UCONN HEALTH

Ray A. Kroc and Robert L. Kroc DEPARTMENT OF NEUROSCIENCE ANNUAL RETREAT

PROGRAM & AGENDA

Thursday, May 19, 2022
9:00 am – 5:00 pm

Registration begins at 8:15 am

UConn Health Academic Rotunda
263 Farmington Avenue
Farmington, CT

UCONN HEALTH

May 13, 2022

Dear Neuroscience Program Faculty, Postdocs, Students and Guests,

Welcome to the 20th annual Neuroscience Program Retreat!

This year's event is being held at the Academic Rotunda, the centerpiece of a 21 million dollar Academic building expansion here at UConn Health. We feel very fortunate to have this space available to us and that the Neuroscience Program Retreat can once again take place.

We would like to bring your attention to a few important things:

Oral presentations - Oral presentations will be made in the rotunda. Speakers **must** upload their files to the computer in the room before the session.

Poster presentations - There will be two poster sessions. Session 1 will be before lunch, Session 2 immediately after. Posters will be displayed along the hallway in the ramp of the Academic Rotunda **for the entire duration of the retreat**. **Each presenter is required to stand beside his/her poster during only one preassigned session.** If you are a poster presenter, please check which session you have been assigned to.

Presentation awards - Presentations by students and postdocs will be judged for awards. You will be judged on several criteria, including the significance and quality of your work, the clarity of your presentation, your knowledge of the subject matter, and your responses to questions. Winners will be announced immediately before the closing remarks.

We hope you will enjoy the day.

Sincerely,

Zhao-Wen Wang, Ph.D.
Professor

Byoung IL Bae, Ph.D.
Assistant Professor

Department of Neuroscience
UConn School of Medicine
263 FARMINGTON AVENUE
FARMINGTON, CT 06030-3401
PHONE 860.679.8787
FAX 860.679.8766
uchc.edu

2022 Meeting Program

Time	Event	Location
8:15 – 8:55 am	**Registration & Sign-in**	
9:00 - 9:10 am	**Welcome Address** Dr. Zhao-Wen Wang	Academic Rotunda
9:10 - 10:00 am	**Symposium A** *Moderator: Dr. Yulan Xiong*	Academic Rotunda
10:00 – 10:50 am	**Symposium B** *Moderator: Dr. Srdjan D. Antic*	Academic Rotunda
10:50 – 12:00 pm	**Poster Session 1**	Rotunda Hallway
12:00 - 1:00 pm	*Lunch*	Academic Rotunda
1:00 - 2:15 pm	**Poster Session 2**	Rotunda Hallway
2:15 – 2:30 pm	**Vendor Recognition Presentation**	Academic Rotunda
2:30 - 3:15 pm	**Symposium C** *Moderator: Dr. Byoung-Il Bae*	Academic Rotunda
3:15 – 3:30pm	*Group picture*	Academic entrance
3:30 – 3:45 pm	*Dairy Bar Ice Cream immediately after photo*	Academic entrance
3:45 - 4:45pm	**Keynote Address:**	Academic Rotunda
	Richard W. Tsien, DPhil Druckenmiller Professor of Neuroscience Chair, Department of Neuroscience and Physiology Director, Neuroscience Institute Scientific Director, Marlene and Paolo Fresco Institute for Parkinson's and Movement Disorders New York University School of Medicine, New York, NY	
4:45 – 5:00 pm	**Presentation of Poster/Oral Awards &** **Closing Remarks**	

Special Thanks

To Our Corporate Partners & Vendor Supporters!

VENDOR REPRESENTATIVES contact information by company and division:

VENDOR	REPRESENTATIVE	CONTACT INFORMATION
BioRad	Julie Brunelle	julie_Brunelle@bio-rad.com
BioRad	Angela Ross	angela_ross@bio-rad.com
Fisher UHART, Trinity	Maria Adinolfi	maria.adinolfi@thermofisher.com
Fisher UConn Health	Kate Courchesne	kate.courchesne@thermofisher.com
Fisher UConn Storrs	Dave Gagnon	david.gagnon@thermofisher.com
Fisher Life Sciences	Alex Chan	alex.chan@thermofisher.com
Fisher Chemical Sciences	Mark Silva	mark.silva@thermofisher.com
Thermo Fisher Genetic Sciences Instruments	Caitlin Huynh	caitlin.huynh@thermofisher.com
Thermo Fisher Genetic Sciences Consumables	Laura Pope	laura.pope@thermofisher.com
Thermo Fisher Centrifugation	Tom Egan	thomas.egan@thermofisher.com
Thermo Fisher Lab Products	Pete Schreck	pete.schreck@thermofisher.com
Thermo Fisher UV-VIS Spectroscopy	Yen Lemire	yen.lemire@thermofisher.com
Thermo Scientific Protein Sciences	Cody Anderson	cody.anderson@thermofisher.com
Thermo Scientific Cell Culture	Eric Jung	eric.jung@thermofisher.com
Thermo Scientific Life Sciences	Brian Nolan	brian.nolan@thermofisher.com
Thermo Scientific Life Sciences	Ari Lechsinska	ari.lechsinska@thermofisher.com

KEYNOTE SPEAKER

“Synapse to nucleus and back again: activity-dependent gene expression keeping neurons in line.”

Richard Tsien, D. Phil.

Druckenmiller Professor of Neuroscience
Chair, Department of Neuroscience and Physiology
Director, Neuroscience Institute
Scientific Director, Marlene and Paolo Fresco Institute for Parkinson’s and Movement Disorders
New York University School of Medicine,
New York, NY

Dr. Tsien, inaugural Director of the Neuroscience Institute at NYU Langone Medical Center, has fostered the careers of many excellent neuroscientists over four decades as a teacher at Yale and Stanford Universities, before joining NYU in 2011.

A cardiac electrophysiologist by training, Dr. Tsien and his colleagues now focus on the dynamics of the synapse and topics ranging from the discovery of calcium channels that trigger neurotransmitter release, to understanding how postsynaptic activity controls nuclear transcription.

He has clarified how ion channels are modulated by transmitters, and in turn, regulate intracellular signaling. His group has developed new optical approaches to peer into single vesicle fusion events and the workings of multiple synaptic inputs in networks. The work has relevance to disease states such as pain, epilepsy, autism and schizophrenia and their amelioration by therapeutic agents.

Dr. Tsien received both an undergraduate and graduate degree in electrical engineering from the Massachusetts Institute of Technology. He was a Rhodes Scholar, graduating with a doctorate in biophysics from Oxford University, England.

Dr. Tsien is a member of the National Academy of Sciences, the Institute of Medicine, and the Academia Sinica. His past honors include the Cole Medal, the Palade Medal, the Cartwright Prize, the Axelrod Prize, the 2014 Bard Lectureship, the 2014 Annual Review Prize and the 2014 Gerard Prize. In 2015, Dr. Tsien was named the inaugural Scientific Director of the Marlene and Paolo Fresco Institute for Parkinson’s and Movement Disorders.

Symposium A: Talk No. 1

Cxcl14 – An Orphan Chemokine for Alzheimer's Disease Pathology

John Zhou[1,2,4], James Galske[3], Jacob Hudobenko[4], Dr. Riqiang Yan[4]
1. Cleveland Clinic Lerner College of Medicine - Neuroscience Department, Cleveland, OH, USA
2. Case Western Reserve University - Molecular Medicine Program, Cleveland, OH, USA
3. University of Connecticut, Storrs, CT, USA
4. University of Connecticut – Neuroscience Department, Farmington, CT, USA

Email: jzhou@uchc.edu

Abstract:

Alzheimer's disease (AD) is the most common age-related dementia characterized by the presence of extracellular amyloid beta plaques, intracellular tau, reactive glia cells, as well as a complex neuroinflammatory response. In the short term, proinflammatory cytokines contribute to increased clearance of amyloid beta, however chronic inflammation can lead to decreased phagocytosis and contribute to further neuron cell death. Therefore, it is critical to understand the role of specific cytokines within the AD-associated inflammatory milieu. Previous research by our lab utilizing single cell RNA sequencing has revealed a unique, C-x-c motif chemokine 14 ($Cxcl14^{hi}$), Clusterin (Clu^{hi}) reactive astrocyte population with enhanced amyloid beta clearance capacity. Cxcl14 is an orphan chemokine with well-studied anti-inflammatory role in the fields of cancer, diabetes, and immune surveillance, but relatively little is known about Cxcl14 function in neurodementia. One possibility is that Cxcl14 plays a beneficial role in the context of AD. Chemotaxis studies reveal the Cxcl14 enhances chemotaxis and transcytosis of both BV2 macrophage cell line, primary microglia, and bone-marrow derived monocytes, cells critical in the clearance of amyloid beta. Cxcl14 also enhances amyloid beta uptake both in vitro. Cxcl14 also appears to play a direct role in protecting neurons from both acute and recurrent amyloid beta insult. Interestingly, we also find that Cxcl14 preserves neuronal mitochondrial dynamics and antioxidant defense from recurrent amyloid beta insult. Altogether this suggests that Cxcl14 may ameliorate AD by enhancing microglial amyloid clearance, as well as preventing neurotoxicity.

Support:
NIH (AG025493, NS074256) and Alzheimer's Association (NPSPAD-10-174543) to Dr Riqiang Yan.

Symposium A: Talk No. 2

Cuprizone-Mediated Demyelination Reversibly Degrades Voiding Behavior in Mice while Sparing Brainstem Reflex

Ramalakshmi Ramasamy[1,3], Cara C. Hardy[1,3], Stephen J. Crocker[1,3,4], Phillip P. Smith[1,2, 3,4,*]

Abstract:

Multiple Sclerosis (MS) is a chronic, progressively debilitating demyelinating disease characterized by several randomly distributed focal lesions in the central nervous system (CNS) caused by a myelin-destructive auto-immune response, resulting in delayed and interrupted neuronal pathways. MS affects more than 2.3 million people worldwide, and nearly 80% of MS patients experience lower urinary tract dysfunction and associated symptoms (LUTD/S) including hesitancy, urgency, frequency and incontinence, early in their diagnosis. This significantly affects the quality of life, and in latter stages of disease is a leading cause of hospitalization. Inflammation and demyelination in the CNS are two important factors that affect functional output in MS patients. While most MS drugs to date reduce inflammation and decrease myelin damage, effective remyelinating drugs and their timely delivery are critical to restoring myelin before axon functionality is permanently lost. Previously, animal models of MS have shown that inflammatory demyelination in the CNS causes profound bladder dysfunction, but the confounding influence of systemic inflammation in all these models limits the potential interpretation of the contribution of CNS demyelination to bladder dysfunction. Furthermore, MS-associated LUTD/S is often clinically attributed to spinal lesions, while the micturition circuit regulating bladder function involves the cortex, brainstem and spinal cord that are all vulnerable to MS-associated demyelination. In fact, cortical demyelination affects decision-making abilities and could be sufficient to significantly affect conscious voiding behavior. Therefore, to address these gaps between research and translation, in this study, we elucidate the impact of cortical demyelination on urinary performance using the cuprizone model, characterized by demyelinating lesions in the cortex and corpus callosum that are independent of T cell-mediated autoimmunity. Herein, we report that a 4-week dietary cuprizone treatment in C57Bl/6J mice induced alterations in voiding behavior with increased micturition frequency and reduced volume voided, that clinically parallels urgency and frequency seen in MS patients. Subsequently, recovery from cuprizone treatment restored normal bladder function. Demyelination and remyelination were confirmed by Luxol Fast Blue staining of the corpus callosum. Additionally, we also determined that an 8-week cuprizone treatment, resulting in chronic demyelination lacking spontaneous remyelination potential, is associated with an exacerbated voiding phenotype. We then used urethane-anesthetized cystometry to evaluate the brainstem and spinal cord reflex. Urethane blocks all thalamocortical inputs to the brainstem reflex thereby allowing the latter to be the sole controller of micturition. Interestingly, while cuprizone-induced cortical demyelination severely affected conscious (cortical) urinary behavior, the autonomous (brainstem and spinal cord) reflex remained unchanged, as confirmed by cystometry. This is the first study to show that cortical demyelination independent of inflammation can negatively impact urinary function.

