygen species-dependent lipid peroxidation. In a bulk RNAseq analysis of 110 VCID and/or AD cases, we found that ferroptosis-related genes were upregulated coincident with increased expression of genes encoding hyaluronan (HA) synthase-1 (HAS1), the HA receptor CD44, and the cell migration-inducing and HA-binding protein (CEMIP) that catabolizes HA. Consistent with these findings, we determined that HA and CD44 are significantly elevated in the perivascular white matter and in blood vessels in cases with AD alone, while CEMIP is significantly elevated in BVECs and HA is absent from the spaces around CEMIP-positive BVECs in AD+VBI cases. Using cultures of human BVECs, we found that high molecular weight (HMW) HA, but not CEMIP-digested HA, blocks erastin-induced ferroptosis through a mechanism involving CD44-dependent increased expression of the ferroptosis inhibitor SLC7A11. Using a novel CEMIP inhibitor, we find that blocking CEMIP activity both rescues BVECs from ferroptosis and promotes remyelination oligodendrocyte progenitor cell maturation into myelinating oligodendrocytes following WMI. These findings suggest that elevated HMW HA in white matter vessels protects BVECs from ferroptosis and that the induction of CEMIP in BVECs, likely through sustained systemic inflammatory signaling, digests HMW and disinhibits ferroptosis, leading to WMI and remyelination failure. Inhibiting CEMIP activity could, therefore, protect patients with AD from VBI.

LBA42

Validation of Proteins Involved in the Insulin Receptor Beta Subunit Patway from Astrocyte Enriched Brain Samples of Overexpressed Insulin Mice

Leopoldine Galopin, Nicholas Wright, Ting-Hsuan Lu, Ruei-Lung Lin, Sophiya Sims, Edmund Rucker, Susan Kraner, Chris Norris and Olivier Thibault

We have previously published that overexpressing a constitutively active form of the insulin receptor beta subunit (IR-β) in hippocampal neurons ameliorates spatial memory performance in the F344 rat model of aging (Frazier et al., 2020). Because astrocytes express IRs and are central to cellular energy and information transfer in the brain, here we focus on the overexpression of IR in astrocytes of the primary somatosensory cortex (S1) in the 5xFAD animal model. The 5xFAD and the IRfl/fl were used to generate unique genotypes IRfl/fl; 5xFAD-/- (WT) and IRfl/fl; 5xFAD+/- (5XFAD). Mice were injected unilaterally in S1 with the HA-Tag-hIRβ AAV to overexpress the IR-β, followed by a 4-week recovery period prior to imaging, behavioral observation, and tissue collection. Magnetic Cell Separation (MCS) was conducted on fresh plugs to create astrocyte enriched (AE) and astrocytes depleted (AD) samples. A western blot analysis was conducted on these samples to visualize and qualify the signal intensity of various proteins related to the IR pathway, including pAKT, AKT, IRS-1, and IGF-1R. The western blot findings are compared to those from two-photon imaging, immunohistochemistry (IHC) and immunofluorescence (IF), and the behavioral analysis (gait) to provide a larger picture of the effects of overexpression (OE) of the IR-β in the brain. Preliminary results indicate no significant changes in the expression of IR-β, pAKT, AKT, IRS-1, or IGF-1R in the AE samples of OR IR-β mice when compared to non IR-β mice (n = 4). In addition, there appears to be a decrease in the signal intensity of IR-β of OE IR-β mice. Further experiments are to be conducted to validate the current findings.

LBA43

Investigation of Boldine and Novel N-Phenyl Acetamide Using Network Pharmacology, Molecular Dynamics, Docking, and *In Vivo* Agonist-Antagonist Models for the Treatment of Major Depression via Modulation of 5-HT_2 and 5-HT_3 Receptors

Likhit Akotkar [1], Urmila Aswar [1], Ankit Ganeshpurkar [2]

[1]*Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune India 411038.*

[2]*Department of Pharmaceutical Chemistry, Central University of Sagar, Sagar. M.P India*

Major depressive disorder (MDD) is a multifactorial neuropsychiatric condition involving dysregulation of serotonergic neurotransmission. This study aimed to evaluate the antidepressant potential of *boldine* and a *novel N-phenyl acetamide derivative of tryptamine (TR_A)* by exploring their interaction with serotonin receptors.

Network pharmacology was conducted using Cytoscape and public databases (KEGG, BindingDB) to map the target network of boldine and TR_A, using tryptamine as the scaffold. ADME profiling, including BBB permeability, was assessed using SwissADME and the BOILED-Egg model. Molecular docking (AutoDock 4.2.6) and 100 ns molecular dynamics simulations (Amber 22) were performed to evaluate binding stability with 5-HT_2A and 5-HT_3 receptors. In vivo pharmacological validation was conducted in mice. Receptor-specific antagonists WAY100635 (5-HT1A, 0.1 mg/kg i.p), ketanserin (5-HT2A, 1 mg/kg i.p), and vortioxetine (5-HT3, 10 mg/kg i.p) boldine (80 mg/kg i.p) and TR_A (30 mg/kg i.p) were used. Behavioral models included the forced swim test (FST), tail suspension test (TST), 5-HTP-induced head twitch response, and reserpine-induced hypothermia, ptosis, and catalepsy.

Network analysis showed significant enrichment in “Neuroactive ligand-receptor interaction” (KEGG: hsa04080, p = 5.20E-49) and “G-protein coupled serotonin receptor signaling” (GO:0098664, p = 1.19E-25). Both compounds showed good BBB permeability and absorption. Docking revealed strong binding affinities for TR_A and boldine with 5-HT_2A (-7.77, -7.9 kcal/mol) and 5-HT_3 (-9.23, -8.81 kcal/mol) receptors. MD simulations confirmed stable protein-ligand complexes with RMSD below 2.5 Å, minimal RMSF in key residues, and stable radius of gyration and solvent-accessible surface area. In vivo, both compounds significantly reduced immobility time in FST and TST, decreased head twitches, and alleviated reserpine-induced symptoms. Pre-treatment with receptor-specific antagonists attenuated these effects, confirming serotonergic involvement.

LBA44

Role of miR-802 in brain insulin signaling and its impact on Down syndrome

Lucrezia Romana Rolfi [1], Eugenio Barone [1], Marzia Perluigi [1], Antonella Tramutola [1]

[1]*Department of Biochemical Science "A. Rossi Fanelli" Sapienza University of Rome.*

Down syndrome (DS) individuals are characterized by a variety of pathological phenotypes that manifest wide variability in the different tissues. At the level of the central nervous system, the accelerated aging phenotype is associated with the risk of developing Alzheimer-like dementia. A central aspect of neurodegeneration is the close association between metabolic disorders and cognitive decline. Multiple studies have suggested a link between metabolic disorder and microRNAs (miR), small non-coding RNAs acting as post-transcriptional regulators of a plethora of genes. Among triplicated miRNAs on chromosome 21, we focus on miR-802 because recent studies demonstrated its association with development on insulin resistance in obesity and diabetes. Considering these findings and based on the "gene dosage hypothesis" of DS, ***the goal of the study is to decipher how miR-802 may contribute to aberrant insulin signaling (IS) and, in parallel to the risk to develop dementia early in life in DS.*** **Methods.** The miR-802 expression, protein levels and activation state of main components of the IS were evaluated (i) in the brain of autoptic cases from DS, DSAD and age-matched controls and (ii) in the brain of euploid and Ts65Dn mice (a model of DS). Further, using bioinformatic tools we identified miR-802 a predicted target genes involved in the IS, such as *GSK-3β*, further validated by luciferase assay. **Results and Conclusions.** The IS alterations worsen in the transition from DS to DS/AD and similar findings were collected inTs65Dn mice, where IS dysregulation persists with aging and is associated with neurodegeneration. Intriguingly, these latter changes were driven by the over-expression of miR-802, which negatively regulates *GSK-3β* mRNA in the brain. In this picture, the identification of specific targets modulated by miR-802 and involved in IS pathway, will provide molecular basis to develop novel therapeutic strategies to prevent/delay the onset of brain insulin resistance in DS.

LBA45

A high-resolution in vivo drug screen identifies novel regulators of axon diameter growth in the CNS of zebrafish

Maria A Eichel-Vogel [1]; Daniel Soong [1,2]; Melody Sequeira [1]; Katy Marshall-Phelps [1];

Jenea Bin [1], David A Lyons [1]

[1]*Centre for Discovery Brain Sciences, University of Edinburgh*

[2]*UK Zebrafish Imaging and Screening Facility.*

Axon diameter varies up to 100-fold between distinct neurons in the central nervous system with larger axons exhibiting faster nerve conduction velocity. It is influencing myelination, is impaired in several injuries or diseases and its dynamic regulation might help fine-tune the timing of signal propagation and thus neural circuit function. Despite its importance, mechanisms regulating its development remain poorly understood. To address this knowledge gap, we utilized the advantages of zebrafish as model system and developed a high-throughput in vivo screening platform to identify pharmacological modulators of axon diameter growth. We focused on the Mauthner neuron, a distinct reticulospinal neuron pair with a large axon that rapidly increases in diameter during early development. We further established an automated image analysis pipeline which enabled a broad chemical-based screening approach of 880 compounds for changes in axon diameter at subcellular resolution in vivo. This screen identified 33 compounds that significantly altered Mauthner axon diameter. We already validated compounds related to beta-2 adrenoceptor and dopamine signaling that show an increase in axon diameter and partially impact locomotion. This work represents the first in vivo screen for axon diameter regulators at subcellular resolution, providing a comprehensive list of novel molecular entry points to study the mechanisms of axon diameter regulation and providing tools to manipulate it and study its role in neuronal function.

LBA46

Pathogenic LRRK2 causes age-dependent and region-specific deficits in ciliation, innervation and viability of cholinergic neurons

Marie L. Mather, Besma Brahmia, Yahaira Naaldijk, Pallabi Sarkar, Loukia Parisiadou and Sabine Hilfiker

Pathogenic activating point mutations in the LRRK2 kinase cause autosomal-dominant familial Parkinson's disease (PD). In cultured cells, mutant LRRK2 causes a deficit in *de novo* cilia formation and also impairs ciliary stability. In brain, previous studies have shown that in PD patients due to the G2019S-LRRK2 mutation as well as in middle-aged G2019S-LRRK2 knock-in mice, striatal cholinergic interneurons show a deficit in primary cilia. Here, we show that cilia loss in G2019S-LRRK2 knock-in mice is not limited to cholinergic striatal interneurons but common to cholinergic neurons across distinct brain nuclei. The lack of cilia in cholinergic forebrain neurons is accompanied by the accumulation of LRRK2-phosphorylated Rab12 GTPase and correlates with the presence of dystrophic cholinergic axons, with these deficits already evident in young adult mutant LRRK2 mice. In contrast, the age-dependent loss of cilia in brainstem cholinergic neurons correlates with an age-dependent loss of cholinergic innervation derived from this brain area. Overall, we find cholinergic cell loss in mutant LRRK2 mice that is age-dependent, cell type-specific and disease-relevant. The age-dependent loss of a subset of cholinergic neurons mimics that observed in sporadic PD patients, highlighting the possibility that these particular neurons may require functional cilia for long-term cell survival.

