UCONN HEALTH

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

PROGRAM & AGENDA

Wednesday, May 8, 2019
8:00 am – 6:15 pm

The Mark Twain House & Museum

351 Farmington Avenue

Hartford, CT 06105

UCONN HEALTH

April 30, 2019

Dear Neuroscience Program Faculty, Postdocs, Students and Guests,

Welcome to the 18th annual Neuroscience Program Retreat!

This year's event is again being hosted at the Mark Twain House in Hartford, which used to be a residence of Samuel Langhorne Clemens (Mark Twain), a great American writer. Mark Twain wrote several of his best-known books here. The Mark Twain House is recognized as a National Historic Landmark.

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

General

1. The address of the Mark Twain House is 351 Farmington, Hartford, CT 06105. There is space for free parking but please make an effort to carpool to save space for other retreat attendees and museum visitors.
2. **You must bring your university I.D.** to allow the Mark Twain House staff and other retreat participants to identify you. Your university I.D. will also entitle you to a 10%-discount at the Mark Twain gift shop.
3. The facility is air-conditioned. You might want to bring a sweater or light jacket in case you find the temperature unpleasant.
4. A **complementary tour** of the Mark Twain House will be available during the lunch break. If you are interested in this event, please sign up when you check in.
5. **Please print or carry an electronic copy of the program brochure with you to the meeting**. Only a printed agenda is available at the check-in desk.
6. Free WiFi will be available courtesy of the Mark Twain House. The WiFi password will be provided at the meeting.

Oral presentations - Oral presentations will be made in the auditorium. Speakers **must** upload their files before the session. All presenters will be using a single computer because the system at the Mark Twain house requires rebooting if computers are switched.

Poster presentations - There will be two poster sessions. Session 1 will be before lunch, Session 2 immediately after. All posters should be on boards during both poster sessions. However, each presenter is required to stand besides 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 a variety of criteria including but not limited to the 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.

Reception - There will be a light reception following the keynote address.

If you have any suggestions for improving the event for the next year, please let us know. We hope you will enjoy the day.

Sincerely,

Zhao-Wen Wang, Ph.D.
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

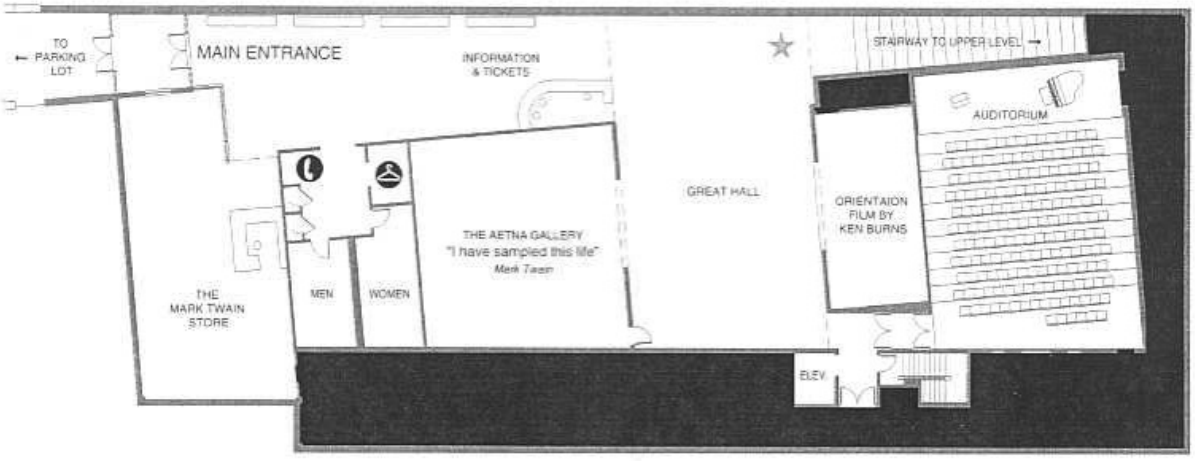
2019 Meeting Program

Time	Event	Location
8:00 – 8:45 am	**Registration & Sign-in** Continental Breakfast	Great Hall
8:45 - 9:00 am	**Welcome Address** Dr. Zhao-Wen Wang	
9:00 - 9:45 am	**Symposium A** *Moderator: Dr. Vishwanatha Rao*	Auditorium
9:45 - 10:00 am	*Coffee Break*	Great Hall
10:00 – 10:45 am	**Symposium B** *Moderator: Dr. Byoung-Il Bae*	Auditorium
10:45 – 12:00 pm	**Poster Session 1**	Classroom
12:00 - 1:30 pm	*Lunch & Tours*	Great Hall
1:30 - 2:45 pm	**Poster Session 2**	Classroom
2:45 - 3:00 pm	**Vendor Recognition Presentation**	Auditorium
3:00 - 3:30 pm	**Symposium C** *Moderator: Dr. Royce Mohan*	Auditorium
3:35 – 3:45pm	**Group picture**	Outside front entrance (staircase)
3:45 - 4:00 pm	*Coffee Break*	Great Hall
4:00 - 5:00pm	***Keynote Address:*** **Mark F. Bear, PhD** Picower Professor of Neuroscience The Picower Institute for Learning and Memory Department of Brain and Cognitive Sciences Cambridge, MA	Auditorium
5:00 – 5:15 pm	**Presentation of Poster/Oral Awards & Closing Remarks**	
5:15 - 6:15 pm	W*ine & Cheese Reception*	Outdoor Terrace*