Support:
National Multiple Sclerosis Society PP-1905-33994
National Institutes of Health R01AG058814
National Institutes of Health R21 NS125332-01

Symposium A: Talk No. 3

Elucidating Retinal Biomarkers of Alzheimer's Disease using Single Cell Transcriptomics

Sarah I. Palko, Marc R. Benoit, and Riqiang Yan

Abstract:

Though Alzheimer's disease affects an estimated 6 million Americans, current diagnostics lack the ability to identify early signs of AD pathology. Amyloid beta (Aβ) plaque deposition and neuronal toxicity can occur as early as 20 years before cognitive symptoms appear, and this caveat prevents effective treatment of the disease. Current technologies such as PET scans, lumbar punctures, and cognitive assessment tools are expensive, invasive and/or ineffective at detecting plaque deposition, therefore, researchers have turned to the retina as a window to detect neurodegeneration in AD. The retina provides a direct extension of the brain that mirrors AD pathology, specifically Aβ plaque accumulation and neuronal loss. However, the mechanisms by which the retina degenerates in AD is unknown. Of note, retinal Müller glia (rMG) have not been adequately characterized in AD despite their integral role as sensors and responders in several ocular and non-ocular neurodegenerative diseases. We aim to elucidate the mechanisms by which the retina degenerates in the APP knock-in NLGF mouse model by investigating changes to rMG. This NLGF mouse model emulates human disease by modulating Aβ deposition to result in plaque saturation in the hippocampus by P90. Using single cell transcriptomics, we investigated retinal MG gene changes preceding plaque deposition in the NLGF mouse model of AD. Using P30 retinas, we discovered an increase in gene expression of ER stress-related genes. More specifically, upregulation of genes in the ATF4 pathway like Atf4, Atf5, Cebpg, Herpud1, and Nupr1 were evident only in rMG in the NLGF retina. Using molecular biology techniques, we confirmed these gene changes at the protein level in P30 retinas. Retinal MG are known to engage ER stress pathways to influence neuronal death in ocular diseases such as glaucoma. These findings implicate rMG in early retinal response to Aβ and could highlight potential early biomarkers of AD pathogenesis in the accessible retina.

Funded by 2R01AG025493-11

Symposium B: Talk No. 4

Functional Coupling of TRPM2 and Extrasynaptic NMDARs Exacerbates Excitotoxicity in Ischemic Brain Injury

Pengyu Zong[1], Jianlin Feng[1], Zhichao Yue[1], Yunfeng Li[2], Gongxiong Wu[3], Baonan Sun[1], Yanlin He[1], Barbara Miller[4], Albert S. Yu, Zhongping Su[1], Jia Xie[1], Yasuo Mori[5], Bing Hao[2], Lixia Yue[1,*]
[1] Department of Cell Biology, Calhoun Cardiology Center, University of Connecticut School of Medicine (UConn Health), Farmington, CT 06030, USA; [2] Department of Molecular Biology and Biophysics, University of Connecticut School of Medicine (UConn Health), Farmington, CT 06030, USA; [3] Department of Medicine, Brigham and Women's Hospital, Laboratory for Translational Research, Harvard Medical School, Cambridge, MA 02139, USA; [4] Departments of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, P.O. Box 850, Hershey, Pennsylvania, 17033, USA; [5] Laboratory of Molecular Biology, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, 615-8510, Japan; The World Premier International Research Initiative-Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, 615-8510, Japan.

Abstract:
Introduction. Ischemic stroke caused a heavy burden on public health, and is a major cause of mortality and disability. NMDA receptor (NMDAR) mediated excitotoxicity has been thought to be the culprit for neuronal death during ischemic stroke for decades. Unfortunately, NMDAR antagonists all failed to show protective effects in human patients. The heat-sensitive ion channel TRPM2 is calcium-permeable and usually activated under oxidative stress conditions. TRPM2 is abundantly expressed in the brain and promotes neuronal death during ischemic stroke. However, the mechanisms underlying TRPM2-mediated deleterious effects in ischemic stroke remain elusive.

Hypothesis. Oxidative stress is a hallmark of brain damage during ischemic stroke. We hypothesized that TRPM2 is important in magnifying NMDAR-mediated excitotoxicity, and neuron specific TRPM2 knockout or uncoupling TRPM2-NMDAR interaction protects mice against ischemic stroke.

Methods. Neuron specific TRPM2 knockout was achieved by crossing *nestin-cre* mice with *Trpm2*$^{fl/fl}$ mice. Middle cerebral artery occlusion (MCAO) and oxygen-glucose deprivation (OGD) were performed to mimic ischemic stroke in vivo and in vitro, respectively. Co-immunoprecipitation and in vitro direct binding assay were used to examine protein-protein interaction. Subcloning and mutagenesis were used to identify interaction details. Interfering peptide specifically disrupting TRPM2-NMDAR interaction was designed and synthesized. Tetrazolium chloride assay was used to evaluate infarct size. Cortical neurons were isolated and cultured, Fura-2-AM and Rhodamine-123 imaging were used to examine calcium overload and mitochondrial dysfunction, respectively. Synaptic and extrasynaptic NMDAR mediated responses were separated, and synaptosome was isolated to examine the influence of TRPM2 on NMDAR and expression of TRPM2 in neurons at different sites, respectively.
Results. Neuron specific TRPM2 knockout protects mice against ischemic brain injury. TRPM2 physically and functionally interacts with extrasynaptic NMDAR, which markedly enhances excitotoxity. The EE_3 motif in TRPM2 directly associates with the KKR motif in GluN2a/2b. Uncoupling of TRPM2-NMDAR association using a disrupting peptide prevents OGD-induced calcium overload and mitochondrial dysfunction in neurons, and protects mice against MCAO-induced brain injury.

Conclusion. Therapeutic interfering peptide TAT-EE_3 designed based on our identified interacting details between TRPM2 and NMDAR attenuates ischemic stroke. Our findings establish that targeting the TRPM2-NMDAR coupling could be a promising strategy for screening more effective therapies for ischemic stroke.

Support. National Institute of Health (R01-HL143750) and American Heart Association

(19TPA34890022) to L.Y. , and Canadian Institutes of Health Research (MOP 86655) to J.V..

Symposium B: Talk No. 5

Contribution of GABA-A receptor subunit deletions to human Angelman syndrome neuronal phenotypes

Tiwanna M. Robinson[1],Stormy J. Chamberlain[2], and Eric S. Levine[1]
[1]Neurosci., [2]Genet. and Genome Sci., Univ. of Connecticut Sch. of Med., Farmington, CT

Email: trobinson@uchc.edu

Abstract:

Maternal deletion of chromosomal region 15q11-q13 causes Angelman syndrome (AS), a genetic disorder in which individuals present with developmental delays, language impairment, intellectual deficits, motor ataxia, and seizures. The gene located in this region that is primarily responsible for AS is *UBE3A*, which encodes a ubiquitin protein ligase. This gene is normally imprinted in the brain, and expression in neurons is only from the maternal allele. Thus, a maternal deletion of this region, which is the most common cause of AS, results in a complete loss of *UBE3A* expression in neurons. Several non-imprinted genes in the region are also affected, including a cluster of GABA-A receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*), which encode the β3, α5, and γ3 subunits, respectively. AS can also be caused by loss of function mutations of *UBE3A*. When AS results from mutations of *UBE3A*, the symptoms of affected individuals pertaining to cognition, motor function, and seizures are less severe compared to the symptoms caused by a maternal deletion. This suggests that in 15q11-q13 deletion patients, the loss of other genes in the region contributes to AS pathophysiology. We have previously shown that human AS neurons containing a maternal 15q11-q13 deletion have impaired maturation of resting membrane potential (RMP) and action potentials (APs), increased excitability, and decreased GABA-mediated synaptic transmission compared to neurons derived from unaffected controls. In the present studies, we used novel isogenic iPSC lines derived from a patient with a *UBE3A* mutation and a CRISPR-corrected counterpart to examine the contributions of *UBE3A* and the GABA-A receptor subunits to AS neuronal phenotypes. Maturation of RMP and AP firing were affected in *UBE3A* mutation neurons to a similar extent as AS deletion neurons. *UBE3A* mutation neurons also displayed increased excitability, but of a lesser magnitude than seen in AS deletion neurons. Spontaneous inhibitory synaptic activity was not altered in *UBE3A* mutation neurons, in contrast to the decreased frequency and amplitude of spontaneous inhibitory synaptic currents seen in AS deletion neurons. We hypothesize that the hemizygous loss of the GABA-A receptor subunit cluster contributes to the more severe cellular phenotypes in AS deletion neurons. Ongoing experiments are exploring whether decreasing the expression of these genes in *UBE3A* mutation neurons using antisense oligonucleotides (ASOs) will mimic the more severe cellular phenotypes seen in AS deletion neurons, including decreased inhibitory synaptic signaling and neuronal hyperexcitability. These studies may identify novel targets for therapeutic development in AS.

This work is supported by NIH R01 and NIH R21.