LBA47

Proteome Signature Of Alzheimer-Like Phenotypes In Frontal Cortices From Young And Old Individuals With Down Syndrome

M Perluigi [1], F Di Domenico [1], V Greco [2,3], A Tramutola [1], E Barone [1], Y Herault [4], E Head [5], A Urbani [2,3]

[1] *Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy.*

[2] *Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy.*

[3] *Institute of Biochemistry and Clinical Biochemistry, Università Cattolica del Sacro Cuore, Rome, Italy.*

[4] *Université de Strasbourg, CNRS, Inserm, Institut de Génetique Biologie Moléculaire et Cellulaire, IGBMC, France.*

[5] *Department of Pathology & Laboratory Medicine, University of California, Irvine, CA, USA.*

Down syndrome (DS) stands out as the most prevalent genetic contributor to intellectual disability, marked by the presence of an extra copy of chromosome 21 (Chr21). Notably, individuals with DS exhibit significant neuropathological changes for a diagnosis of Alzheimer's disease (AD) typically by the age of 50ys. To search and identify biomarkers crucial for detecting and understanding the mechanisms involved in DS neuropathology, we delved into the analysis of protein expression on post-mortem brain samples. We evaluated the frontal cortex of post-mortem brain samples from patients with DS both before and after the onset of AD pathology (DSAD), in comparison with age-matched healthy patients (HD Young and Old). Employing a comprehensive label-free shotgun proteomics approach, we sought to gain a deeper understanding of the intricate protein profiles associated with DS and its progression into DSAD. Collected results have been analyzed using different databases and analysis software to understand relevant pathways, networks, and function associated with experimental data. These observations reveal a genotype-associated alteration in protein expression profile that further progresses with aging. Notably, the affected signaling pathways encompasses energy-related processes, synaptic transmission, and stress response. Particularly, in the comparison between DS and age-matched healthy individuals (HD), we discern that genotype plays a pivotal role in driving mitochondrial dysfunction, impairing insulin signaling and oxidative phosphorylation, and inducing aberrant autophagy and Nrf2-mediated antioxidant response. Furthermore, the influence of aging within both the DS and HD groups significantly impacts the regulation of proteostasis and synaptogenesis.

LBA48

Effect of neurodevelopmental disorder associated G3BP1 mutations on stress granule assembly and function.

Meghal Desai [1], Manasi Agrawal [1], Shruti Ghumra [1], Bonnie Firestein [2], Pabitra K. Sahoo [1]

[1]*Department of Biological Sciences, Rutgers University – Newark, Newark, NJ, USA.*

[2] *Department of Cell Biology and Neuroscience, Rutgers University -New Brunswick, Piscataway, NJ*

Neurodevelopment is a meticulously organized process involving cell migration, roliferation, and differentiation, collectively regulating nervous system development. Any disruption in these processes can lead to neurodevelopmental disorders (NDDs), resulting in impairments in personal, social, academic, or occupational functioning. During neuronal development, local protein synthesis plays key roles in processes such as neurite growth, establishing dendrite-axon polarity, and the formation of functional synapses. Multiple pieces of evidence show that specific mRNAs are stored in dendrites and axons in a translationally suppressed state, only to be translated when specific stimuli trigger this process. Stress granules (SGs) are dynamic ribonuclear protein complexes formed under stressful conditions to store and protect mRNAs. Contrary to popular belief, we have shown that SG-like structures are present in the axons of both the central nervous system (CNS) and peripheral nervous system (PNS) neurons under physiological conditions, providing a mechanism to regulate localized protein synthesis. Ras GTPase-activating protein-binding protein 1 (G3BP1) is a core protein that has been shown to nucleate SG assembly. Recent studies have identified *de novo* mutations in *G3BP1* in individuals with NDDs. While this finding suggests the potential role of G3BP1 in the etiology of NDDs, the molecular mechanisms of how NDD-associated G3BP1 mutants cause neurodevelopmental defects remain largely unknown. We find that G3BP1 granules are present in the synapse potentially playing a role in synapse development and function. The soluble G3BP1 pool in synapses is post-translationally modified. The

NDD-associated mutations (R132I and S208C) in G3BP1 impair its ability to oligomerize, leading to defective SG core formation. Overall, our data show that the G3BP1 granules are present in the synapses and potentially regulate local protein synthesis, which is defective in individuals with NDD-associated *G3BP1* mutations.

LBA49

Comparative Analysis of Single Cell Transcriptomes in Preclinical Models of Multiple Sclerosis (MS) and MS Tissue

Meghan R. Bullard [1], George S. Melchor [1], Jeffrey K. Huang [1]

[1]*Biology Department, Georgetown University, Washington, DC, USA*

Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system (CNS) that affects over 2.8 million people worldwide. Although the etiology of MS is unknown, demyelinated lesions represent the fundamental pathological feature of the disease. After demyelinating events, spontaneous remyelination occurs, and this biphasic relapsing remitting cycle can last for years before remyelination eventually fails, leading to progressive MS. Identifying the factors that leading to remyelination failure is at the forefront of MS research. In MS lesions, immune cells such as microglia exhibit diverse activation states ranging from pro-inflammatory to pro-regenerative phenotypes, influencing both demyelination and repair processes. In addition, oligodendrocyte lineage cells show stage-specific transcriptional profiles that reflect their capacity for proliferation and myelination during spontaneous remyelination. Understanding the dynamic roles and molecular states of these cell types in lesions is essential for developing effective therapies to promote remyelination and protect against remyelination failure. To better understand the molecular pathways that govern the demyelinating vs. a remyelinating environment, we performed a comprehensive analysis of multiple publicly available single-nucleus RNA sequencing (snRNA-seq) datasets from preclinical models of MS, and further compared the preclinical data with a human snRNA-seq dataset from MS post-mortem brain tissue. Bioinformatic analysis revealed conserved and model-specific gene expression associated with oligodendrocyte loss and immune activation during demyelination and remyelination. Notably, we observed the upregulation of pathways associated with phagocytosis and ferroptosis, along with others, that may play an active role in the demyelinating environment in MS. The results of this comparative study will aid in the identification of novel therapeutic strategies aimed at preventing demyelination, promoting remyelination, and ultimately protecting the CNS from progressive degeneration in MS.

LBA50

Synaptic plasticity during fear memory destabilization/reconsolidation is affected by acute stress

Melisa Riva Gargiulo [1,2,3], Ramiro G. Comas Mutis [1,2], Crhistian L. Bender [1,2], Gaston D. Calfa [1,2], Lucas Pozzo Miller [3]

[1]*Institute of Experimental Pharmacology of Córdoba-CONICET. School of Chemical Sciences.*

[2] *Department of Pharmacology Otto Orsingher, National University of Córdoba, Córdoba, Argentina.*

[3] *Department of Pediatrics & Human Development, College of Human Medicine, Michigan State University., USA*

Conditioned memories enter a labile state during the presentation of the conditioning stimulus (US), followed by a new process of stabilization called re-consolidation. Because the initial consolidation is mediated by plastic changes at excitatory synapses (long-term potentiation, LTP, of AMPAR-mediated synaptic currents), their weakening by long-term depression (LTD) could underlie the re-consolidation phase. In addition, emotionally relevant experiences, such as stress prior to contextual fear conditioning produce an atypical resistance to re-consolidation, with the consequent loss of the adaptive plasticity of fear memories. In the present study, we tested if a single stressful event influences synaptic function in CA1 pyramidal neurons of the mouse dorsal hippocampus (dHIP) during the destabilization/re-consolidation phase of contextual fear memories. C57BL/6J male mice were exposed to restrained stress, 24 hours later fear conditioned and one day after reactivated in the same conditioning context. Mice were sacrificed before or 60 min after fear memory retrieval, and *ex vivo* slices of dHIP were used for either Western immunoblotting for surface and total levels of GluA2 subunits of AMPRs, or intracellular recordings of spontaneous miniature excitatory postsynaptic currents (mEPSCs). Our preliminary results suggest that stress exposure prevents the retrieval-specific endocytosis of GluA2 subunits and increases the frequency, amplitude and charge of mEPSCs in CA1 pyramidal neurons, which can result in altered CA1 pyramidal neuron activity and dHIP network function.

LBA51

Astrocytic and vascular changes associated with neocortical vulnerability in Alzheimer's disease

Merina T. Varghese [5], Alec K. McKendell [1], Sofia Magalhaes [1], Aurelien M. Badina [2], Elizabeth McDonough [3], Lisa Lowery [3], Benjamin B. Tournier [2], Dan E. Meyer [3], Jennifer I. Luebke [4], Patrick R. Hof [1],

[1] *Nash Family Department of Neuroscience, Friedman Brain Institute, and Ronald M. Loeb Center for Alzheimer's Disease, Icahn School of Medicine at Mount Sinai, New York, NY 10019, USA*

[2] *Department of Psychiatry, University Hospitals of Geneva and University of Geneva, Geneva, Switzerland*

[3] *GE HealthCare Technology & Innovation Center, Niskayuna, NY 12309, USA*

[4] *Department of Anatomy and Neurobiology, and Center for Systems Neuroscience, Boston University School of Medicine, Boston, MA 02118, USA*

[5] *George & Anne Ryan Institute for Neuroscience, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881, USA*

Background: Alzheimer's disease (AD) is the most common form of dementia. AD follows a regional pattern of pathology and neurodegeneration that affects cognition and memory. Specific neocortiocal neurons are vulnerable to AD pathology and degeneration while others remain resistant. Changes of astrocytic and vascular cells have been implicated in AD pathological changes. Novel spatial imaging methods, like highly multiplexed immunofluorescence (MxIF), together with computational analysis can identify how glial and vascular cells interact with vulnerable neurons in AD.

Methods: We performed MxIF staining for 26 markers of cell types, states, and features as well as AD neuropathology. We used postmortem human brains selecting an AD-susceptible area, the dorsolateral prefrontal cortex (PFC) and an AD-resistant area, the primary visual cortex (V1). Groups compared included AD (n = 3, clinical dementia rating CDR 3, Thal stages 3-4, Braak stages V-VI), mild cognitive impairment (MCI, n = 4, CDR 0.5, Thal 1-3, Braak I-V), and age-matched neurologically healthy females and males (n = 5, CDR 0, Thal 0-1, Braak I-II). Using the image analysis program *QuPath*, MxIF images were segmented for aldehyde dehydrogenase 1 family member L1 (all astrocytes), glial fibrillary acidic protein (reactive astrocytes), collagen IV (vasculature), and amyloid β peptide (Aβ).

Results: Reactive astrocytes were increased near amyloid-beta (Aβ) plaques in the V1 with worsening CDR ($p = 0.022$). Juxtavascular reactive astrocytes were decreased in layer 1 of the PFC ($p = 0.029$) and increased in the V1 white matter ($p = 0.036$) with worsening Thal

stage. Characterizing astrocytes specifically around Aβ-laden vessels will help determine if this is a specific response to cerebral amyloid angiopathy.