*Weather permitting – alternate location: Great Hall

The Mark Twain House & Museum

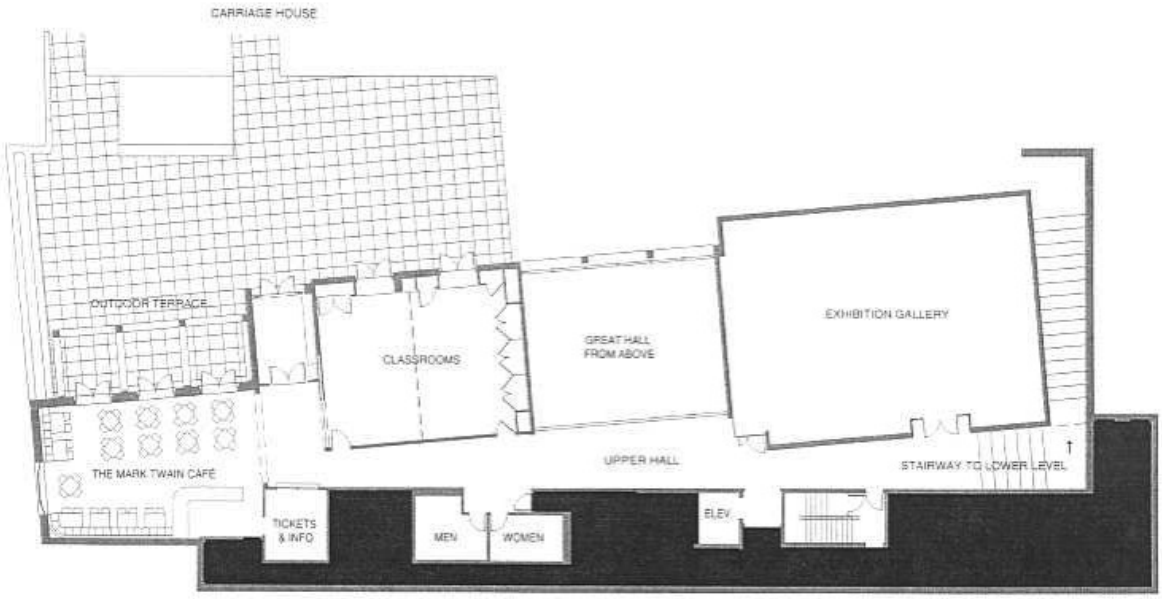
Lower Level / Main Entrance



★ TOURS START HERE

Upper Level

↑ TO THE MARK TWAIN HOUSE



3rd Floor–Research Library (By Appointment Only)

Special Thanks

To Our Corporate Partners & Vendor Supporters!

VENDOR REPRESENTATIVE(s) contact information (alphabetical order):

VENDOR	REPRESENTATIVE	CONTACT INFORMATION
BioRad	Julie Brunelle	Julie_Brunelle@bio-rad.com
BioTek Instruments	Jayme Phillips	phillipsj@BioTek.com
Brady Corp	Mark Bishop	mark_bishop@bradycorp.com
Corning	Max Appelt	appeltmt@corning.com
DWK	Soo Shin	Soo.Shin@dwk-lifesciences.com
New England Biolabs	Ryan Aruck	aruck@neb.com
Eppendorf	John Bee	bee.john@eppendorf.com
Fisher – UCONN Health & Storrs	Yen Lemire	yen.lemire@thermofisher.com
Fisher – UCONN Storrs	Shawn Rodriguez	shawn.rodriguez@thermofisher.com
Fisher – UHart & Trinity	Maria Adinolfi	Maria.Adinolfi@thermofisher.com
GE Healthcare	Kara Gallo	kara.gallo@ge.com
Thermo Fisher Scientific Life Science	Michelle Steele	Michelle.Steel@thermofisher.com
Thermo Scientific	Brian Nolan	brian.nolan@thermofisher.com
Thomas Scientific	Matt Kusiak	matthew.kusiak@thomassci.com
USA Scientific	Thomas Takacs	ttakacs@usascientific.com cell: 203-521-6282

Driving Directions to:

The Mark Twain House & Museum
351 Farmington Avenue
Hartford, CT 06105

From Springfield and Points North

Take I-91 South to I-84 West in Hartford. Take Exit 46, Sisson Avenue. At the traffic light, turn right (north) onto Sisson. Continue four blocks to the end of Sisson Avenue. At the traffic light, turn right (east) onto Farmington Avenue. Continue three blocks (approx. 2/10 mile) on Farmington and look for the sign on the right at the entrance to the museum's free parking lot. ***Note:*** The entrance to the parking lot is one block before The Mark Twain House.