Symposium B: Talk No. 6

Regulation of BK channel endocytosis by an RNF-145/EFA-6/ARF-6 molecular pathway

Michal Ragan[1], Long-Gang Niu[1], Jeremy Balsbaugh[2], Bojun Chen[1], Zhao-Wen Wang[1]

[1]Department of Neuroscience, University of Connecticut School of Medicine, Farmington, CT; [2]UConn Proteomics & Metabolomics Facility, University of Connecticut, Storrs, CT

Email: ragan@uchc.edu

Abstract:

BK channels (Slo1) are high-conductance K^+ channels that are activated by membrane depolarization and cytosolic Ca^{2+}. In neurons, BK channels colocalize with voltage-gated Ca^{2+} channels at presynaptic sites and negatively regulate neurotransmitter release. The function and expression of BK channels *in vivo* depends on many other proteins. To identify novel proteins required for BK channel function, we performed a genetic screen for mutants suppressing a sluggish phenotype caused by a hyperactive or gain-of-function (*gf*) SLO-1, which is *C. elegans* BK channel. Three loss-of-function (*lf*) mutants of *rnf-145* were isolated from the genetic screen. *rnf-145* encodes a RING finger protein with an E3 ubiquitin ligase domain. Like *slo-1(lf)* mutants, *rnf-145(lf)* mutants display a higher degree of head bending, and increased amplitude of evoked postsynaptic currents (ePSCs) and an increased frequency of miniature postsynaptic currents (minis) at the neuromuscular junction (NMJ) compared with wild type, suggesting that RNF-145 is required for SLO-1 function *in vivo*. How might RNF-145 contribute to SLO-1 function? Since ubiquitin E3 ligases generally promote target protein degradation, we reasoned that RNF-145 might ubiquitinate a negative regulator of SLO-1. To identify the putative negative regulator, we performed mass/specs analyses to detect proteins that are upregulated in *rnf-145(lf)* mutants compared with wild type. One of the highly upregulated protein in the mutants was EFA-6/Efa6, which is a guanine nucleotide exchange factor for the small GTPase ARF-6/Arf6 (ADP-ribosylation factor). It catalyzes ARF-6/Arf6 from a GDP-bound inactive state to a GTP-bound active state to promote endocytosis of membrane proteins. We therefore hypothesize that RNF-145 promotes SLO-1 function by ubiquitinating EFA-6 to inhibit an EFA-6 and ARF-6 endocytosis pathway of SLO-1. This hypothesis is supported by all the behavioral and electrophysiological data that we have collected up to this stage, including decreased locomotor activities, and reduced amplitude of ePSCs and a reduced frequency of minis at the NMJ in *efa-6(lf)* and *arf-6(lf)* mutants compared with wild type. Experiments are underway to determine whether SLO-1 surface expression *in vivo* is decreased in *rnf-145(lf)* mutant but increased in *efa-6(lf)* and *arf-6(lf)* mutants, and whether human Slo1 in transfected HEK293 cells is also endocytosed through the EFA6 and ARF-6 pathway. The results of this study will likely establish endocytosis as a novel molecular mechanism for regulating SLO-1/Slo1 physiological functions.

This work was supported by NIH R01MH085927 (Z.W), R01NS109388 (Z.W), and R35GM139620 (B.C.).

Symposium C: Talk No. 7

Paternal *UBE3A* is silenced by *UBE3A-ATS* through transcriptional interference

Dea Gorka[1], Noelle Germain[1], Leighton Core[2], and Stormy J. Chamberlain[1,3]

[1]Department of Genetics and Genome Sciences, UConn Health Center, Farmington CT, USA
[2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA
[3]Institute for Systems Genomics, University of Connecticut, Storrs, CT, USA

Email: gorka@uchc.edu

Abstract:

Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by motor dysfunction, intellectual disability, severe seizures, absent speech, and a happy demeanor. The disorder occurs approximately one in every 15,000 live births. AS is caused by loss of function from the maternally inherited allele of *UBE3A*. In most cell types, *UBE3A* is expressed from both the maternal and paternal alleles. In mature neurons, *UBE3A* is only expressed from the maternally-inherited allele. Thus, loss of function from the maternal allele leads to nearly complete loss of *UBE3A* RNA and protein. Imprinted (maternal-only) expression of *UBE3A* occurs because the paternal allele of *UBE3A* is silenced by a long non-coding antisense RNA, termed *UBE3A-ATS*. Activation of paternal *UBE3A* through the suppression of *UBE3A-ATS* transcription is a promising therapeutic strategy for AS, however the exact mechanism underlying *UBE3A* imprinting by *UBE3A-ATS* is not fully understood. We have used patient-derived human iPSCs and their neural derivatives to carefully dissect the mechanisms by which *UBE3A-ATS* silences paternal *UBE3A* and antisense oligonucleotides (ASOs) unsilence paternal *UBE3A.* Our data strongly support a model whereby RNA Polymerase II (RNA Pol II) machineries transcribing *UBE3A-ATS* on the plus strand obstruct RNA Pol II on the minus strand from transcribing the 3' end of *UBE3A*, thus preventing full transcription of *UBE3A* mRNA. The transcribing polymerase, rather than the *UBE3A-ATS* RNA itself, leads to silencing of *UBE3A* through transcriptional interference. Moreover, we demonstrate that ASOs activate paternal *UBE3A* by disengaging the RNA Pol II machineries transcribing *UBE3A-ATS*. ASOs that fail to terminate transcription do not activate paternal *UBE3A*, thus providing a framework for the design of ASOs as potential therapeutic approaches to restore *UBE3A* expression in individuals with AS. Altogether, these discoveries epitomize the power of human pluripotent stem cells for disease modeling and drug discovery. These studies also reveal important factors to consider when designing and implementing ASOs and other RNA cutting molecules as viable therapeutics.

Support/Funding:
National Institutes of Health grant R01HD094953
Angelman Syndrome Foundation

Symposium C: Talk No. 8

FOXP Genes regulate cerebellar development by controlling the diversification of Purkinje Cells

Nagham Khouri-Farah[1], Qiuxia Guo[1], and James Y.H. Li[1,2]

[1]Department of Genetics and Genome Sciences, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030-6403, USA

[2]Institute for Systems Genomics, University of Connecticut, 400 Farmington Avenue, Farmington, CT 06030-6403, USA

Email: khourifarah@uchc.edu

Abstract:

Purkinje cells (PC) are the cornerstone of the cerebellar circuitry – PCs orchestrate circuit assembly by regulating the number of other cell types, layering of the cerebellar cortex, and topographic connectivity. Through single-cell RNA sequencing, we have uncovered the remarkable heterogeneity of PCs in the embryonic mouse cerebellum. Using fluorescent immunostaining and 3D imaging, we mapped PC subtypes and found that their positioning coincided with the anteroposterior and mediolateral patterning of the developing cerebellum. Remarkably, PC subtypes display distinctive combinatorial expression patterns of *Foxp1*, *Foxp2*, and *Foxp4*, which encode a subfamily of the forkhead box transcription factors. Cerebellum-specific knockouts of *Foxp1* and *Foxp2* disrupt specific subgroups of PCs and the formation of the cerebellar hemisphere, which is an innovative feature of mammals and involved in higher cognitive function. Mice lacking either *Foxp1* or *Foxp2* in the cerebellum exhibit severe ultrasonic vocalization defects, demonstrating an important role of Foxp-regulated cerebellar circuits in vocalization. Together, our findings show that the combinatorial function of Foxp genes coordinates the diversification of Purkinje cells, which in turn instruct cerebellar development.

This work was supported by grants from the NIH to James Y.H. Li (R01 NS106844 and R01 NS120556) and Nagham Khouri-Farah (1F31NS124264).

Symposium C: Talk No. 9

Post-injury born oligodendrocytes integrate into the glial scar and inhibit growth of regenerating axons by premature myelination

Jian Xing, Bruce A. Rheaume, Juhwan Kim, Agnieszka Lukomska, Muhammad S. Sajid, Ashiti Damania, and Ephraim F. Trakhtenberg.

Department of Neuroscience, University of Connecticut School of Medicine

Abstract:

The failure of mature central nervous system (CNS) projection neurons to regenerate axons over long distance often results in permanent functional deficits and limits the recovery after CNS injuries and diseases. A major barrier to CNS axonal regenerative research is that the regenerative axons triggered by experimental treatments stall growth before reaching their post-synaptic targets. Here we test the hypothesis that premature, de novo, myelination of regenerating axons stalls their growth, even after bypassing the glial scar. To test this hypothesis, we used single cell RNA-seq (scRNA-seq) and immunohistological analysis to investigate whether post-injury born oligodendrocytes integrate into the glial scar after optic nerve injury. We also used a multiple sclerosis model of demyelination concurrently with the stimulation of axon regeneration by Pten knockdown (KD) in projection neurons after optic nerve injury. We found that post-injury born oligodendrocytes integrate into the glial scar, where they are susceptible to the demyelination treatment, which prevented premature myelination, and thereby enhanced Pten KD-stimulated axon regeneration. We also present a website for comparing the gene expression of scRNA-seq-profiled optic nerve oligodendrocytes under physiological and pathophysiological conditions.

Support: The University of Connecticut School of Medicine, Start-Up Funds (to E.F.T.), the BrightFocus Foundation (Grant G2017204, to E.F.T.), and the National Institutes of Health (NIH) (Grant R01-EY029739, to E.F.T.).

Poster Session 1 / No. 1

Investigating Changes in Bladder Tissue Function in *App^{NL-G-F}* Mouse Model of Alzheimer's Disease

Alya Alobaidi[1,4] , Ramalakshmi Ramasamy[2,4], Jenna Bartley[4], Cara Hardy[2,4], Phillip P. Smith[2,3,4]
MS Neuroscience Program[1],University of Hartford, West Hartford, CT; Departments of Neuroscience[2], Surgery[3], and UConn Center on Aging[4], University of Connecticut School of Medicine, Farmington, CT, USA

Email: alobaidi@uchc.edu/alyasaeed24@gmail.com

Abstract:

Alzheimer's disease (AD) is a degenerative neurological condition caused by plaque buildup. AD and urinary incontinence (UI) frequently coexist and are common in AD patients. Indeed, AD patients have a higher rate of UI than those without AD. However, the link between AD and UI has yet to be established. It is possible that plaque aggregates in the brain impact the micturition regions leading to UI; however, it's also possible that there are bladder-specific alterations in responses. To investigate the effect of AD pathology on bladder tissue physiology, we used a knock-in (KI) AD mouse model (APPNL-G-F) with three distinct mutations for familial AD. Wild type C57Bl/6 (WT, homozygous KI (KIKI), and heterozygous KI (KIWT) mice were sacrificed at 3.5, 5.5, and 9.5 months and bladder function was analyzed via strip tension experiments and electrical field stimulation to investigate adrenergic and muscarinic receptor postsynaptic signaling. Interestingly, at 3.5 months of age, when plaque starts to accumulate, KIKI mice were more responsive to a muscarinic agonist drug (carbachol, 10μM) and less responsive to adrenergic agonist drug (isoproterenol, 0.1 μM) than WT mice, while older ages showed no differences in muscarinic or adrenergic responses. It's possible that compensatory mechanisms may occur over time to equalize responses via decreasing muscarinic receptors in the bladder as plaque accumulates. More research is necessary to determine how adrenergic and muscarinic responses are impacted by AD.