Conclusions: These preliminary results suggest that astrocytic response near vasculature and Aβ deposits is heterogenous between neocortical regions with varying vulnerability to AD. We are further investigating morphology and cell states to assess how astrocytic populations vary across the neocortex with worsening cognition and how these may contribute to selective neuronal vulnerability in AD.

LBA52

Regulation of glutamate-ammonia metabolism through astrocytic Hippo-YAP pathway

Miho Terunuma [1], Yusuke Nasu [1]

[1] *Div. of Oral Biochemistry, Fac. of Dent. & Grad. Sch. of Med. and Dent. Sci., Niigata Univ, Japan*

Astrocytes are the major glial cells in the brain that take up glutamate and ammonia and convert them into glutamine. This function is carried out by the enzyme called glutamine synthetase (GS), which is a well-known marker for astrocyte. It has been reported that GS expression is significantly reduced in the epileptic brain, which in turn causes glutamate elevation, persistent seizures, and neuronal cell death. However, the regulatory mechanism of GS expression is ill defined. Here, we aimed to identify the regulatory mechanism of GS expression in astrocytes. We found that YAP, a well-known transcriptional coactivator that promotes tissue growth, regulate GS expression. Treatment of cultured astrocytes with verteporfin, an inhibitor of YAP nuclear translocation reduced GS, whereas YAP overexpression increased GS expression. Both the Hippo- and Wnt/β-catenin- pathways were found to be involved in GS expression in astrocytes, however, when astrocytes were exposed with glutamate and ammonia, a condition which mimics epileptic patients' brain, GS was reduced due to the activation of Hipposignaling pathways, which in turn inhibited YAP nuclear translocation and promoted YAP phosphorylation for degradation. Of note, we found that XMU-MP-1, an inhibitor of Hippo pathway kinases MST1/2 that enhances YAP activation and gene transcription, was able to recover YAP nuclear translocation and upregulate GS under high glutamate/ammonia condition. In the epileptic mouse hippocampus induced by the injection of kainic acid, the GS expression in astrocytes was decreased, accompanied with elevated glial fibrillary acidic protein (GFAP), reduced YAP in the astrocyte nucleus, and reduced NeuN-positive cells in the CA1 pyramidal cell layer. In contrast, XMU-MP-1 enhanced YAP nuclear expression and recovered GS expression. In addition, altered GFAP and NeuN positive cells found in epileptic mice were not observed in XMU-MP-1 treated mice, suggesting that XMU-MP- 1 suppressed neuroinflammation and neuronal death. Finally, we confirmed that a high mortality rate of kainic acid administered mice was diminished by XMU-MP-1 treatment. In summary, our studies provide novel insight into how astrocytic GS expression is regulated, and the activation of Hippo-YAP could be the potential mechanism of pathophysiology observed in epileptic brain.

LBA53

Fine structure of the para-axon initial segment of the Purkinje cells in aged mouse

Miki Furusho [1], Mark Terasaki [1]

[1]*Dept. of Cell Biology, Univ. Connecticut Med. School, Farmington, CT, USA*

The neuronal action potential initiates at the axon initial segment (AIS) and is rapidly and efficiently propagated along the myelinated axon by the Nodes of Ranvier. Both the AIS and Node of Ranvier feature high-density ion channels. While the nodes are specialized for reliable propagation, the AIS plays a key role in the initiation of action potentials, and the neuron may have the capability to modify the properties of the AIS to fine-tune their excitability. Recently, using serial section electron microscopy, we discovered agedependent myelin remodeling in the ventral spinal cord neurons of 3-month-old mice (J. Comp. Neurol., 532(2):e25574(2024)). In the para-AIS region, we identified four types of alterations. We proposed that around the time of adulthood, a glial process can extend into the paranode region, disrupting the tight connections between the myelin loops and axon. The inner tongue, which was previously the leading edge of the oligodendrocyte lamella wrapping around the axon, becomes locally reactivated, extends beneath the glial foot process, and makes a new myelin sheath on the naked AIS. Our original study focused on the spinal cord. Now we examine the fine structure of the para-AIS in Purkinje neurons of the cerebellum and find similar age-dependent myelin remodeling in 12-month-old mice in that region. This data suggests that myelin at the para- AIS of some brain neurons may also undergo age-dependent modifications.

LBA54

Modulating ESCRT Components Alters NMDA Receptor Trafficking and Pharmacological Profile: A New Target for Antipsychotic Drug Design

Shalaby M, [1] Kantamneni S, [1] McLean SL. [1]

[1]*School of Pharmacy and Medical Sciences, University of Bradford, UK.*

Recent evidence supporting the NMDA receptor hypofunction hypothesis in schizophrenia has prompted renewed interest in targeting glutamatergic signaling to develop novel therapeutic strategies. In this study, primary rat cortical neurons were transduced with lentiviral vectors expressing either short-hairpin RNA (shRNA) targeting TSG101 or a dominant-negative (DN) construct of VPS4a to perturb the function of the endosomal sorting complex required for transport (ESCRT). This approach was used to investigate the impact of disrupted endosomal sorting on the expression, trafficking, and pharmacological properties of glutamate receptors. Receptor expression levels were quantified by Western blot, while receptor surface localization was assessed through live-cell biotinylation and immunocytochemistry.

Functional analysis was performed by applying NMDA and kainate agonists, followed by measurement of intracellular calcium dynamics using calcium fluorescence imaging with Rhod-2 AM and whole-cell patch-clamp electrophysiology. These assays revealed significant increases in calcium influx and channel currents following agonist stimulation. Notably, TSG101 knockdown resulted in a substantial enhancement of NMDA receptor-mediated calcium responses (405.64 ± 34.12 RFU versus 297.23 ± 19.8 RFU in control conditions, $P < 0.05$), suggesting an increase in receptor activity. Furthermore, the pharmacological profile of the NMDA receptor antagonist phencyclidine (PCP) was altered, with a marked increase in the IC50 from 19.6 ± 1.6 μM to 73 ± 6.1 μM ($n = 3$, $P < 0.01$), indicating a shift in receptor sensitivity following TSG101 knockdown. The surface expression of the NMDA receptor subunit NR2a was also significantly enhanced in TSG101 knockdown neurons, with a 38.1% increase in immunocytochemical staining intensity and a 12.68% increase in biotinylation signals ($P < 0.05$). In contrast, overexpression of a mutant GluK2 receptor, which is resistant to ESCRT-mediated sorting, led to reduced calcium signaling in response to high concentrations of kainate, suggesting a potential neuroprotective effect against excitotoxicity at elevated agonist levels. Together, these findings highlight TSG101 as a critical modulator of glutamate receptor trafficking and function in primary neurons, and suggest that targeting ESCRT components, such as TSG101 and VPS4a, could represent a novel therapeutic approach for restoring glutamatergic signaling in neurodegenerative and psychiatric disorders, including schizophrenia.

LBA55

Sex-specific neurochemical remodeling of Lateral Hypothalamic Perineuronal Nets following olfactory-conditioned stress: Behavioral and structural evidence

Nezha Bouhaddou[1],2, Brittany N. Kuhn[1], Eric Dereschewitz[1], Ritchy Hodebourg[1], Peter W. Kalivas[1]

[1]Department of Neuroscience, Medical University of South Carolina, Charleston, SC 29425, USA
[2]Physiology and Physiopathology Research Team, Faculty of Sciences, University Mohamed V in Rabat, Rabat, Morocco

The neurochemical mechanisms underlying stress memory consolidation remain incompletely understood, particularly regarding sex-specific adaptations in extracellular matrix plasticity. Perineuronal nets (PNNs) represent critical neurochemical regulators of synaptic stability that may mediate stress-induced behavioral phenotypes.
Objective: We investigated sex-dependent alterations in defensive behaviors and PNN density within the lateral hypothalamus (LH) following olfactory-paired restraint stress in rats.
Adult male and female Long-Evans rats (n=10 per group) underwent two 2-hour restraint sessions paired with essential oil exposure (0.5 mL, 100% pure) (conditioned), restraint without odor pairing (neutral), or odor exposure without restraint (control). Following 21-day consolidation, animals were re-exposed to the conditioned stimulus. Behavioral assessments included freezing, stress zone occupancy, defensive burying duration, and grooming frequency. Plasma corticosterone was measured and PNN density was quantified in LH subregions (rostral, medial, caudal) using Wisteria floribunda agglutinin (WFA) histochemistry and confocal microscopy.
Our preliminary results show that conditioned females demonstrated significantly elevated freezing responses compared to males (0.4% vs 0.2%, respectively). Stress zone occupancy showed treatment effects with conditioned animals displaying increased avoidance, particularly in females (17% vs 12% in males). Grooming behavior revealed robust sex differences, with females exhibiting higher frequencies across all conditions (females: 35-40%; males: 30-33%). When normalized to habituation, conditioned females showed dramatic increases in freezing (>250%) and stress zone occupancy (>1500%). Notably, PNN density analysis revealed region-specific alterations: rostral and caudal LH showed decreased PNN density in conditioned animals of both sexes, while medial LH demonstrated increased density in conditioned females but decreased density in conditioned males, indicating sexually dimorphic neurochemical responses.
These findings establish distinct sex-specific neurochemical signatures in LH PNN remodeling following stress conditioning. The bidirectional PNN changes in medial LH between sexes suggest fundamentally different neurochemical adaptation strategies. Female rats exhibited heightened behavioral responsivity coupled with region-specific PNN increases, while males showed more uniform PNN reductions. This sexually dimorphic neurochemical remodeling underscores the importance of sex-stratified approaches in developing PNN-targeted therapeutics for stress-related disorders. Our results position the LH as a critical neurochemical hub where sex hormones may interact with extracellular matrix components to shape stress memory consolidation.

LBA56

Investigating the Effect of Astrocytic Insulin Receptor Overexpression on Beta-Amyloid Load and Gait in a Model of Amyloidosis

Nicholas A. Wright* [1], Leopoldine B. Galopin* [1], Ruei-Lung Lin,[1] Sophiya Sims, [1] Ting-Hsuan Lu, [1] and Olivier Thibault [1]

[1]*University of Kentucky, Lexington, KY 40506*

Gait dysregulation is a hallmark of Alzheimer's Disease (AD) pathology, which affects the primary somatosensory cortex (S1), and can serve as a potential early indicator. An amyloid mouse model (5xFAD) was selected to investigate the correlation between the accumulation of amyloid-beta plaques and gait dysregulation in young and aged mice. Evidence from the literature suggests that insulin receptors of astrocytes may play a critical role in downstream signaling and function. To explore the pathology of amyloidosis across age, genotype, and sex, immunofluorescence (IF) was performed on mouse brain sections containing the somatosensory cortex (S1) region to visualize reactive astrocytes, plaque load, and insulin receptor function in 5xFAD mice compared to wild type (WT). Mice were singly or dually injected via craniotomy with a combination of AAV5-Gfa104-GCaMP8f and AAV5-Gfa104- Luciferase or AAV5-Gfa104-hIRbeta-HA in the S1 region 4 weeks prior to walking on a homemade, three-plane visualization gait apparatus. Matlab and ImageJ were utilized to quantify gait parameters. Coordinates acquired from each mouse were used to determine the average stride length, stride length deviance, average speed, stride time deviance, paw precision index, deviance from center, number of steps per cm, and number of steps per second. Perfused brains were then sectioned at 40 μm using a cryostat. Immunofluorescence was conducted on sections, probing for GFAP, amyloid-beta, HA-tag/IR-beta, and insulin degrading enzyme (IDE). Sections were Z-stacked using a confocal and condensed to measure plaque area. An ROI containing the section was chosen to quantify plaque area, measuring three sections per mouse. Significance was determined using 3-way and 2-way ANOVAs.

Significant genotype differences were found in almost every measure taken from the gait analysis, along with an increase in plaque load, for the 5xFAD versus the WT models. 5xFAD mice appear to have a larger deviation from WT mice, especially in females, aligning with clinical data. Our preliminary data suggest that IROE-injected 5xFAD mice appear to have no reduction in overall gait function when compared to their luciferase counterparts. The impact of IROE on inflammation and insulin signaling function is ongoing work.

LBA57

Phosphorylation status of the astrocyte gap junction protein Cx43 influences seizure susceptibility after brain injury

Owen Leitzel [1], Carmen Muñez-Balleste [1, #], Samantha Golf [1], Stefanie Robel [1,2]

[1] *Cell, Developmental and Integrative Biology Department, University of Alabama at Birmingham, Birmingham, 21353, AL*

[#] *Currently: Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, 21250, Maryland*

[2] *Department of Physical Medicine and Rehabilitation, University of Alabama at Birmingham, Birmingham, 35212, AL*

Astrocytes couple into networks of hundreds of cells via gap junctions (GJs). These GJs, composed of connexins, gate the passage of ions, messengers, and metabolites between astrocytes. We previously showed that dysregulation of Connexin-43 (Cx43), the most abundant GJ protein in astrocytes, occurred within hours after mild traumatic brain injury (mTBI). One subset of "atypical" astrocytes lacked Cx43 altogether and was uncoupled from the astrocyte network. These atypical astrocytes were correlated with the development of late, spontaneous post-traumatic seizures. However, atypical astrocytes are a small subset after mTBI. In this study, we investigated changes in Cx43 expression, distribution, and regulation after mTBI across all astrocytes and how these changes relate to seizure susceptibility. We found substantial heterogeneity in Cx43 levels among individual astrocytes and overall Cx43 protein levels were increased three days after mTBI. At the subcellular level, hemichannels and/or cytoplasmic Cx43 were increased while junctional Cx43 protein remained unchanged. Super-resolution microscopy revealed no changes in Cx43 GJ plaque size or density, further demonstrating that the increase in Cx43 protein was not due to increased Cx43 GJs. Next, we found an increase in phosphorylation at Cx43 serine 368 ($Cx43^{S368}$), a key regulatory site which, in cardiomyocytes of the heart, closes Cx43 GJs, a molecular mechanism to functionally uncouple cells. Here, I tested the hypothesis that TBI causes neuronal hyperexcitability due to $Cx43^{S368}$ phosphorylation-mediated astrocyte uncoupling. To test this hypothesis, I used a mouse model where $Cx43^{S368}$ is converted to an alanine (S368A mice), preventing phosphorylation at this site. To assess changes in seizure susceptibility, I administered subthreshold doses of pentylenetetrazol (PTZ) to S368A and control mice starting 3 days after mTBI/Sham. As expected, C67Bl/6 TBI mice had a higher seizure susceptibility than Shams. However, seizure susceptibility in S368A mice was lower even after mTBI, indicating that mTBI-induced $Cx43^{S368}$ phosphorylation increases neuronal hyperexcitability. Super-resolution microscopy determined that S368A mice had reduced Cx43 GJ plaque density but similar GJ size. These findings implicate Cx43 GJ plaque density, but not size, and $Cx43^{S368}$ phosphorylation in seizure susceptibility after brain injury.

LBA58

Iron metabolism in astrocytes: Implications for neuroinflammation and demyelination in a mouse model of multiple sclerosis

Pablo M. Paez[1], Karishma Kedari[1], Veronica T. Cheli [1]

[1] Institute for Myelin and Glia Exploration, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, The State University of New York, University at Buffalo, Buffalo, New York.

Ceruloplasmin (Cp) is a ferroxidase enzyme that is essential for cell iron efflux. The absence of this protein in humans and rodents produces progressive neurodegeneration with brain iron accumulation. Astrocytes express high levels of Cp and iron efflux from these cells has been shown to be central for oligodendrocyte maturation and myelination. Here we investigate whether astrocytic iron release mediated by Cp modulates neuroinflammation, oligodendrocyte survival, and oxidative stress following demyelination. To this aim, we conditionally knock out Cp in astrocytes and induce experimental autoimmune encephalomyelitis (EAE), an autoimmune-mediated model of demyelination. Cp ablation in astrocytes increased the severity of disease in both the acute and chronic phases. The day of onset, peak disease severity, and cumulative clinical score were all significantly increased in Cp KO animals. This corresponded to worse performance on the rotarod and decreased mobility in Cp KO mice. Furthermore, the spinal cord of Cp KO mice display increased numbers of reactive astrocytes, activated microglia, and infiltrating lymphocytes. Correspondingly, the size of demyelinated lesions, iron accumulation, and oxidative stress were exacerbated in the CNS of Cp KO subjects, particularly in white matter regions of the spinal cord. Thus, deleting Cp in astrocytes increased neuroinflammation, oxidative stress, and myelin deterioration in EAE animals. Collectively, these findings suggest that iron release from astrocytes is a potential therapeutic target to lessen CNS inflammation and myelin loss in autoimmune demyelinating diseases.

LBA59

Progranulin deficiency in microglia and its impact in neurodegeneration

Polanco P. [1,2], Van den Biggelaar D. [1,2], Meese T. [1,2], Perdok A. [1,2], Premereur J.[1,2], Lastra Osua M. [1,2], Asselbergh B.2, Mancuso R.[1,2]

[1] *Microglia and Inflammation in Neurological Disorders (MIND) Lab, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium*

[2] *Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.*

Microglia are the immune cells of the brain and they play a key role in neurodegeneration. This is the brain cell that expresses the highest levels of progranulin (GRN), hence maintaining healthy levels of GRN in microglia is crucial for neuroprotection and overall cellular health. Loss of function mutations in GRN are the second most common gene(c cause of frontotemporal lobar degeneration (FTLD), and reduced levels of GRN are associated with increased risk for other neurodegenerative disorders like Alzheimer's Disease or Parkinson's Disease. The functional consequences of GRN deficiency on human microglia biology remain vastly unknown. There are extensive differences in the transcriptomic and proteomic profiles of human and mouse microglia as well as their response to disease, hence it is essential to use human/humanized systems to understand the impact of disease-causing mutations on microglial homeostasis and their contribution to disease. We generated two homozygous and heterozygous GRN deficient induced pluripotent stem cell (iPSC) lines using CRISPRCas technology. We have differentiated into microglia the homozygous GRN KO lines and xenotransplanted them into Wt mice or APPN-L-G-F mice to explore their reaction under an inflammatory challenge by using our MIGRATE protocol. Xenotransplanted GRN-deficient microglia display a disease activated profile when transplanted into Wt mice. On the contrary, under amyloid pathology, GRN-deficient microglia display an inverted phenotype with lack of activation. We have performed a series of in vitro experiments exploring whether GRN deficiency leads to functional alterations in the endo-lysosomal pathway. GRN-deficient microglia displays an over-reactive functional profile with increased expression of inflammatory molecules, increased uptake and deficits in degrading phagocytosed material. We found that this over-reactive responses, in combination with an abnormal lysosomal fitness, results in microglial cell death. Our work shows for the first -me that GRN induces primarily a microgliopathy, and opens new avenues in the way we understand the role of microglia in neurodegeneration.

LBA60

Exocytosis of ATP in astrocytes regulates amyloid-beta pathology

Qian Huang [1], Chase J. Heim [1], Hiu Ham Lee [2], Sohyun Moon [2], Addison Li [2], Min Seong Kim [2], Matthew Jiang [2], Shan Jin [2], Jerry Y. Zhao [2], and Weikang Cai [1]

[1] *Department of Molecular and Cellular Biochemistry, University of Kentucky College of Medicine, Lexington, KY, U.S.A.*

[2] *Department of Biomedical Sciences, New York of Technology College of Osteopathic Medicine Old Westbury, NY, U.S.A*

Alzheimer's disease (AD) is characterized by β-amyloid (Aβ)-containing senile plaques and tau-containing neurofibrillary tangles in the brain. Excessive deposition of toxic Aβ peptides is believed one of the important mechanisms contributing to AD. Emerging evidence demonstrate that astrocyte dysfunction may be involved in the progression of AD. Here, we show that release of purinergic molecules from astrocytes plays an important role in the progression of amyloid pathology. Aβ42 induced adenosine triphosphate (ATP) release in primary astrocytes. An ATP analogy, 2-Me-SATP, triggered a largely overlapping transcriptional response in astrocytes comparable to those treated with Aβ42, exemplified by the induction of inflammation, suppression of extracellular matrix production, and regulation on phagocytosis. These strongly suggest that in response to Aβ42, astrocytes may release ATP to trigger functional alterations. To further test this hypothesis, we used a unique genetic tool to specifically target ATP exocytosis in astrocytes. Slc17a9 gene encodes the vesicular nucleotide transporter (Vnut), which is essential for loading cytosolic ATP into the secretory vesicles. With the transgenic mouse model carrying floxed cassette flanking the exon 1 of Vnut gene, we were able to specifically eliminate exocytosis of ATP from astrocytes. In primary astrocytes, Vnut deletion reduced vesicular ATP release by ~50%. Notably, loss of Vnut significantly increased the uptake of HiLyte647-conjugated Aβ42 by primary astrocytes. This is strongly supported by the transcriptomic signature of VnutKO astrocytes, showing increased expression of genes involved in phagocytosis. In agreement with the loss-of-function model, overexpression of Vnut inhibited Aβ42 uptake by astrocytes. To further examine the role of astrocytic Vnut in AD pathology in vivo, we crossed the VnutAldh1l1KO mice with the 5xFAD mice. Loss of Vnut in astrocytes of the female 5xFAD mice dramatically reduced Aβ plaques by ~50% at 6 months of age. The inductions of Gfap expression and key proinflammatory cytokines were blunted in VnutAldh1l1KO/5xFAD mice. More importantly, loss of astrocytic Vnut greatly improved cognitive deficit in the female mice with 5xFAD background. Together, our results suggest that Vnut-mediated vesicular release of ATP plays an important role for regulating astrogliosis, neuroinflammation, and Aβ pathology.