Funding and acknowledgements
NIH R21 AG061609-01
NIG R01 R01AG058814
APP^{NL-G-F} mice were a generous gift from Dr. Xiangyou Hu and Dr. Riqiang Yan in the Neuroscience department at UConn Health

Poster Session 1 / No. 2

Elucidating the role of GABA-A receptor overexpression in Dup15q Syndrome using human stem cell derived neurons

Deepa Anjan Kumar, Marwa Elamin, Tiwanna Robinson, Eric S. Levine

Abstract:

Chromosome 15q11-q13 duplication syndrome (Dup15q) is a neurodevelopmental disorder caused by maternal, but not paternal duplication of this region. It is characterized by motor deficits, hypotonia, intellectual disability, epilepsy, and a majority of individuals with this syndrome meet the criteria for autism spectrum disorder (ASD). Of the genes in this region, *UBE3A* is the only maternally imprinted gene and is thought to be the major driver for the ASD phenotype. Previous studies from our lab have demonstrated that overexpression of *UBE3A* alone, however, mimics some but not all of the cellular phenotypes observed in Dup15q patient-derived neurons. There are several other genes in the duplicated region that are expressed biallelically, including a cluster of GABA-A receptor subunits, which are also implicated in neurodevelopmental disorders. Using human patient-derived stem cell lines along with isogenic, corrected control lines, our goal is to identify genes in addition to *UBE3A* that contribute to the phenotypes observed in Dup15q neurons. With the help of antisense oligonucleotides (ASOs), we will normalize the expression of specific GABA-A receptor subunits (*GABRB3, GABRA5, GABRG3*) singly or in combination to determine if knocking down these subunits affects cellular and synaptic phenotypes observed in Dup15q neurons. mRNA levels, protein levels, and electrophysiological characteristics will be assessed after knockdown and compared to the isogenic control.

Supported by grants from the NIH and the Eagles Autism Foundation.

Poster Session 1 / No. 3

Intersectional genetic access to transcriptionally-distinct melanin-concentrating hormone neuron subpopulations in the lateral hypothalamus

Monica S. Antony[1], Alexander C. Jackson[1,2,]

Affiliations: [1]Department of Physiology and Neurobiology, [2]Institute for the Brain and Cognitive Sciences, University of Connecticut, Storrs, CT

Email: monica.antony@uconn.edu

Abstract:

The lateral hypothalamic area (LHA) coordinates crucial innate behaviors through diverse and poorly understood neuronal populations. *Pmch*-expressing neurons, synthesizing melanin-concentrating hormone protein (MCH), comprise a distinct cell population in the LHA and are key contributors to this complex physiological control. Overall, MCH neurons have been implicated in sleep, feeding, memory, reproduction, stress, as well as many other innate behaviors. Prior anatomical and transcriptomic data reveals that MCH neurons can be parsed into two subclusters- one that robustly expresses the *Tacr3* gene, encoding the tachykinin-3 receptor (NK3R), and another sub-cluster that does not. However, these studies have been limited by the lack of genetic reagents to further explore these *Tacr3*-defined MCH subpopulations. Our project seeks to gain genetic access to *Tacr3*-defined MCH subpopulations through the characterization of a novel *Tacr3*-FlpO transgenic mouse line used in conjunction with a *Pmch*-Cre transgenic mouse line. Through fluorescence *in situ* hybridization (FISH) and immunohistochemical techniques, we demonstrate that these transgenic lines are valid and specific in their recombinase expression. We then used these mutant lines to deliver recombinase-specific viruses in the LHA through stereotaxic injection, and discovered LHA *Tacr3*+ and *Pmch*+ fibers present in the lateral septum, medial septum, nucleus of the diagonal band, basal forebrain, and periaqueductal grey. We also discovered *Pmch*+ only fibers in the inferior and superior colliculi. Ultimately, we aim to characterize the use of *Pmch*-Cre and *Tacr3*-FlpO transgenic mouse lines to be able to gain intersectional genetic access to *Tacr3*-defined MCH subpopulations using multiple viral strategies, in order to better understand the diversity of circuit-level synaptic and behavioral mechanisms mediated by transcriptionally-distinct MCH subpopulations.

Support: Project supported by the National Institute of Mental Health (NIMH) R01MH112739 (to A.C.J.)

Poster Session 1 / No. 4

Uncovering the mechanism of neuronal CX3CL1 back-signaling using single-nuclear RNA sequencing

Marc Benoit, Manoshi Gayen, Riqiang Yan

Abstract:

Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting 10-30% of the population >65 years of age. The pathological hallmark of AD is the accumulation of extracellular beta amyloid (Aβ) plaques, intracellular neurofibrillary tangles, and massive neuronal death primarily localized to the hippocampus, frontal, and temporal cortices. Current therapeutic efforts targeting the production and clearance of amyloid plaques from the extracellular space have continually failed to improve outcomes, thus novel approaches to treating neurodegenerative disease must be pursued. Therapies aimed at preventing and/or replacing the death of neurons have yet to be fully investigated.

It is widely accepted neural stem cell (NSC) niches in the sub-granular zone of the hippocampus (SGZ) and sub-ventricular zone (SVZ) exist in the healthy adult mammalian brain and continually give rise to newborn neurons, referred to as adult neurogenesis. Work in mouse models of AD and in diseased human brain tissue have shown a reduction in the neurogenic stem cell pool. It is unclear how growth-promoting signals stimulate the production and survival of adult-born neurons, and whether stimulating the neurogenic stem cell pool will slow or reverse the progression of neurodegenerative disease.

The neuronal chemokine CX3CL1, or fractalkine, is a type 1 transmembrane protein that plays a central role in neuron-microglia communication by binding its cognate receptor, CX3CR1, exclusively expressed by microglia. A recent study has uncovered a novel mechanism by which the CX3CL1 intracellular c-terminal domain (CX3CL1-ICD), upon cleavage from the membrane via alpha-, beta- and gamma-secretases, localizes to the nucleus and triggers a neuronal proliferative program independent of CX3CR1. This chemokine back-signaling is a novel and exciting discovery. Transgenic overexpression of only the c-terminal fragment in mice induces an increase in newborn neurons in the SGZ and SVZ, and has profound positive effects on Aβ plaque deposition, lifespan, and cognitive deficits in mouse models of AD. We aim to understand how CX3CL1 enhances neurogenesis through its c-terminal domain, and how this ameliorates AD phenotypes. We hypothesize CX3CL1-ICD triggers a neuronal survival transcriptional program upon translocating to the nucleus by interacting with transcription machinery, resulting in upregulated pro-survival signals that increase the number of adult-born neurons and ameliorate Alzheimer's disease pathology.

Our study takes a bioinformatic approach via single-nuclear RNA sequencing to answer the following questions: **1) what CX3CL1-induced changes to the transcriptome explain the increased survival of neurons in the adult mouse brain in response to Alzheimer's disease pathology**, and 2), **is the effect of CX3CL1-ICD overexpression cell-autonomous?** The targets we have identified using this bioinformatic approach has allowed us to use biochemical assays to further understand the mechanism of action of this novel and exciting signaling pathway.

Support: RF1AG058261- Riqiang Yan - National Institute on Aging (NIA)

Poster Session 1 / No. 5

LRP10 knockout mice as a novel model of Parkinson's disease and other neurodegenerative diseases

Shifan Chen[1], Jianzhong Yu[2], Yulan Xiong[1]

[1] Department of Neuroscience, University of Connecticut School of Medicine, Farmington, CT 06030, USA

[2] Department of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269 USA

Email: schen@uchc.edu

Abstract:

Loss-of-function variants in the low-density lipoprotein receptor-related protein 10 (LRP10) gene have been associated with autosomal-dominant Parkinson's disease (PD), PD dementia, and dementia with Lewy bodies (DLB). Moreover, LRP10 variants have been found in individuals diagnosed with progressive supranuclear palsy and amyotrophic lateral sclerosis, and LRP10 is a driver of a specific molecular subtype of Alzheimer's disease (AD). This provides evidence of potential roles for LRP10 across a broader spectrum of neurodegenerative diseases. Despite these genetic evidences and initial function studies, little is known about LRP10 function and no LRP10 animal models have been developed and characterized. We have generated LRP10 knockout (KO) mice. LRP10 KO mice exhibit a battery of behavioral deficits including locomotor function defects, cognitive changes and social interaction deficits. Preliminary studies show that knockout of LRP10 also promotes periphery pathology. Accompanying with the behavioral deficits and periphery pathology, the neurodegeneration is being characterized. To further investigate the cellular functions or pathways that LRP10 is involved, proteomic analysis is performed to identify the dysregulated proteins and pathways. Thus, these mice provide the first LRP10 mouse model to investigate the loss of function of LRP10 in neurodegenerative diseases and an important platform to study the molecular mechanisms of how LRP10 induces neurodegeneration.

Funding: This work was supported, in part, by grants from NIH/NINDS R01NS112506, NIH/NIA K01AG046366 award, Parkinson's Foundation Stanley Fahn Junior Faculty award PF-JFA-1934, UConn Startup fund.