LBA61

Trafficking of AMPA receptor subunits in Par1c/MARK1 knockout mice

Rebecca Shear[1], Emily C. Kelly-Castro[1], Miao Sun[1], and Huaye Zhang[1]

[1] *Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, 675 Hoes Lane West, Piscataway, NJ 08854*

Dendritic spines are sites of postsynaptic excitatory input that are critical for learning and memory. Abnormalities in dendritic spine dynamics and morphology have been linked to neurodevelopmental disorders such as autism spectrum disorder (ASD). Our laboratory has recently generated a forebrain-specific conditional knockout (cKO) of partitioning defective 1 c (Par1c), also known as microtubule affinity regulating kinase 1 (MARK1), which is a serine/threonine kinase linked to ASD and bipolar disorder. Importantly, genetic evidence supports that Par1c functions in higher level cognition. Furthermore, MARK1 is highly expressed in forebrain pyramidal neurons and exhibits human-specific accelerated evolution, suggesting its importance in the development of cognition. We found that Par1c cKO mice show dendritic spine and behavioral abnormalities in vivo. To examine how loss of Par1c affects the postsynaptic receptors and scaffolding proteins, we performed Western blot analysis of synaptosomes. Interestingly, we found a significant increase in synaptic levels of the AMPA receptor subunit GluR2. To probe for the underlying mechanisms, we performed phosphoproteomic analysis of WT and Par1c cKO hippocampi. We discovered a significant decrease in the phosphorylation of S843 of RalGAPα1 in Par1c cKO hippocampi, which is a site that matches the Par1 phosphorylation consensus sequence. Preliminary in vitro kinase assay data support that RalGAPα1 is a direct Par1c substrate. RalGAPα1 is appreciated for its role in neurodevelopmental disorders and is known to regulate membrane trafficking. Interestingly, preliminary data suggest expression of a nonphosphorylatable (S843A) RalGAPα1 significantly increases surface GluA2 in primary hippocampal neurons and N2a cells. Thus, our current experiments are aimed at better establishing RalGAPα1 as a Par1c substrate, and determining whether RalGAPα1 regulates GluA2 trafficking downstream of Par1.

LBA62

Effects of Vitamin B12 on synthesis of D-serine and myelin proteins in NSC-34 motor neuron-like cells

Ritsuko Fuji[1, 2], Natsumi Kawamura[1], Natsuki Omoto[1] , Miho Fukuyama-Hadano [1]

[1] *Department of Nutrition, Faculty of Human Sciences, Hiroshima Bunkyo University, Japan*

[2] *RIRBM Hiroshima University, Japan*

Motor neuron-like cell line NSC-34 has widely been used to investigate molecular events linked to neurodegeneration of differentiated neurons. NSC-34 cells can be differentiated by serum deprivation and additional treatment with all-trans retinoic acid (RA). And the differentiation of NSC-34 cells is characterized by neurite outgrowth and increased expression of neuronal and cholinergic markers such as SMI-32, MAPT, glutamate receptor subunits, choline acetyltransferase. It has been well documented that vitamin B12(cobalamin)-deficiency leads to defective DNA synthesis and megaloblastic anemia which is known to cause myelopathy and neuropathy associated with loss of myelin in the spinal cord and the peripheral nerves including motor neurons. To gain insights of vitamin B12-dependent prevention of myelopathy, differentiated NSC-34 cells were treated with vitamin B12(VB12) and vitamin B1(VB1) as control. First, we investigated the morphological changes and viability of the differentiated cells treated with VB12. Within 6-7days after RA-treatment started, neurite outgrowth of the NSC-34 cells reached at their peak, however the differentiated cells cultured without VB12-treatment started to degenerate. The differentiated cells cultured in the presence of VB12 maintained the neurites and their branches on laminin-coated dishes over 20 days, and axon-like long protrusions were dominantly observed VB12-treated cells while the control VB1-treated cells showed short protrusions on the cell body. Immunocytochemistry and western blot analyses showed that mature peripheral axonal marker protein NF-H expression level was significantly increased in VB12-treated differentiated NSC-34 cells, however dendrites/immature neuronal marker MAP2 expression level was not changed. We also discovered that VB12-treatment lowered the level of D-serine production in NSC-34 cells after the cell hyper-depolarization as results of balancing activities and expression of serine racemase and DAO (D-amino acid oxidase). These observations suggest that vitamin B12 is required for maintenance of the axon-like neurites of the NSC-34 cells through the regulation of myelin protein synthesis and also D-serine production.

LBA63

Astrovascular coupling in awake 5xFAD mice: relationship to ambulation status, calcium, sex, and aging

Ruei-Lung Lin[1], Sophiya L. Sims[1], Nicholas A. Wright[1], Leopoldine B. Galopin[1], Ting-Hsuan Lu[1], and Olivier Thibault[1]

[1] *Department of Pharmacology and Nutritional Sciences, University of Kentucky, 780 Rose Street, Lexington, KY 40536*

Evidence points to dysregulated calcium homeostasis in neurons and astrocytes in models of amyloidosis. While most of these data have been obtained in vitro or in vivo during anesthetized conditions, less work has investigated these variables in awake ambulating mice with a focus on astrovascular coupling.
Here we studied astrovascular coupling in the 5xFAD animal model of amyloidosis (IRfl/fl / 5xFAD+/-) and their control (IRfl/fl / 5xFAD-/-) across age (3-4 months & 9-10 months old) and sex. The somatosensory cortex (S1) astrocytic calcium response (AAV5-Gfa104-GCaMP8f), vasoreactivity (rhodamine dextran) and animal velocity were obtained simultaneously in freely ambulating mice using two-photon microscopy and the Neurotar Mobile Home Cage. Single-cell resolution calcium fluctuations from each astrocyte were further extracted using continuous wavelet transform (CWT) to calculate pairwise correlations from the power of the extraction across frequency domains from 0.02-14 Hz. Cross correlations comparisons and single astrocyte calcium intensity as well as vasoreactivity measured were analyzed with respect to the ambulatory status of the animal. While the calcium signatures (rise time, decay time, amplitude and area-under-the-curve) were not altered in 5xFAD group compared to the control group, we found that the CWT-derived network functional properties such as functional connectivity, pairwise correlations, and network synchronicity were significantly reduced in the older 5xFAD mice with greater decreases in older females. Measures of vasoreactivity, including proportions of vessels in the field of view that were either dilating, constricting, responsive bidirectionally, or inactive did not show significant sex, age or genotype differences. However, strong main effects of genotype and sex were noted in astrocyte-related activity measures (i.e., calcium), including the reductions of astrocytic-vasoreactivity and astrocytic-velocity correlations in older 5xFAD female.
The results provided here align robustly with the notion that age and sex are major risk factors for AD but appear to exist independently from changes in astrocytic calcium kinetics.

LBA64

The human vermiform appendix is a reservoir for aggregation-prone alpha-synuclein proteoforms

Zameer S[1], Li P[1], Gordevicius J [3] Schilthuis M[3], Pinho M[3], Pritzkow S[3], Soto C, Brundin P [4], Brundin L[1], Killinger BA[1, 5] Anis E[1]

[1] *Center for Neurodegenerative Science, Van Andel Research Institute, Grand Rapids, MI, USA*
[2] *Vugene LLC, Grand Rapids, MI, USA*
[3] *Mitchell Center for Alzheimer's Disease and Related Brain Disorders, University of Texas McGovern Medical School, Houston, Texas, USA.*
[4] *Roche Pharma Research and Early Development (pRED), NRD, Roche Innovation Center Basel, Basel, Switzerland.*
[5] *Department of Neurological Sciences. Rush Medical College. Chicago, IL, USA*

Parkinson's disease (PD) is a neurodegenerative disease characterized by dopaminergic neuron loss in the substantia nigra (SN) and pathological accumulation of alpha-synuclein. While PD is considered a brain disorder, increasing evidence suggests that PD may originate in the gut, with alpha-synuclein potentially propagating to the brain via the vagus nerve. Evidence suggests the vermiform appendix is particularly susceptible to alpha-synuclein aggregation and appendectomy may impact the risk and onset of PD. However, the molecular basis of alpha-synuclein aggregation in the appendix remains poorly understood. To investigate this, we performed total RNA-sequencing and differential bisulfite-hybridization based methylation analysis in postmortem appendix tissue from healthy controls and subjects with PD. We also assessed the presence of pathological alpha-synuclein in the appendices using the alpha-synuclein seed amplification assay (alpha-synuclein-SAA). Additionally, top-down mass spectrometry (TD-MS) to catalog alpha-synuclein proteoforms from postmortem SN and appendix tissues. Our RNA-sequencing and methylation analyses revealed significant dysregulation in PD appendix samples, notably in genes linked to protein folding and degradation, inflammation, and ciliary function. The alpha-synuclein-SAA was positive for 68.8% of PD appendices and 6.6% of controls. TD-MS identified 65 distinct alpha-synuclein proteoforms in the SN and appendix, including 9 that were unique to the appendix. These findings suggest that the vermiform appendix harbors a distinct pool of alpha-synuclein proteoforms, with increased aggregation potential in PD appendix, alongside widespread transcriptomic and epigenetic changes. While these findings reveal unique molecular features of the appendix in PD, further investigation is required to determine their causal role in disease pathogenesis. In conclusion, the appendix may serve as a valuable site for studying early alphasynuclein aggregation and the molecular underpinning of synucleinopathies.

LBA65

Storage and translational regulation of axonal App mRNA by 3BP1granules

Shruti Ghumra[1], Manasi Agrawal[1], Meghal Desai[1], Wilma Friedman[1], Pabitra K. Sahoo[1]

[1] *Rutgers University – Newark, Newark, NJ, USA.*

Traumatic brain injury (TBI) to the cortex has been shown to induce an increase in the levels of pro-nerve growth factor and pro-brain derived neurotrophic factor (proNGF and proBDNF) in the injured cortex, leading to local p75 neurotrophin receptor (p75NTR)-mediated loss of neurons in the penumbra of the injury. Basal forebrain cholinergic neurons (BFCNs) express p75NTR throughout life and extend long projections to multiple targets, including the cortex, and injury to the target region of these neurons affects their viability *in vivo*. Our recent study showed that cortical TBI not only promotes the loss of cortical neurons in the penumbra of the injury but also promotes retrograde axonal degeneration of BFCNs via the proNGF→p75NTR signaling. While our data show that local axonal protein synthesis, especially local translation of *App* mRNA, is necessary for proNGF-induced axon degeneration and cell death, our understanding of how and where the App mRNA is stored in the BFCN axons is far from clear. Stress granules (SGs) are molecular condensates of ribonucleoproteins (RNPs) formed to temporarily store and protect mRNAs during cellular stress. Previously, we have shown that in axons of both the peripheral nervous system (PNS) and central nervous system (CNS) neurons, the core SG protein Ras GTPase-activating protein-binding protein 1 (G3BP1) forms SG-like structures under physiological conditions to store and regulate translation of specific mRNAs. Here we show that the G3BP1 granules are also in BFCN axon, and interact with *App* mRNA. Our super-resolution studies show colocalization between G3BP1 granules and p75NTR. Overall, our findings show that the G3BP1 granules potentially store and regulate the translation of *App* mRNA in the axons of BFCNs.

LBA66

2-photon imaging of astrocyte metabolic activity in an awake mouse using the PercevalHR and Peredox nanosensors

Sophiya Sims[1], Ruei-Lung Lin[1], Ting-Husan Lu[1], Leopoldine Galopin[1], Nicholas Wright[1], and Olivier Thibault[1]

[1] *Department of Pharmacology & Nutritional Sciences*

By characterizing brain energy dynamics, metabolic processes integral to maintaining and regulating homeostatic equilibrium can be addressed in healthy and diseased states. Indeed, the disruption of key energetic pathways and vascular function are hallmarks of cognitive decline (i.e. Alzheimer's disease and related dementias). With the advent of commercially available nanosensors used to evaluate metabolic processes with high spatial and temporal resolution, and the accessibility of two-photon microscopy, energy dynamics can be investigated in live, awake animals. However, there is still currently a paucity of research dedicated to investigating neurometabolic processes *in vivo*. We used PerecevalHR (ATP:ADP) and Peredox (NADH:NAD+) to assess metabolic status in astrocytes in control and 5xFAD mice during rest and movement using 2-photon imaging. Concomitantly, a fluorescent dextran was used to visualize the vasculature to measure changes in vessel tone. Craniotomies were performed on all animals included in the study. A circular window was installed above the center of the S1 region (AP: -0.5 mm, ML: ± 1.62 mm). Animals were either injected with PercevalHR (1 uL or 2 uL; GFAP promoter, AAV2) or Peredox (1 uL; Gfa104 promoter, AAV2). Following the injection, a head plate was mounted. 3 weeks following surgery, animals were acclimated to the 2-photon environment. On the day of imaging, animals were anesthetized briefly, received an intra-orbital injection of rhodamine dextran, and head-fixed under the objective lens. Awake animals were then imaged across excitation wavelengths (790 nm – 975 nm). During imaging, measures of sensor fluorescence intensity and animal velocity were captured. All data were assessed across rest and ambulation. Correlation coefficients between astrocytic fluorescence intensity and velocity were calculated to evaluate signal associations with movement.
Unexpectedly, across 20 mice treated and imaged with PercevalHR and 12 mice treated imaged with Peredox, no significant correlations between ambulation and fluorescence intensity signal were detected in either 5xFAD or control mice. This suggests that with current methods and resolution ATP or NADH levels are stable in astrocytes during ambulation, perhaps highlighting a solid metabolic phenotype in these animals.

LBA67

2-photon Osteopontin-mediated modulation of dopaminergic circuits in NeuroHIV: Insights from humanized mouse models

Srivaishnavi Loganathan[1], Menghan Sun[1], Catalina Argandona Lopez[1], Jasmine Wangui[1], Amanda M. Brown[1]

[1] *Department of Neurology – Neuroimmunology and Neurological infection, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*

NeuroHIV, a spectrum of neurological complications affecting people living and aging with HIV (PWH), is characterized by attention deficits, cognitive decline, motor impairments and other neuropsychiatric comorbidities that persist despite antiretroviral therapy (ART). Alterations in dopaminergic circuitry are observed in a subset of PWH, and mirrored in rodent models, often accompanied by motivational and apathetic behavioral alterations. Osteopontin (OPN), encoded by SPP1 (secreted phosphoprotein 1) is a multifunctional protein with cytokine-like activity that is highly expressed in the central nervous system (CNS) and elevated in neurodegenerative diseases including Alzheimer's, Parkinson's, multiple sclerosis and NeuroHIV. Previous studies from our laboratory suggest neuroprotective roles for OPN including the regulation of neurite outgrowth and neuroinflammation. Interestingly, by immunohistochemistry we found activated microglia in close association with a subset of translocator protein (TSPO) positive dopaminergic neurons in the substantia nigra. Whether OPN modulates dopaminergic circuits in health or disease remains undefined. We hypothesized that systemic inflammation following HIV infection disrupts dopaminergic signaling, and that local OPN expression is required for dopaminergic circuit homeostasis. Using HIV-infected humanized NOD/Scid IL-2y (hu-NSG) mice, a model that replicates key aspects of infection, we assessed the impact of HIV and systemic OPN knockdown on locomotion and anxiety-like behavior, motivation, cognition and the expression of tyrosine hydroxylase (TH), TSPO, and OPN in the midbrain. Behavioral assays revealed that HIV infection impaired cognitive function compared to uninfected mice and that the phenotype could be rescued by OPN knockdown. Interestingly, motivational deficits emerged only in HIV-infected mice lacking OPN, while locomotor and anxiety-related behaviors remained unaffected across groups. Immunofluorescence analysis revealed robust OPN expression in a subset of cells near the ventral tegmental area (VTA), with elevated levels in HIV-infected mice and reduced expression following OPN knockdown. While TH+ cell numbers remained unchanged, OPN knockdown led to decreased TH expression regardless of HIV status. These findings highlight a complex, context-dependent role for OPN in modulating dopaminergic circuits and behavior during HIV infection. Ongoing studies aim to identify OPN-expressing midbrain cell types and their involvement in circuit-specific regulation. These studies are expected to provide deeper insights into OPN's role in systemic HIV infection and their impact on CNS function.

LBA68

Mechanisms associated with impairments in trafficking and activity of glucose transporter 1 in astrocytes: Implications for Alzheimer's disease

Steven W. Barger[1,2], Nazneen Begum[1]

[1] *Department of Geriatrics*

[2]*Department of Neuroscience, University of Arkansas for Medical Sciences; Little Rock AR 72205, USA*

Dementia is strongly correlated with reductions in the cerebral metabolic rate of glucose (CMRglc); in Alzheimer's disease this appears to result from aberrant trafficking of glucose transporter 1 (GLUT1) in astrocytes. This deficit is modulated by genotype of the apolipoprotein E gene (APOE), as the ε4 allele is associated with greater impairments in CMRglc. Attenuated trafficking of GLUT1 to the plasma membrane (PM) can also be reproduced in cultured astrocytes by the application of amyloid β-peptide (Aβ) or proinflammatory cytokines. Glucose-regulated protein 78 (GRP78) facilitates GLUT1 PM trafficking in a manner dependent upon extracellular glucose levels. Here, we investigated relevant mechanisms. Cultures were established from wild-type rats and from mice bearing targeted replacement of the murine Apoe gene with sequences expressing human ApoE3 or ApoE4; the latter produced lower rates of GLUT1 PM trafficking and glucose uptake. A relatively short treatment (4 h) with a combination of interleukin 1β (IL1β) and tumor necrosis factor (TNF) diminished GLUT1 PM trafficking and glucose uptake in all cells. We previously showed that ApoE4 specifically binds a DNA sequence that is otherwise bound by members of the MiT/TFE family of transcription factors for the induction of autophagy-related genes. To test the role of this mechanism in regulation of GLUT1, we used an AAV viral vector to overexpress TFE3 in cultured astrocytes established from mice expressing human ApoE3 or ApoE4 in place of the murine ApoE. TFE3 overexpression elevated the PM fraction of GLUT1 in ApoE4-expressing cells. However, this phenomenon may not be specific to ApoE4, as TFE3 prevented the drop in glucose uptake triggered by IL1β/TNF in cells of both genotypes. HA-15, an GRP78 inhibitor, diminished glucose uptake, producing a state recalcitrant to IL1β/TNF. These findings suggest that the effects of ApoE genotype on glucose transport in astrocytes are distinct from those of proinflammatory cytokines, which are modulated by TFE-dependent gene regulation.

LBA69

Interactions of Quinone Derivates with Human Organic Cation Transporters 1-3 and Plasma Membrane Monoamine Transporter: Implications for Antimalaria Drug Pharmacokinetics

Thomas J. F. Angenoorth[1], Ena Baralic[1], Stevan Stankovic[1], Marion Holy[1], Jae-Won Yang[1], Harald H. Sitte[1] and Julian Maier[1]

[1] *Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Währingerstraße 13A, 1090, Vienna, Austria*

Interactions between quinone derivatives and human OCT1-3 (SLC22A1-3) and PMAT (SLC29A4) may influence antimalarial drug pharmacokinetics, as many prescribed drugs interact with these transporters. This study examined the inhibitory and substrate properties of 17 quinone derivatives on hOCT1-3 and PMAT using radiotracer-based uptake inhibition assays and HPLC/LC-MS-based uptake assays in HEK293 cells stably expressing these transporters.

All quinones moderately to strongly inhibited hOCT1, with IC50 values from 2.14 to 37.17 µM. hOCT2 was similarly inhibited, except by dihydroartemisinin. Most compounds also inhibited MPP^+ uptake via hOCT3. In contrast, PMAT showed moderate to no inhibition. Regarding substrate properties, most quinones were not transported by hOCT1-3 or PMAT. However, some uptake was observed: primaquine was taken up by all four transporters; cinchonidin by hOCT2, hOCT3, and PMAT; and mepacrine by hOCT2.

These findings suggest that quinone-transporter interactions may affect drug disposition and therapeutic efficacy in malaria treatment and could play a role in drug-drug interactions or side effects. Further research is needed to confirm the involvement of these transporters in antimalarial drug action. Overall, this study provides valuable insight into transporterquinone interactions and supports the development of selective inhibitors for hOCT1-3 and PMAT, contributing to more targeted drug design and improved therapeutic strategies.

The work underlying the current abstract is supported by FWF/Austrian Science Fund, grant DOI: 10.55776/PAT6080523

LBA70

Antiseizure Drugs For Neuroprotective Prophylaxis Against Traumatic Brain Injury

Tiffany M. Rodriguez[1,2]; Anika Sanjana[4,5,6]; Katherine Bayona[1]; Rawnaa Elshita[1]; Pernashee Dave[1]; Gillian Pavia[3]; Laura Kot[1]; Ewa Ozga Stasiewicz[1]; Mariola Sobolewska[3]; Eden Vaknine[1]; Michael Morris[3]; Douglas S.F Ling[4,5,6], Peter A. Serrano[1,2,3]

[1] Department of Psychology, Hunter College, City University of New York, New York, NY, 10065, USA.
[2] Ph.D. Program in Biochemistry, The Graduate Center of CUNY, New York, NY, 10016, USA.
[3] Master's Program in Cognitive Neuroscience, The Graduate Center of CUNY, New York, NY, 10016, USA.
[4] Department of Physiology and Pharmacology, SUNY Downstate Health Sciences University, 450 Clarkson Ave, Brooklyn, NY 11203, USA.
[5] Program in Neural and Behavioral Science, SUNY Downstate Health Sciences University, 450 Clarkson Ave, Brooklyn, NY 11203, USA.
[6] Robert F. Furchgott Center for Behavioral and Neural Science, SUNY Downstate Health Sciences University, 450 Clarkson Ave, Brooklyn, NY 11203, USA.