Poster Session 1 / No. 6

Effect of Age and stroke on Purinergic receptor P2X4 (P2X4R) expression in Human monocyte subset

Daylin Gamiotea-Turro[1], Sharon DiMauro[2], Pranay Srivastava[1], Sanjay Mittal[3], Ketan R Bulsara[4], and Bruce Liang[2] Rajkumar Verma[1]

[1]Department of Neurosciences, [2]Calhoun Cardiology Center, [3]Department of Neurology and [4]Division of Neurosurgery, School of Medicine, UConn Health, Farmington, CT 06032, USA

Email: gamioteaturro@uchc.edu

Abstract:
An acute ischemic stroke (AIS) triggers rapid mobilization of immune cells from bone marrow, and the infiltration of circulating immune cells in the brain. P2X4R, a receptor for adenosine triphosphate ATP, regulate activation of circulating myeloid immune cells e.g. monocytes after stroke injury. Over-stimulation of P2X4R contributes to ischemic injury. Monocytes, a heterogeneous population of primary myeloid cells, can be divided into three subsets based on expression of the low-affinity FcγRIII: $CD14^{++}CD16^{-}$ classical, $CD14^{++}CD16^{+}$ intermediate, and $CD14^{+}CD16^{++}$ non-classical monocytes. Alterations in activity of circulating monocyte subsets may independently predict pathogenesis of AIS, however, the role of P2X4R in the activation of these monocyte subsets is not known. Therefore, in this study we analyzed the effect of P2X4R activation on monocyte subsets after AIS.
Consecutive AIS patients (60-90 years) undergoing endovascular clot retrieval and healthy control subjects both young (18-45 years) and aged (60-90 years) of both sexes were recruited and informed consent obtained. Flow cytometric analysis of whole blood derived monocytes at 0–2 days (acute, n=15), 3–7 days (subacute, n=14), and 65±20 days (chronic, n=8) after stroke onset were compared with healthy subjects (n=9-10/ age group). A pure monocyte or P2X4R +ve monocyte population was separated using a side and forward scatter plot with cell-surface markers, such as P2X4R, HLA-DR, CD45, CD14 and CD16, as well as a negative gating strategy.
Both number of total monocyte counts and P2X4R intensity significantly increase with age when compared between healthy young and aged control ($P<0.05$). Total monocyte count progressively increased during recovery in AIS patient ($P<0.05$). No. of $CD14^{++}CD16^{+}$ intermediate monocytes were significantly reduced after stroke ($p<0.05$). Both $CD14^{++}CD16^{+}$ intermediate, and $CD14^{+}CD16^{++}$ non-classical monocytes showed a significant increased median fluorescent intensity ($P<0.01$) of P2X4R at subacute and chronic time after AIS. In summary, P2X4R expression increases with age and after stroke. Disappearance of the $CD14^{+}CD16^{++}$ non-classical monocyte subpopulation from circulation during stroke recovery suggests potential migration of these cells to the site of injury, consistent with their potential role in inflammation/phagocytosis. Increased P2X4R expression in intermediate and non-classical monocytes subpopulation suggest its specific role in selective activation of these monocytes subtype. Detailed molecular characterization of P2X4R response in intermediate and non-classical monocyte subpopulations may provide novel insights into P2X4R's therapeutic potential during AIS.

Support: This work was supported by AHA Career Development award 18CDA34110011 and UConn OVPR (to Rajkumar Verma)

Poster Session 1 / No. 7

Beta-secretase-1 (BACE1) modulates cognition and neuronal excitability in the hippocampal CA1 region in a cell-autonomous manner

Annie Yao[1], Riqiang Yan[1]
[1] University of Connecticut Health Center, Department of Neuroscience

Email: ayao@uchc.edu

Abstract:

Alzheimer's Disease (AD), the most common cause of dementia, is a debilitating disease that leads to progressive memory loss, cognitive impairment, and ultimately death. Pathological hallmarks of AD include extracellular amyloid beta (Aβ) plaques. β-secretase-1 (β-site APP cleaving enzyme 1, BACE1) is the rate-limiting enzyme of toxic Aβ generation. Transgenic BACE1 KO mouse models of AD led to suppression of AD pathology, which suggests that inhibiting BACE1 may be a rational strategy for AD treatment. However, human clinical trials have shown that BACE1 inhibitors are inefficacious, even worsening cognitive function, among AD patients. This benchtop-to-bedside translational failure is due to our incomplete understanding of BACE1's physiological function. In particular, the mechanisms underlying neuronal and synaptic impairments in BACE1 deficiency or inhibition is poorly understood. To investigate how BACE1 regulates excitability in excitatory pyramidal neurons (PNs) without the potential compensatory or compounding effects of BACE1 deletion in inhibitory neurons, we have adopted a mouse model (Nex1-cre$^{+/wt}$;Bace$^{fl/fl}$) that induces BACE1 deletion specifically in forebrain excitatory neurons after early developmental stages. Whole-cell patch-clamp recordings of hippocampal CA1 PNs in brain slices prepared from 1.5-month-old mutant mice display increased excitability in both active and passive membrane properties, including a more depolarized resting membrane potential (RMP), increased evoked action potential (AP) spiking, reduction in spike frequency adaptation, and higher input resistance compared to controls. AP waveform features were also altered, consistent with possible alterations of ion channel activity. A battery of behavioral testing demonstrated that BACE1 deletion in excitatory neurons is sufficient to produce cognitive impairment on open field and fear conditioning tests that indicate a hyperactive phenotype and impaired associative learning and memory. These findings provide evidence that selective BACE1 deletion in excitatory neurons leads to neuronal hyperexcitability, suggesting that BACE1 deletion disrupts intrinsic neuronal function in a cell-autonomous manner in the hippocampus, a major substrate of memory storage derailed by AD. Ultimately, these findings will provide insight into BACE1's function in regulating the activity of excitatory neurons, synapses, and circuits in the hippocampus, which will provide critical insight into our fundamental understanding of learning and memory, synaptic transmission and plasticity, and the development of AD therapeutics.

Support: This project is funded by R01 NS074256, National Institute of Neurological Disorders and Stroke (NINDS) and NIA.

Poster Session 1 / No. 8

Studying Canonical Cortical Circuitry – Ex Vivo Voltage Imaging – Genetically Encoded Indicators

Darko D. Lovic, Mei Hong Zhu, Brianna Barbeau, Kumiko Watanabe and Srdjan D. Antic;
Neuroscience, UConn Health, School of Medicine, Farmington, Connecticut, 06030, USA.

Email: lovic@uchc.edu

Abstract:

Neurophysiological and neuroanatomical studies have found that some basic structural and functional organization of neuronal circuits repeat throughout the cortex. This kind of cortical organization, termed canonical circuit, has been functionally demonstrated in brain slices. In a typical ex vivo electrophysiology experiment, synaptic stimulus is delivered in a cortical layer (1–6) and neuronal responses are recorded with electrodes, or calcium imaging, to investigate the local cortical circuitry. We recreated this standard electrophysiological paradigm in brain slices of mice expressing genetically encoded voltage indicators (GEVIs). This allowed us to monitor membrane voltages in the target cortical layers. Pyramidal neurons have complex dendritic trees that span multiple cortical layers, while the horizontal dendritic span is roughly identical to the width of a cortical column. By combining whole-cell with GEVI imaging, we obtained a crude distribution of activated synaptic afferents in respect to the dendritic tree of a hypothetical pyramidal cell, and in respect to a hypothetical cortical column. Synaptically evoked voltage waves propagating through the cortical neuropil (dendrites and axons) were not static but rather they changed on a millisecond scale. Voltage imaging can identify areas of brain slices in which the neuropil was depolarized by glutamatergic synapses, as well as the area of the slice in which neuronal membranes were depolarized directly from the stimulation electrode while glutamatergic AMPA synapses were blocked (AMPA receptor antagonist DNQX [10 μM]). To rule out artifacts from the voltage indicator used, or transgenic animal used, we explored three voltage indicators (chi-VSFP, ASAP2s, and di-4-ANEPPS) with different optical sensitivity, optical response speed, labeling strategy, and a target neuron type. All three imaging methods work in our hands. We continue to analyze the canonical cortical circuit by applying synaptic stimulation in cortical layers L5, L4 or L2/3, in the absence and then in the presence of drugs that block fast synaptic transmission (glutamatergic or GABAergic).

Support:
This research was funded by the National Institute of Mental Health award (U01-MH109091) to SDA; National Institute of Neurology and Stroke award (U01-NS099573) to SDA; Cure Alzheimer's Fund award (number N/A) to SDA and RY; the National Institute on Aging grant (R21-AG064554) to SDA and RY; and the UConn Alcohol Research Center (ARC) / Kasowitz Medical Research Fund grant (P50-AA027055) to SDA.

Poster Session 1 / No. 9

Presenilin promotes neurotransmitter release through ryanodine receptors in *C. elegans*

Longgang Niu, Xinran Du, Zhao-Wen Wang
Department of Neuroscience, UConn Health, Farmington, CT 06030

Email: niu@uchc.edu

Abstract:

Mutations in the presenilin genes, *PS1* and *PS2*, are the most common cause of familial Alzheimer's disease. Presenilins function as the catalytic subunit of a γ-secretase protein complex to cleave the amyloid precursor protein. Presenilin mutations may cause accumulation of amyloid β peptides and impair synaptic functions. However, the underlying molecular mechanisms remain elusive. The SEL-12 protein is a *C. elegans* presenilin. Here we found that loss-of-function (*lf*) mutation of *sel-12* inhibited the motility of *C. elegans* significantly at the Day 1 adult stage, produced a greater inhibitory effect at the Day 2 adult stage, but had no significant effect at the L4 larval stage, suggesting that neuronal functions might be impaired by *sel-12(lf)* in an age-dependent manner. To explore the molecular mechanisms of *sel-12(lf)* on neuronal functions, we began by analyzing miniature and evoked postsynaptic currents (minis and ePSCs) at the *C. elegans* neuromuscular junction. We found that both the mean frequency of minis and the amplitude of ePSCs were greatly reduced in *sel-12(lf)* compared with wild type at the Day 1 adult stage, and that the mutant synaptic phenotypes could be reversed by cell-targeted expression of wild-type SEL-12 in neurons but not muscle cells. Because neurons and muscle cells are the pre- and postsynaptic cells, respectively, with respect to the neuromuscular junction, these results suggest that SEL-12 plays an important role in regulating neurotransmitter release. Intriguingly, the synaptic phenotypes of *sel-12(lf)* resembled those caused by *lf* mutations of *unc-68*, which is the sole ryanodine receptor gene in *C. elegans*, and were not aggravated in *sel-12(lf);unc-68(lf)* double mutant, suggesting that SEL-12 might regulate UNC-68-mediated calcium release from the endoplasmic reticulum. Experiments are underway to determine whether and how SEL-12 regulates UNC-68 function or expression. The potential results may shed light on how presenilin mutations cause Alzheimer's disease.

Supported by NIH R01MH085927 and R01NS109388

Poster Session 1 / No. 10

Optimization of Fixatives for Electron Microscopy and immunohistochemistry of neuronal tissue

Emily Parrish-Mulliken, Janeth Perez Garza, Linnaea E Ostroff
Affiliations: University of Connecticut

Email: emily.parrish@uconn.edu

Abstract:

Electron microscopy allows scientists to obtain high resolution images of biological tissue and to visualize ultrastructural characteristics of cells. This tool has been utilized for decades to view neuronal tissue and analyze ultrastructural characteristics and changes which occur in response to different behavioral paradigms. This is valuable for studying paradigms such as Pavlovian threat conditioning, which are known to increase synaptic plasticity. By utilizing electron microscopy, we can visualize these synaptic changes and better understand ultrastructural changes caused by synaptic plasticity. However, electron microscopy requires multiple steps to process tissue in order to stabilize it under the electron beam, including fixation, dehydration, and embedding. Due to these processing steps, the tissue viewed in the electron microscope is no longer an exact representation of the native tissue when the animal is alive. One such issue is the loss of extracellular space, giving the appearance that cell membranes are always flush with one another, which is not the case in unfixed tissue. Furthermore, some of these processing techniques, especially fixation, minimize the effectiveness of immunolabeling. For example, fixation with glutaraldehyde is known to interfere with immunohistochemistry. Here, we show progress in optimizing fixation techniques used in EM to help preserve the native form of neuronal tissue as well as enable better immunolabeling. We discuss key features of synaptic ultrastructure which are impacted by different fixation protocols. This work will help us to get closer to understanding the true native structure of synapses and different ultrastructural characteristics of synapse types.