Traumatic brain injury (TBI) promotes excessive glutamate release, leading to the overactivation of glutamatergic receptors and disruption of intracellular calcium (Ca^{2+}) homeostasis. Dysregulated Ca^{2+} is a key mediator of neuronal dysfunction and death in the hippocampus and prefrontal cortex, contributing to the development of post- traumatic epilepsy (PTE)—a mechanism associated with exacerbated short-term working memory deficits and anxiety-like behaviors. To date, no effective therapy has been established to prevent PTE, which is often irreversible and resistant to current anti-seizure medications (ASMs), potentially due to missed critical therapeutic windows. Early post-TBI administration of synaptic vesicle 2A (SV2A) ligand-ASMs, levetiracetam (LEV) and brivaracetam (BRV), reduces epileptiform activity 30–60 min post-TBI via glutamate inhibition. We investigated whether prophylactic administration of LEV (150 mg/kg) or BRV (2.1 mg/kg) 15 minutes prior to TBI prevents PTE onset and improves spatial working memory and anxiety-like responses 1-month post-injury using a controlled cortical impact (CCI) model in male Sprague Dawley (SD) rats. We hypothesize that prophylactic LEV or BRV will elicit neuroprotective effects, preventing PTE development and reducing spatial working memory deficits and anxiety-like behavior post-1 month. Male SD rats received a CCI injury (6.0 mm diameter; 2.0 mm depth) at postnatal day 39–65. Animals were randomly assigned to TBI + LEV, TBI + BRV, TBI + Vehicle (0.9% saline), or No TBI + Vehicle groups. The radial 8-arm maze (RAM) assessed spatial working memory errors, and the elevated plus maze (EPM) evaluated anxiety-like behavior 1-month post-TBI. LEV pretreatment significantly decreased working memory errors compared to TBI + Vehicle, suggesting a protective effect. In EPM, TBI + Vehicle animals spent significantly more time in closed arms compared to No TBI controls, indicating heightened anxiety. Conversely, TBI + LEV and TBI + BRV rats spent more time in open arms and less in closed arms than TBI + Vehicle animals, suggesting reduced anxiety-like behavior and increased exploratory activity. Notably, BRV produced a stronger effect, likely due to its ~20-fold higher binding affinity for SV2A. Ongoing studies will assess the dose-response relationship of BRV to determine the optimal neuroprotective dose and evaluate efficacy at one-week post-injury.

LBA71

Cell-Penetrating Peptides Improve Efficacy of Exendin-4 Treatment Following Photothrombotic Stroke Model

Todd C. Peterson[1], Paul T. Marron[1], Lev P. Oykhman[1], Matthew Wells[1], Noemi P. Meza[1], Quinn L. Morgan[1],
Bella Readling[1], Jake D. Ludington[1], Milena E. Lorang[1], Ying Wang[1],

[1] *University of North Carolina Wilmington*

Strokes are one of the leading causes of death and disability worldwide, with limited options for treatment available. A major obstacle to recovery after stroke is chronic neuroinflammation, which is driven by proinflammatory cytokines. One critical inflammatory pathway is mediated by activated microglia, which release TNF, IL-1α, and C1q to induce a neurotoxic astrocyte subtype that perpetuates the injury. GLP-1 receptor agonists can suppress such neuroinflammation and hold promise for post-stroke treatment. To achieve this therapeutic effect, the peptide drug in the blood circulation must cross the blood-brain barrier (BBB). We propose that conjugating exendin-4 (Ex4), a common GLP-1 agonist, to a brain-targeting cellpenetrating peptide (CPP) may enhance BBB penetration and improve therapeutic outcomes following stroke. We designed three CPP-conjugated Ex4 (Ex4-CPP1, Ex4-CPP2, and Ex4-CPP3) compounds capable of facilitating transport across the BBB via receptor-mediated endocytosis. These peptides were synthesized by solid-phase peptide synthesis. We evaluated the functional recovery of mice treated with these compounds by administering them following induction of photothrombotic stroke in the mouse motor cortex. Compound administration of Ex4, Ex4-CPP1, Ex4-CPP2, Ex4-CPP3, or a vehicle occurred daily for 10 days following stroke. Functional recovery was assessed 1-, 3-, 7-, and 10-days post-stroke using three motor behavior tasks: tapered beam, rotating beam, and grid walk, before sacrifice on day 11 post-stroke. Our results showed significant improvements in functional recovery over unconjugated Ex4 with the Ex4-CPP1 and Ex4-CPP2 compounds. Treatment with Ex4-CPP1 and Ex4-CPP2 also resulted in a significant reduction of post-stroke weight loss, an indication of decreased stroke severity resulting from those treatments. This study is the first to demonstrate that conjugating Ex4 to a CPP enhances its efficacy in a stroke model, providing insight into how CPPs can improve the clinical utility of GLP-1 receptor agonists for post-stroke recovery.

LBA72

Chemogenetic manipulation of oligodendrocyte development and function

Veronica T. Cheli[1], Pablo M. Paez[1]

[1] Institute for Myelin and Glia Exploration, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, The State University of New York, University at Buffalo, Buffalo, New York.

Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic technologies represent valuable platforms for manipulating neuronal and non-neuronal signal transduction in a cell-type-specific fashion. In this work, DREADDs were used to manipulate Ca++ signaling in oligodendrocyte progenitor cells (OPCs) and thus the development of these cells in the postnatal as well as in the adult mouse brain. Via Cre-mediated recombination, we expressed in OPCs an excitatory G-protein-coupled receptor based on the human muscarinic M3 receptor (hM3Dq). Activation of hM3Dq in OPCs induces the release of Ca++ from internal stores and increases Ca++ influx mediated by voltage-gated and glutamate channels. In vitro, hM3Dq activity promoted OPC proliferation and reduced oligodendrocyte maturation and myelin protein synthesis. In vivo, hM3Dq activation in NG2- or Sox10-positive OPCs during early postnatal development significantly delayed the myelination process, reduced the density of mature oligodendrocytes, and increased the number of proliferating OPCs in several brain areas. In contrast, hM3Dq activation in mature oligodendrocytes induced myelin loss and oligodendrocyte apoptotic cell death in the adult brain. RNA-seq analysis performed in hM3Dq-expressing OPCs revealed dysregulated genes involved in OPC proliferation, cyclin-dependent protein serine/threonine kinase function, and potassium channel regulatory activity. Activation of hM3Dq in cortical OPCs also disrupted the expression of genes associated with oligodendrocyte maturation and signal transduction molecules modulated by the tumor suppressor p53. Together, these results indicate that OPC Ca++ signaling can be effectively modulated by DREADDs and that receptors such as hM3Dq could be employed to control OPC development and proliferation.

LBA73

Micro-Rna Based Gene Therapy To Promote Neuroprotection And Axon Regeneration In The Central Nervous System

Victor Ulysses de S. Matos[1], Raquel Alves de A. Costa[1], Carol Gomes Ferreira[1], Marco Túlio Rodrigues Alves[1], Lucas Caldi-Gomes[2], Pedro Pires Goulart Guimarães[1], Marina Pimenta Braga[1], Isabela Borges Hubner[1], Uwe Michel[3], Frederico Marianetti Soriani[1], Cleiton Lopes Aguiar[1], Vinicius Toledo Ribas[1]

[1] *Institute of Biological Sciences, Federal University of Minas Gerais, Brazil*
[2] *Technical University of Munich, Germany*
[3] *University Medicine Göttingen, Germany*

Axon degeneration is an early event that occurs in many neurodegenerative disorders, including spinal cord injury (SCI), and severely impair the neuronal network leading to neurological deficits, which are often permanent. For axonal regeneration to occur neuronal gene expression must be modulated. Thus, studying molecules that can regulate gene expression, such as microRNAs, is important to develop new therapeutic strategies do neurodegeneration. An interesting microRNA is miRNA-146a, which has been involved in neuroinflammation and axon outgrowth in the peripheral nervous system, but its role in the CNS is not completely understood. We aim to evaluates whether the overexpression of miR-146a, mediated by adeno-associated virus (AAV) vectors, is capable of promoting neuroprotection and axon regeneration in the CNS, using in vitro models of axonal injury and in in vivo model of traumatic SCI. The overexpression of miR-146a was induced using AAV vectors (AAV.miR-146a). As control we used an AAV vector expressing only the reporter gene EGFP (AAV.CTRL), which is also expressed by the AAV.miR-146a vector. For the in vitro analysis, cortical neurons from embryonic Wistar rats were plated in different conditions to evaluate neurite and axon outgrowth and regeneration. For the in vivo experiments, adult female Wistar rats were subjected to stereotaxic injections of the same AAV vectors into the sensoriomotor cortex to transduce corticospinal neurons neurons. Then, a SCI was done and soma atrophy of corticospinal neurons in brain sections, as well as, degeneration, sprouting and regeneration of corticospinal tract (CST) axons were analyzed by fluorescence microscopy. In addition, some animals underwent a gait test to identify motor alterations. It was seen that transduction of neurons in vitro with the AAV.miR-146a enhances neurite outgrowth, arborization complexity and regeneration, compared with the AAV.CTRL group. Most important, our results showed a greater regeneration specifically of axons by AAV.miR-146a in the in vitro experiments. In the in vivo SCI model, preliminary results suggest that AAV.miR-146a protects neuronal soma from atrophy, attenuates axon degeneration, and stimulated adaptive axonal sprouting responses of corticospinal neurons. The presented study suggests that miR-146a could be an interesting target to promote neuroprotection and regeneration in CNS.

LBA74

Non-Mutated Human Tau Stimulates Alzheimer's Disease-Relevant Eurodegeneration In A Microglia-Dependent Manner

Wei Cao[1], Ethan R. Roy[1], Qiang Wang[1], Kexin Huang[2], Sanming Li[1], Shuning Huang[3], Juan J. Herrera[3], Clare Pridans[4], Xiaobo Zhou[2],

[1] Department of Anesthesiology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA.
[2] McWilliams School of Biomedical Informatics at UTHealth Houston, Houston, TX, USA.
[3] Department of Diagnostic & Interventional Imaging, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA.
[4] University of Edinburgh Centre for Inflammation Research, The Queen's Medical Research Institute, Edinburgh, UK

The accumulation of abnormal, non-mutated tau protein is a key pathological hallmark of Alzheimer's disease (AD). Despite its strong association with disease progression, the mechanisms by which tau drives neurodegeneration in the brain remain poorly understood. Here, we selectively expressed non-mutated or mutated human microtubule-associated protein tau (hMAPT) in neurons across the brain and observed neurodegeneration in the hippocampus, especially associated with non-mutated human tau. Single-nuclei RNA sequencing confirmed a selective loss of hippocampal excitatory neurons by the wild-type tau and revealed the upregulation of neurodegeneration-related pathways in the affected populations. The accumulation of phosphorylated tau was accompanied by cellular stress in neurons and reactive gliosis in multiple brain regions. Notably, the lifelong absence of microglia significantly and differentially influenced the extent of neurodegeneration in the hippocampus and thalamus. Therefore, our study established an AD-relevant tauopathy mouse model, elucidated both neuron-intrinsic and neuron-extrinsic responses, and highlighted critical and complex roles of microglia in modulating tau-driven neurodegeneration.