Support:
NIH MH119617
NSF 2014862.

Poster Session 1 / No. 11

HMGB1 directly inhibits oligodendrocyte progenitor cell differentiation and impairs CNS remyelination

Megan E. Rouillard, Jingwen Hu, Pearl A. Sutter, Hee Won Kim, Jeffrey K. Huang, Stephen J. Crocker

Email: rouillard@uchc.edu

Abstract:

HMGB1 is a highly conserved, ubiquitous protein in eukaryotic cells. HMGB1 is normally localized to the nucleus, where it acts as a chromatin associated non-histone binding protein. In contrast, extracellular HMGB1 is an alarmin released by stressed cells to act as a danger associated molecular pattern (DAMP). We have recently determined that progenitor cells from multiple sclerosis patients exhibit a cellular senescent phenotype and release extracellular HMGB1 which directly impaired the maturation of oligodendrocyte progenitor cells (OPCs) to myelinating oligodendrocytes (OLs). Herein, we report that administration of recombinant HMGB1 into the spinal cord at the time of lysolecithin administration resulted in arrest of OPC differentiation *in vivo*, and a profound impairment of remyelination. To define the receptor by which extracellular HMGB1 mediates its inhibitory influence on OPCs to impair OL differentiation, we tested selective inhibitors against the four primary receptors known to mediate the effects of HMGB1, the toll-like receptors (TLRs)-2, -4, -9 or the receptor for advanced glycation end-products (RAGE). We found that inhibition of neither TLR9 nor RAGE increased OL differentiation in the presence of HMGB1, while inhibition of TLR4 resulted in partial restoration of OL differentiation and inhibiting TLR2 fully restored differentiation of OLs in the presence of HMGB1. Analysis of transcriptomic data (RNAseq) from OPCs identified an overrepresentation of NFκB regulated genes in OPCs when in the presence of HMGB1. We found that application of HMGB1 to OPCs in culture resulted in a rapid and concentration dependent shift in NFκB nuclear translocation which was also attenuated with coincident TLR2 inhibition. These data provide new information on how extracellular HMGB1 directly affects the differentiation potential of OPCs. Recent and past evidence for elevated HMGB1 released from senescent progenitor cells within demyelinated lesions in the MS brain suggests that a greater understanding of how this molecule acts on OPCs may unfetter the endogenous remyelination potential in MS.

Funding was provided by the National MS Society.

Poster Session 1 / No. 12

Hippocampal function in a changing aversive-appetitive environment

Ryan Troha, Bailey Morte, Sucika Perumulla, Etan Markus Ph.D.

Abstract:

The hippocampus has long been known to be important for learning and memory. Functional differentiation has been studied between the dorsal and ventral hippocampal subregions of the hippocampus. The dorsal hippocampus is heavily involved in spatial memory and navigation, while the ventral hippocampus is more involved in stress and emotional memory. Within these two regions, place cells code for the specific location of animals as they traverse an environment. However, less is known regarding place cells in the ventral hippocampus compared to the dorsal hippocampus. Strong connectivity between the dorsal and ventral hippocampus subregions has also been shown, but few studies have examined the physiological interaction between these two subregions.

The current study plans to train animals to run back and forth for a food reward on a U-shaped maze. Animals will also learn to associate a tone that is paired with an active shock region, which the animals must cross in order to obtain food on the other side of the maze. Therefore, animals are exposed to a changing environment which switches between “safe” and “unsafe” trials. While animals perform this task, a microelectrode array will record activity from single units in the ventral hippocampus. In addition, chemogenetics will be used to transiently inactivate the dorsal hippocampus.

Therefore, this study allows for examination of ventral hippocampal activity in a changing emotional context, both with and without normal input from the dorsal hippocampus. This study will further our understanding of hippocampal function in a changing context. Furthermore, this study will provide clarity on the interplay along the longitudinal axis of the hippocampus. These results could also have important implications for psychiatric disorders involving fear and anxiety.

Poster Session 2 / No. 13

Evidence of Tinnitus-Specific Differences in Stimulus-Evoked Brainstem Potentials

Emily M. Fabrizio-Stover, Christopher M. Lee, Alice L. Burghard, Douglas L. Oliver

Abstract:

Tinnitus, a phantom perception of sound with no external auditory stimulus, is experienced by over 50 million Americans. Behavioral tests are used to assess tinnitus in laboratory animals for research, but this process is expensive, time consuming, and often subjective as testing protocols differ between research groups. An objective, electrophysiological test would bypass these difficulties and allow for more efficient tinnitus research. Here, we tested animals with and without behavioral signs of tinnitus for changes in the brainstem electrophysiological activity after application of a novel auditory stimulus paradigm (NSP) (patent pending). Tinnitus was induced in awake CBA/CaJ mice by unilateral continuous sound exposure. Tinnitus status was determined behaviorally 8 weeks after exposure. Evoked potentials like auditory brainstem responses (ABR) were collected in response to our NSP. This included responses to pure tone pips at three or more frequencies delivered to each ear. For tinnitus mice, the frequencies were determined by the tinnitus frequency indicated by behavioral testing, plus stimuli ±1 octave above and below. Non-tinnitus and control mice were tested with frequencies matching the sound exposure frequency plus stimuli ±1 octave. We measured peak and trough amplitudes in the tone-pip evoked potentials produced by our NSP to calculate the tinnitus score for each waveform. When comparing the peak-trough scores, the interaction between tinnitus and exposure was significant (2-way ANOVA, $p=0.0027$). Specifically, the non-tinnitus exposed ear scores were significantly less than those in the tinnitus exposed ear (Tukey test, $p=0.0056$). This effect was significant at higher frequencies and for later ABR waves. There was no significant difference between the tinnitus exposed and unexposed ears. However, there were significant differences between non-tinnitus exposed and unexposed ears (Tukey test, $p=0.011$). This indicates that although sound exposure may affect tinnitus score, there may be evidence of a global change in tinnitus animals. A bootstrapping method that randomly compares multiple evoked potentials and correlates them was used to compare responses to our NSP. The non-tinnitus exposed ears were significantly lower than the tinnitus exposed, non-tinnitus unexposed, and control ears. This suggests that sound exposure results in a less correlated ABR that is somehow ‘brought back to baseline’ in tinnitus animals. Taken together, these data suggest that our NSP is a good candidate for generating measurable differences in evoked responses between tinnitus and non-tinnitus subjects.

Support: This work was supported by a grant from the DOD | United States Army | MEDCOM| Congressionally Directed Medical Research Programs - W81XWH-18-0135

Poster Session 2 / No. 14

The Roles of Neuron-derived longevity factor Klotho on CNS metabolism by the control of VDAC1 expression and Lactate Secretion

Yinzheng Guan,MS[1] and Riqiang Yan, PhD[1,2]

[1]Department of Biological, Geological and Environmental Sciences, Cleveland State University
[2]Neuroscience Department, University of Connecticut Health Center

Abstract:

Klotho (KL), an anti-aging protein, has well-known neuroprotective effects. Animals with a mutated hypomorphic KL gene have altered glycemia regulation, suggesting a role of KL in the metabolic regulation. KL is expressed by neurons in some brain regions, but its function in the central nervous system (CNS) remains to be fully understood. In this study, we demonstrated that neuron-derived KL controls Voltage Dependent Anion Channel 1 (VDAC1) expression to promote lactate production. We generated a novel transgenic mouse model, with an inducible expression of KL in the nervous system. By treating mice with doxycycline (Dox), expression of KL will be inducibly expressed. We also examined mice with KL deficiency (KL knockout) for comparison. We discovered that high levels of lactate and pyruvate were detected in the hippocampal region of KL transgenic mice while KL knockout mice exhibited a reduction in lactate and pyruvate production in the hippocampus region. Typically, pyruvate crosses the outer mitochondrial membrane (OMM) to reach the intermembrane space (IMS), probably through the large, relatively non-specific, voltage-dependent anion channel (VDAC), which prompted us to determine VDAC1 levels in transgenic and knockout mouse brains. We found that KL elevation reduced VDAC1 protein expression, whereas KL knockout elevated VDAC1 protein expression in their brains. We indeed found that reduction in VDAC1 levels by KL increased lactate secretion. Since VDAC1 is also a component of mitochondria permeability transition pore (mPTP), we found that KL regulated mPTP and mitochondria membrane potential through changes in VDAC1 levels. In KL knockout mice, upregulation of VDAC1 promoted mitochondrial fission, reflected by altered mitofusin-1 and -2 (MFN1 and MFN2) levels. We further showed that VDAC1 reduction restricted mitochondria calcium levels to exert the neuroprotective effects. Mechanistic study revealed that the regulation of VDAC1 by KL was mediated by the Akt/GSK3β/CREB/miR-7a-5p signaling pathway. Together, our findings suggest that KL plays a coordinating role in the regulation of brain metabolism. By controlling VDAC1 expression levels, KL promotes lactate production and achieves beneficial effects on healthy aging.

Funding: This study was supported by grants from the National Institutes of Health to R Yan (NS074256).

Poster Session 2 / No. 15

Neuroanatomical analysis of lateral hypothalamic GABAergic neuron projections and role in anxiety-like behavior

Yi Huang[1], Emma.Burleigh[1] and Alexander C. Jackson[1,2]
[1]Department of Physiology and Neurobiology, [2]Connecticut Institute for the Brain and Cognitive Sciences, University of Connecticut, Storrs, CT

Email: yi.2.huang@uconn.edu

Abstract:

The lateral hypothalamic area (LHA) is a hub for central and peripheral signals throughout the central nervous system as well as being an important modulator of sleep-wake states, feeding, and stress/reward behavior. Previous research has demonstrated robust long-range outputs from the LHA to diverse regions of the brain. Importantly, the LHA projects to major monoaminergic, neuromodulatory regions that regulate behavioral state, including the tuberomammillary nucleus (TMN), ventral tegmental area (VTA), dorsal raphe (DR), locus coeruleus (LC) and periaqueductal gray (PAG) among other regions. Previous work has demonstrated that a large subpopulation of LHA neurons express markers for the synthesis and release of the fast inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and that optogenetic or chemogenetic activation of these LHA GABAergic neurons elicits robust behavioral activation (increased arousal, appetitive and consummatory behaviors). The robust behavioral responses elicited by activation of LHA GABAergic neurons may be explained by their widespread projections to diverse brain regions associated with the control of behavioral state, however, the exact targets of LHA GABAergic neurons are not well-understood.
In this work, we examine LHA GABAergic projections to the TMN, VTA, DR, LC and PAG through neuroanatomical tract-tracing and immunohistochemistry. Of particular interest are projections to the pons region of the brainstem, which contains the LC. Previous work has demonstrated that LHA GABAergic neurons project to the LC. However, the concentration of fibers seems to suggest that it is in fact more strongly innervating a region more medial to the LC, known as Barrington's nucleus (Bar). The LC plays an important role in modulating arousal, stress and autonomic function and although Bar is critical in micturition, there is evidence that Bar neurons modulate LC activity in the context of stress responses. We hypothesize that these LHA GABAergic neurons are not in fact projecting to the LC but to Bar. To test this hypothesis, we carried out a systematic neuroanatomical mapping of LHA^{GABA} neurons using transgenic mouse lines (VGAT-Cre) and viral anterograde tracing to study the location of these genetically labeled neurons in the LHA and their descending projections to key monoaminergic cell populations. Furthermore, given the role of both the LHA and dorsal region of the pons in arousal and anxiety-related behavior, we further examined the effect of chemogenetic activation of LHA GABAergic neurons in two assays of anxiety-related behavior.
Financial support: Project supported by the National Institute of Mental Health (NIMH) R01MH112739 (to A.C.J.)

Poster Session 2 / No. 16

LRRK2 regulates an AP2M1 phosphorylation cycle to mediate endocytosis and dopaminergic neurodegeneration

Qinfang Liu[1, 3], Judith Bautista-Gomez[4], Daniel A. Higgins[4], Jianzhong Yu[2, 3], Yulan Xiong[1,3]

[1] Department of Neuroscience, University of Connecticut School of Medicine, Farmington, CT 06030, USA

[2] Department of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269 USA

[3] Department of Anatomy & Physiology, Kansas State University College of Veterinary Medicine, Manhattan, KS 66506, USA

[4] Department of Chemistry, Kansas State University, Manhattan, KS 66506, USA

Email: qiliu@uchc.edu

Abstract:

Recent genetic evidence revealed that endocytic pathway plays a major role in Parkinson's disease (PD) risk. However, the molecular mechanism of how endocytic defects contribute to dopaminergic neurodegeneration in PD is poorly understood. Here we report that LRRK2, the mutations of which are the most genetic causes of PD, binds to and phosphorylates AP2M1, the core component of endocytosis that has been recently implicated in PD risk. Our study revealed that abnormal AP2 phosphorylation cycle, regulated either by knockout or overexpression of LRRK2, causes endocytic defects. Our study also uncovered a novel tissue-specific regulation of AP2 phosphorylation by LRRK2. Further, we found that LRRK2 phosphorylation on AP2M1 mediates LRRK2-induced neuronal toxicity both *in vitro* in neuronal cultures and *in vivo* in *Drosophila* dopamine neurons. Importantly, AP2M1 phosphorylation levels are elevated in patient fibroblasts of both LRRK2-associated PD and sporadic PD, suggesting the clinical relevance of our finding in PD. Together, our study provides a direct mechanistic link between LRRK2, AP2 and endocytosis in PD pathogenesis.

Funding: This work was supported, in part, by grants from NIH/NINDS R01 NS112506, NIH/NIA K01 AG046366 award, Parkinson's Foundation Stanley Fahn Junior Faculty award PF-JFA-1934, American Parkinson Disease Association (APDA) Research Grant, NIH/NIGMS P20 GM113109 pilot grant, SUCCESS-FYI Intramural Grant from Kansas State University College of Veterinary Medicine, UConn Startup fund.

Poster Session 2 / No. 17

Serial-section multiplex labeling for imaging brain biomolecules at high-resolution

Janeth Pérez-Garza[1], Zachary Deane[2], Ethan Gasteyer[1], Mariel Agbim[1], Linnaea Ostroff[1]
[1] Department of Physiology and Neurobiology. University of Connecticut, Storrs, CT. USA.
[2] Salk Institute for Biological Studies. La Jolla, CA. USA

Email: janeth.perez_garza@uconn.edu

Single cell approaches are crucial to elucidate the spatial distribution of biomolecules in the brain's cellular and subcellular level. Individual neurons vary, not only by molecular distribution, but morphological and structural characteristics also delineate the cell identity. Simultaneous detection of proteins and RNA molecules can be achieved by fluorescent labeling, but limitations on current methods, such as the number of targets visualized, reduces the high throughput of multiplex labeling in single cells. Resin embedding of biological tissues is widely used for electron microscopy, obtaining nanometer scale tissue sections with detailed structural information of single neurons. However, resin on tissue interferes with molecular labeling, unless is fully removable and entirely expose tissue to labeling molecules. Our goal is to accomplish ultrathin serial sectioning for multiplex labeling of proteins and RNA in intact brain tissue. Here, we embedded rat brain tissue in an acrylic resin that maintained intact tissue structure and allowed serial nanometer sectioning to use for fluorescent labeling of proteins and RNA. Furthermore, native fluorescent viral tracers are preserved and can be visualized in tissue sections. Individual nanometer sections are collected and selected for specific labeling methods, immunofluorescence or *in situ* hybridization. This resin is fully dissolved after sections are placed on coverslips. After sectioning, histological stains are used to visualize tissue quality and macrostructural morphology of neurons. With this approach, we can perform immunofluorescence labeling for neurotransmitters, such as gamma-aminobutyric acid (GABA), or cytoplasmic proteins, such as calcium binding proteins, calbindin (CalB), parvalbumin (PV), and calretinin (CalR), and localize their distribution in the brain. Individual compartments, such as axons and dendrites can also be identified by targeting specific markers for these processes. Furthermore, we have achieved *in situ* labeling to total mRNA, and by the creation of specific cell-type markers probes, RNA and protein organization can be discerned at the single cell level. These labeling approaches can be combined with viral fluorescent tracers to help understand the structural and molecular components associated to a specific neural circuit. With this serial multiplex approach, sensitive labeling using antibodies or RNA probes is achieved on ultrathin sections, where multiple targets can be visualized in single neurons. Notably, serial sections can be perfectly aligned to resolve large-scale, single cell mapping in the mammalian brain.

We gratefully acknowledge support from NIH MH119617 and MH123994
.

Poster Session 2 / No. 18

Depletion of CD8+ T-cells prevents clinical disease and CNS myelin pathology in a mouse model of Globoid Cell Leukodystrophy

Pearl A. Sutter[1,4], Antoine Menoret[2], Evan Jellison[2], Ernesto Bongarzone[3], Anthony T. Vella[2], Stephen J. Crocker[1,2]

Departments of Neuroscience[1] and Immunology[2]
University of Connecticut School of Medicine, Farmington CT
Department of Anatomy and Cell Biology[3], University of Illinois at Chicago, Chicago, IL

Email: psutter@uchc.edu

ABSTRACT:
Globoid Cell Leukodystrophy (GLD) is a demyelinating central nervous system (CNS) disease that results in death in 99% of children before the age of 5 years old. Loss of function mutation in galactocerebrosidase (*galc*) in GLD leads to a toxic build-up of the lipid psychosine, which is currently thought to underlie the development of this disease. The rapid progression of behavioral and cognitive deficits present in GLD is devastating for both patients and families, however current treatments have limited success at modulating these symptoms. Therefore, it is critical to further understand the complex cellular changes associated with the pathology of this disease to develop successful therapies for these patients. Neuropathology in GLD is marked by profound demyelination and inflammation. To better understand the complex cellular changes related to inflammation that transpire at the time of symptom onset (p21), we performed single-cell RNA sequencing on the brains of Twitcher (Twi) mice, an authentic mouse model of GLD, and wildtype littermates. These analyses identified profound differences in T-cell populations in the Twi central nervous system (CNS) compared to age-matched wildtype controls. Specifically, we identified a 9-fold increase in CD8+ T-cells with a transcriptional signature for cytotoxic CD8+ T-cells. Cytotoxic CD8+ T-cells are known to mediate tissue specific injury in a range of autoimmune diseases, but their role in GLD has not been previously reported. To test the functional contribution of CD8+ T-cells, we administered anti-CD8α antisera to mice beginning one week before symptom onset and measured disease progression compared to control IgG-treated Twi mice. Mice treated with anti-CD8 antisera exhibited greatly diminished clinical symptomatology, as measured by disease scoring, compared to controls. Importantly transmission electron microscope analysis of anti-CD8α antisera treated mice revealed that anti-CD8α treatment completely prevented CNS demyelination as measured by axon g-ratio analysis. Furthermore, anti-CD8α antisera treated animals also had reduced inflammatory cytokine levels both in the CNS as well as in peripheral blood samples when compared to controls. Lastly, we confirmed co-localization of CD8+ T-cells in the CNS of human GLD neurospecimens in association with sites of demyelination. Taken together, these data reveal a previously unrecognized role for CD8+ T-cells as key regulators of the neuroinflammatory response and CNS demyelination present in GLD. Ongoing studies are expected to rigorously test the functionality of GLD CD8+ T-cells, which are expected to fill an important gap in our understanding on the fundamental cellular pathological mechanisms underlying the development of GLD. Moreover, a pathogenic role for CD8+ T-cells in GLD could support repurposing of available immunomodulatory therapies as treatments for GLD.

This work was supported by funding from the NIH (R56 NS 0999359) and the UConn School of Medicine.

Poster Session 2 / No. 19

Molecular mechanisms of BK channel regulation by melatonin

Kiranmayi Vedantham, Longgang Niu, Bojun Chen, and Zhao-Wen Wang

Email: vedantham@uchc.edu

Department of Neuroscience, University of Connecticut School of Medicine, Farmington, CT 06030, USA

Melatonin, secreted by the pineal gland, produces its sleep-promoting effect via two G protein-coupled receptors: MT_1 and MT_2. These two receptors are expressed in a variety of brain structures including the cerebral cortex, the suprachiasmatic nucleus (SCN) of the hypothalamus, and hippocampus. However, the downstream molecular target(s) mediating the sleep effect of melatonin has remained enigmatic. In our recent study with *C. elegans* (Niu et al., PNAS 2021), we found that melatonin promotes sleep by activating the BK channel SLO-1 through a specific melatonin receptor, and that melatonin secretion and activation of the melatonin receptor play a permissive role in SLO-1's physiological functions of regulating neurotransmitter release and promoting sleep. In addition, we found that the human BK channel Slo1 may be activated by melatonin in the *Xenopus* oocyte expression system when it is co-expressed with MT_1 but not MT_2, and that the activation results from the action of Gβγ subunits. These results suggest that mammalian Slo1 might also be important in mediating melatonin's cellular effects. To determine why melatonin activates Slo1 through MT_1 but not MT_2 in *Xenopus* oocytes, we tested the effects of chimeras of mouse MT_1 and MT_2 on mouse Slo1 single-channel open probability (P_O). We found that substituting the N- but not C-terminus of MT_1 with that of MT_2 abolished the activating effect of melatonin on Slo1, suggesting that the N-terminus of MT_1 is required for the activation of Slo1. Slo1 channels are enriched at neuronal presynaptic sites, where they co-localize with voltage-gated Ca^{2+} channels and negatively regulate neurotransmitter release. A recent study shows that MT_1 is a component of presynaptic protein complexes. The presence of both MT_1 and Slo1 at presynaptic sites in neurons suggests that that they might have physical and functional interactions. Our recent co-immunoprecipitation experiments with transfected HEK293T cells indicate that MT_1 and Slo1 physically interact through discrete domains. Slo1 co-immunoprecipitated with MT_1 receptor, and this physical interaction depends on an intracellular loop (S0-S1 loop) of Slo1. The primary physiological functions of Slo1 in mammalian neurons are to regulate excitability and neurotransmitter release. To determine whether melatonin may regulate these functions of Slo1, we performed electrophysiological experiments with brain slices containing SCN neurons from the melatonin-proficient CBA/CaJ mice. In preliminary studies, we observed that administration of melatonin to the extracellular solution led to hyperpolarized resting membrane potential, reduced action potential firing rate, and increased paired pulse ratio of excitatory postsynaptic potentials. In contrast, administration of paxilline, a selective BK channel blocker, showed opposite effects, and prevented the effects of melatonin. These results support our hypothesis that melatonin can activate Slo1 to inhibit neuronal excitability and neurotransmitter release.