LBA75

Neuron-specific NF-κB activation attenuates neurodegeneration during experimental autoimmune encephalomyelitis

Wensheng Lin[1,2], Zhixin Lei[1,2]

[1] *Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota, USA*
[2] *Institute for Translational Neuroscience, University of Minnesota, Minneapolis, Minnesota, USA*

Multiple sclerosis (MS) is chronic inflammatory demyelinating and neurodegenerative diseases of the CNS. Neurodegeneration is considered the primary driver of chronic disability in MS; however, the underlying mechanisms of neurodegeneration remain poorly understood. Data indicate that NF-κB participates in regulating the viability and function of neurons under normal and disease conditions. Although it is well documented that NF-κB is activated in neurons by inflammation in MS and its animal model experimental autoimmune encephalomyelitis (EAE), the cell-autonomous role of NF-κB activation in neurons in these diseases remains controversial and poorly defined. In this study, we showed that selective enhancement of NF-κB activation in neurons attenuated EAE disease severity and mitigates EAE-induced axonal degeneration, neuronal loss, and demyelination. Conversely, inhibition of neuronal NF-κB activation exacerbates disease severity and promotes neurodegeneration in EAE. These findings indicate that neuronal NF-κB activation plays a protective role in MS and EAE by promoting neuronal and axonal resilience in the face of inflammatory injury.

LBA76

PAK1 and its Kinase Activity Regulates OPC Proliferation and Repopulation During Development and After Demyelination

Yan Wang [1,] Xiangyi Shi[1], Bokyung Kim[1], , Fuzheng Guo[1]

[1] *Department of Neurology, UC Davis School of Medicine; Institute for Pediatric Regenerative Medicine (IPRM), Shriners Hospitals for Children, Sacramento, CA 95817.*

Gain-of-function mutations in PAK1 (p21-activated kinase 1) are associated with intellectual disability, macrocephaly, and white matter abnormalities in children, yet the mechanisms underlying these phenotypes remain unclear. PAK1 is a serine/threonine kinase activated by Cdc42/Rac1 and is known to regulate cell proliferation, cytoskeletal dynamics, and migration in both cancer and neurodevelopment. While extensively studied in neurons, the role of PAK1 in oligodendroglial lineage development is incompletely defined.

This study investigates whether and how PAK1 controls oligodendrocyte progenitor cell (OPC) proliferation during early postnatal development and whether it is required for OPC repopulation after white matter injury. Methods: Using genetic mouse models (including Pak1-floxed and loxP-STOP-loxP-Pak1 inhibition peptide [LSL-PID]) crossed with OPC-specific Cre drivers (Pdgfra-CreERT2), we selectively deleted or inhibited PAK1 kinase activity in OPCs. In vivo phenotyping, in vitro cultures, and lysolecithin-induced demyelination models were used to assess OPC proliferation, differentiation, and repopulation capacity. Molecular signaling downstream of PAK1 was also examined.

We found that OPCs, but not differentiating oligodendrocytes, exhibit high levels of PAK1 kinase activity during early postnatal development. PAK1 maintained OPCs in a proliferative state by modulating PDGFRα-mediated mitogenic signaling and acted as a brake on premature differentiation. PAK1 deletion or kinase inhibition reduced OPC proliferation and population expansion in a cell-autonomous manner. Mice with OPCspecific PAK1 deletion or inhibition exhibited fewer OPCs during development and impaired OPC repopulation following demyelination. These deficits were accompanied by reduced intra-lesional cell density and delayed remyelination. Conclusion: Our findings reveal a previously unrecognized role for PAK1 kinase activity in maintaining the OPC pool during development and after injury. PAK1 dysregulation likely contributes to the hypomyelination observed in children with PAK1-activating mutations. Furthermore, PAK1 may represent a therapeutic target for enhancing OPC repopulation and promoting remyelination in demyelinating diseases such as multiple sclerosis.

LBA77

Repetitive stimulation modifies network characteristics of neural organoid circuits

Yoshiho Ikeuchi[1,2,3,5], Siu Yu A. Chow[1,] Huaruo Hu[1,2], Tomoya Duenki[1,2,3], Takuya Asakura[4], Sota Sugimura [4],

[1] *Institute of Industrial Science, The University of Tokyo, Tokyo, Japan.*
[2] *Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, Tokyo, Japan.*
[3] *LIMMS, CNRS-Institute of Industrial Science, IRL 2820, The University of Tokyo, Tokyo, Japan.*
[4] *Research Institute of Advanced Technology, Softbank Corp., Tokyo, Japan.*
[5] *Institute for AI and Beyond, The University of Tokyo, Tokyo, Japan.*

Neural organoids develop complex networks but lack the external stimuli and hierarchical structures essential for refining functional microcircuits. We investigated whether connecting multiple organoids could enhance network refinement in response to external stimuli. Using high-density microelectrode arrays, we cultured networks comprising one, two, or three organoids and applied repetitive stimulation at two distinct input locations. We then monitored the emergence of output signals and assessed stimulus location decoding using machine learning algorithms. Following two weeks of daily stimulation, three-organoid networks demonstrated significantly higher stimulus decoding capability compared to simpler one- or two-organoid configurations. This long-term stimulation induced substantial changes in the three-organoid networks, including altered response patterns, modified spontaneous activity, and restructured inter- and intra-organoid functional connectivity. These findings highlight how hierarchical network organization—particularly the creation of distinct subnetworks with specialized roles—facilitates the development of circuits with robust input-output functionality in response to stimuli.

LBA78

Modulatory Effects Of GLT-1 Enhancer, MC-100093, On Glutamate Uptake And Associated Signaling Pathways In Female And Male Alcohol Preferring Rats Exposed To Ethanol

Youssef Sari[1], Ahmed Alotaibi[1], Khokon Kanti Bhowmik[1], Woonyen Wong[1], Adil Shareef Mohammed[2], Magid Abou-Gharbia [2], Wayne Childers[2]

[1] Department of Pharmacology and Experimental Therapeutics, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH, USA.
[2] Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA, USA.

Ethanol consumption disrupts the glutamate homeostasis in several brain regions. The uptake of extracellular glutamate is regulated in majority by the astrocytic glutamate transporter 1 (GLT-1), and cystine-glutamate exchanger (xCT) contributes to this regulatory effect. Chronic ethanol consumption is well known to downregulate GLT-1 expression in several reward brain regions, including the nucleus accumbens (NAc). Recently, we reported that a novel beta-lactam, MC-100093, attenuated ethanol consumption and normalized the expression of GLT-1 in the subregions of the NAc. Based on these findings, we aimed in this study to determine the dose-dependent effect of MC-100093 in attenuating ethanol consumption and whether this attenuating effect is associated with restoration of glutamate homeostasis, which was assessed using a radioactive glutamate uptake assay. Additionally, we focused on whether the effects of MC-100093 on GLT-1 are mediated through the mTOR, Akt, and NFkB signaling pathways. Male and female alcohol-preferring (P) rats had free access to ethanol (15% and 30% v/v) for five weeks. On Week 6, rats received i.p. MC-100093 at a dosage of 100 mg/kg or 150 mg/kg, or saline, for five days. MC-100093 treatment resulted in reduced ethanol drinking in male and female P rats, however, no sex difference effect was found on ethanol consumption. MC-100093 was associated with an increase in Na+-dependent and Na+-independent glutamate uptake. Furthermore, MC-100093 treatment attenuated ethanol-induced decrease in GLT-1, xCT, NFkB, and p-Akt expression in the NAc. These findings demonstrate that MC-100093 attenuated

LBA79

A novel degradative pathway in lysosomes and its implication to neuronal diseases and their treatment

Yuuki Fujiwara [1, 2], Yinping Zhou [1], Ichizo Nishino [1], Keiji Wada [1], Ko Miyoshi [2], Satoru Noguchi [1], Tomohiro Kabuta [1], Taiichi Katayama [2]

[1]United Graduate School of Child Development, Osaka University, Osaka, Japan
[2] National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan.

The constitutive degradation of intracellular components plays a crucial role in the homeostasis of the organism. Lysosomes are the major intracellular site for degradation of macromolecules, and abnormalities or dysfunction in lysosomal degradation systems are related to diverse diseases including neurodegenerative disorders and other neurological diseases. The mechanism that translocates intracellular substances to lysosomes is broadly and collectively referred to as autophagy. While the pathway known as macroautophagy has been intensively studied, other pathways, or in other words noncanonical autophagic pathways, have been relatively poorly studied. Against this background, we have discovered a new translocation and degradation pathway, which is a direct uptake of nucleic acids and proteins into the lumen by lysosomes, and have investigated the mechanism and pathophysiological significance of this pathway. Since various disease-associated aggregative proteins are degraded by this pathway and dysfunction of this pathway causes neuromuscular diseases with accumulation of aggregating proteins, we speculate that the regulation of this pathway can improve intracellular protein homeostasis and is a candidate target for the prevention and treatment of these diseases. In this presentation, we will present our recent findings on these topics.

LBA80

Lanthionine ketimine ethyl ester induces oligodendrocyte progenitor cell maturation mediated by collapsin response mediator protein 2.

Zane Deliu [1,2], Sergey Kalinin [2], Douglas Feinstein [2]

[1]*College of Medicine, Central Michigan University, Mount Pleasant, MI 48859, United States*
[2] *Department of Anesthesiology, University of Illinois, Chicago, IL 60612, United States*

Lanthionine ketimine ethyl-ester (LKE) is a synthetic derivative of lanthionine ketimine (LK), a naturally occurring metabolite of the nonproteinogenic amino acid lanthionine. Previously we showed that LKE increases differentiation of oligodendrocyte precursor cells (OPCs) in vitro, reduces clinical scores in the EAE mouse model of multiple sclerosis, and increases remyelination in the Cuprizone chemically-induced model of demyelination. The mechanisms of action of LKE are not fully known, but it has been reported that LKE regulates the activity of collapsin response mediator protein-2 (CRMP2) by blocking its phosphorylation. In the current study we tested if CRMP2 mediates LKE effects using mice with conditional knockout (cKO) from OPCs and in cells with CRMP2 depletion. To generate OPC specific CRMP2 cKO we crossed CRMP2 floxed mice with PDGFRa-CreER mice. To confirm cell specificity, we crossed PDGFRa-CreER mice to TdTomato expressing reporter mice. Following treatment with tamoxifen, the PDGFRa-Cre-ER : TdTomato mice showed expressed Tomato reporter in OPCs, confirmed by colocalization staining with PDGFRa but not with GFAP or Iba1. CRMP2 cKO and wild type (Cre-) littermates were administered CPZ for 3 weeks then analyzed for glial cell activation and myelin loss. The cKO mice showed increased GFAP and Iba1 expression, however myelin expression (MBP and BlackGold staining) was not altered. Acutely isolated OPCs from these mice were treated in vitro with 4-hydroxy tamoxifen to deplete CRMP2, then grown in differentiation media for up to 3 days. In contrast to the wildtype cells, CRMP2 cKO OPCs showed increased differentiation (increased MBP expression) which was not affected by treatment with LKE. These results suggest that LKE acts via inhibition of CRMP2 activity, such that depletion of CRMP2 replicates LKE actions. This work was funded in part by grants from the National MS Society and the Department of Veterans Affairs.