Supported by NIH R01MH085927 (Z.W), R01NS109388 (Z.W), and R35GM139620 (B.C.).

Poster Session 2 / No. 20

Role of Neurospecific Reticulons on Mammalian Neurodevelopment

John Zhou[1,2,3], Yingying Ge[3], Riqiang Yan[3]
1 Cleveland Clinic Lerner College of Medicine - Neuroscience Department, Cleveland, OH, USA
2 Case Western Reserve University - Molecular Medicine Program, Cleveland, OH, USA
3 University of Connecticut – Neuroscience Department, Farmington, CT, USA

Email: jzhou@uchc.edu

Abstract:

Reticulons are a family of proteins critical for the shaping of endoplasmic reticulum (ER). In the central nervous system, reticulon 1 (Rtn1) and reticulon 3(Rtn3) are critical in the formation of tubular ER found in axons. Tubular ER may play a role in vesicular transport, as well as cell to cell communication, but the role of tubular ER on axonal development is currently unkown. In our recent study of mice with double-knockout of reticulon 1 (RTN1) and RTN3 (R1R3dKO mice), we found neurofilament light chain (NFL), an intermediate filament found in axons, was missing or significantly reduced in a regiospecific manner. In fact, mice deficient in only RTN1 or RTN3 have no discernible phenotypes, suggesting the redundant functions of these two proteins. Thus, it appears that in neurons, both of these proteins are expressed and required to orchestrate neurofilament organization. Electron microscopy further revealed a significant reduction in tubular ER and axon size in areas without NFL. Furthermore, R1R3dKO mice are almost entirely penetrant for perinatal lethality most likely due to reduced acetycholine receptors found in the diaphragm of these mice. Together this suggests that neurospecific reticulons play a critical role in development of mammalian tubular ER formation, neurofilament organization, and axonal morphology.
Support:
Alzheimer’s Association (AARF-17-504724) to Dr Riqiang Yan.

Poster Session 2 / No. 21

Evoked Cortical Depolarizations Before and After the Amyloid Plaque Accumulation

Mei Hong Zhu, Aditi H. Jogdand, Brati Das, Riqiang Yan and Srdjan D. Antic; Neuroscience, UConn Health, School of Medicine, Farmington, Connecticut, 06030, USA.

Email: mzhu@uchc.edu

Abstract:

In Alzheimer's disease (AD), synaptic dysfunction is thought to occur many years before the onset of cognitive decline. Detecting synaptic dysfunctions at the earliest stage of AD would be desirable in both clinic and research settings. Knowing what these changes are and how to detect them, may be useful for development of timely preventive/causal therapies. Here, we asked if changes in brain function could be detected in the AD model mice. To measure synaptically evoked depolarizations, we expressed fluorescent voltage indicators in nerve cells of the AD model mice. Population voltage imaging allows monitoring of synaptic depolarizations, to which calcium imaging is relatively blind. We developed an AD mouse model (APPswe/PS1dE9 background) expressing a genetically encoded voltage indicator (GEVI) in the neocortex. GEVI was restricted to the excitatory pyramidal neurons (unlike the voltage-sensitive dyes that bind all membranes indiscriminately). The expression of GEVI was stable in both AD model mice and Control (healthy) littermates (CTRL) over 247 days postnatal. Around postnatal age 150 days (P150) and especially at P200, synaptically evoked voltage signals were weaker in the AD groups vs. the age- and sex-matched CTRL groups, suggesting an AD-mediated synaptic weakening that coincides with the accumulation of amyloid plaques. However, at the youngest ages examined, P40 and P80, the AD groups showed differentially stronger signals, suggesting "hyperexcitability" prior to the formation of plaques. From the evoked voltage waveforms, we extracted several parameters for comparison AD vs CTRL. Some parameters (e.g. temporal summation, refractoriness, and peak latency) were weak predictors, while other parameters (e.g. signal amplitude, attenuation with distance, and duration (half-width) of the evoked transients) were stronger predictors of the AD condition. Together, our results indicate subtle alterations in cortical physiology in AD model mice, occurring both prior (P40-80) and after (P150-200) the amyloid deposition. Voltage imaging showed that prior to formation of AD plaques, the evoked brain signals are slightly stronger (hyperexcitation). After the plaque accumulation, signals become slightly weaker (synaptic or circuit weakening). The new AD-GEVI mouse model allows for physiological investigations in the neocortex.

Support:
This research was funded by the Cure Alzheimer's Fund award (number N/A) to SDA and RY; the National Institute on Aging grant R21-AG064554 to SDA and RY; and the UConn Alcohol Research Center (ARC) / Kasowitz Medical Research Fund grant P50-AA027055 to SDA.

Poster Session 2 / No. 22

UNDERSTANDING HOW HUMAN NEURONS REGULATE UBE3A-ATS EXPRESSION

Dea Gorka[1,*], Jack S. Hsiao[1], Noelle Germain[1], Eric Levine[2], Stormy Chamberlain[1,3]

[1]Department of Genetics and Genome Sciences, UConn Health, Farmington, CT 06030-6403
[2]Department of Neuroscience, UConn Health, Farmington, CT, USA
[3]Institute for Systems Genomics, University of Connecticut, Storrs, CT 06269-3197

Email: gorka@uchc.edu

Abstract:

Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by motor dysfunction, intellectual disability, severe seizures, absent speech, and a happy demeaner. The disorder is caused by a deletion or mutation of the maternally inherited allele of *UBE3A*. In mature neurons, the paternally inherited allele of UBE3A undergoes tissue-specific silencing via genomic imprinting by the UBE3A antisense (UBE3A-ATS) transcript, leading to loss of UBE3A expression. The UBE3A-ATS transcript is initiated from the promoter of SNURF/SNRPN and normally terminated before UBE3A, however, in neurons the transcription extends to the UBE3A locus and silences UBE3A. Our goal is to identify the underlying mechanism of how the neuron-specific *UBE3A-ATS* transcript is regulated in a cell-type specific manner using AS patient derived induced pluripotent stem cells (iPSCs) and their neuronal derivatives as the model system. We have recently reported a bipartite chromatin boundary that stops the transcription of UBE3A-ATS in human pluripotent stem cells and therefore restricts UBE3A imprinting to mature human iPSC-derived neurons. This discovery revealed that UBE3A imprinting requires both the removal of boundary function as well as sufficient expression of UBE3A-ATS. We hypothesize that one part of this boundary, a pair of CTCF binding sites, is primarily responsible for the timing of UBE3A imprinted expression. We hypothesize that the expression level of UBE3A-ATS is regulated by increased usage of alternative SNRPN promoters in neurons. Here, we test these hypotheses using monolayer neuronal cultures as well as 3D cerebral organoids derived from AS iPSCs. We will furthermore determine the influence that altered timing of UBE3A imprinted expression has on the development of cellular phenotypes in human AS neurons. These data will provide important insights into AS therapeutics and the underlying physiological deficits in AS neurons.

Support: RO1 HD068730, R21 HD019823, Angleman Syndrome Foundation (ASF), CT DPH Stem Cell Research Program

Poster Session 2 / No. 23

PAD4 governs Muller cell citrullination and is a potential druggable target for Age-related Macular degeneration

Sarah I. Palko[1], Nicholas J. Saba[1], Benjamin D. Nicholas[2], Yosuke Nagasaka[3], Jayakrishna Ambati[2,3], Bradley D. Gelfand[2,3,4], Akihito Ishigami[5], Paola Bargagna-Mohan[1], and Royce Mohan[1]
[1]University of Connecticut Health Center, Farmington, Connecticut, 06032
[2]Department of Ophthalmology, University of Virginia Health System, Charlottesville, VA 22908
[3]Center for Advanced Vision Science, University of Virginia Health System, Charlottesville, VA 22901
[4]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908
[5]Molecular Regulation of Aging, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan

Abstract:

Retinal scarring with vision loss continues to be an enigma in individuals with advanced age-related macular degeneration (AMD). A major player in the development of fibrotic scarring in AMD are Müller glial cells, as they initiate and perpetuate reactive gliosis. During retinal gliosis, glial fibrillary acidic protein (GFAP) expression increases very early and rapidly undergo the posttranslational modification citrullination. Given that hypercitrullination may be associated with fibrotic disease, we investigated how citrullination temporally impacts Müller cell gliosis in the murine laser-induced model of AMD. We also used human AMD retinal tissue to examine hypercitrullination in Muller glia. Citrullinated species and the responsible enzyme peptidyl arginine deiminase-4 (PAD4) were localized to GFAP filaments early after laser injury, and hypercitrullination was sustained even 30 days post-injury. In glial-specific conditional PAD4 knockout mice subjected to injury, there was a stark reduction of citrullinated GFAP filaments and overall lesion size, as well as a reduction in fibronectin deposition in the sub-retinal space. Strikingly, the AMD human retinas showed hypercitrullinated forms of GFAP in the Müller end feet, indicating a role of the end feet in initiating and sustaining citrullination in the human disease. Taken together, these findings reveal that PAD4 mediates Müller cell-specific hypercitrullination and influences subretinal fibrosis.

THANK YOU for attending this year's retreat.

Special thanks to all trainees, judges and vendors for their time and contributions to this event.

We hope you enjoyed yourselves!

LOOK FORWARD TO SEEING YOU NEXT YEAR!

Spring 2023