From New York City, New Haven and Points South

Take I-91 North to I-84 West in Hartford. Take Exit 46, Sisson Avenue. At the traffic light, turn right (north) onto Sisson. Continue four blocks to the end of Sisson Avenue. At the traffic light, turn right (east) onto Farmington Avenue. Continue three blocks (approx. 2/10 mile) on Farmington and look for the sign on the right at the entrance to the museum's free parking lot. ***Note:*** The entrance to the parking lot is one block before The Mark Twain House.

From Waterbury and Points West

Take I-84 East to Hartford; take Exit 46, Sisson Avenue. At the traffic light, turn right onto Sisson. Continue four blocks to the end of Sisson Avenue. At the traffic light, turn right (east) onto Farmington Avenue. Continue three blocks (approx. 2/10 mile) on Farmington and look for the sign on the right at the entrance to the museum's free parking lot. ***Note:*** The entrance to the parking lot is one block before The Mark Twain House.

From Boston and Points East

Take I-84 West to Hartford; take Exit 46, Sisson Avenue. At the traffic light, turn right onto Sisson. Continue four blocks to the end of Sisson Avenue. At the traffic light, turn right (east) onto Farmington Avenue. Continue three blocks (approx. 2/10 mile) on Farmington and look for the sign on the right at the entrance to the museum's free parking lot. Note: The entrance to the parking lot is one block before The Mark Twain House.

For Passengers with Limited Mobility or LOADING DOCK

(65 Forest Street Entrance)

Follow the above directions, but continue east on Farmington Avenue and go past The Mark Twain House. Take your first right onto Forest Street and look for the sign on the right for the entrance to the Museum Center's rear parking lot.

KEYNOTE SPEAKER

“Synaptic Plasticity and Amblyopia.”

Mark F. Bear, PhD

Picower Professor of Neuroscience
The Picower Institute for Learning and Memory
Department of Brain and Cognitive Sciences
Cambridge, MA

Mark F. Bear received his Ph.D. in neurobiology from Brown University. He took postdoctoral training from Wolf Singer at the Max Planck Institute for Brain Research in Frankfurt, Germany, and from Leon Cooper at Brown. He joined the faculty of the Brown University School of Medicine in 1985 and was named a Howard Hughes Medical Investigator in 1996. At Brown, he was awarded the 2000 Elizabeth H. Leduc Award for teaching excellence in the life sciences, and the Class of 2000 Barrett Hazeltine Citation for teaching excellence. In 2003, he was appointed Picower Professor of Neuroscience at The Picower Institute for Learning and Memory in the Department of Brain and Cognitive Sciences at MIT.

How is the brain modified by experience, deprivation and disease?

Our overarching interest is in the question of how experience and deprivation modify synaptic connections in the brain. Experience-dependent synaptic plasticity is the physical substrate of memory, sculpts connections during postnatal development to determine the capabilities and limitations of brain functions, is responsible for the reorganization of the brain after damage, and is vulnerable in numerous psychiatric and neurological diseases and contributes to their symptoms.

Historically, our major efforts to address this question have been focused on the visual cortex and hippocampus. The visual cortex is a site of robust experience-dependent synaptic plasticity, exemplified by the consequences of temporary monocular deprivation (MD) during childhood. MD sets in motion a stereotyped choreography of synaptic modification whereby the deprived-eye inputs to visual cortex rapidly lose strength and, with a delay, the open-eye inputs undergo a compensatory gain in strength. The behavioral consequence of this plasticity is severe visual impairment in the deprived eye. In humans, this condition is called amblyopia, responsible for loss of vision in over 1% of the world population. Thus, the visual cortex is an excellent preparation to connect the elementary molecular mechanisms of synaptic plasticity to their behavioral consequences. Further, insights into how synapses depress or potentiate have potential clinical applications for the treatment of amblyopia.

The hippocampus is a cortical structure that is critical to forms of learning and memory. The simple cellular architecture of the hippocampus also makes it amenable to electrophysiological investigations of synaptic plasticity that are much more difficult in other parts of the brain. In the early 1990's we applied insights gained from a theoretical analysis of synaptic plasticity to establish a phenomenon called homosynaptic long-term depression (LTD). LTD is the functional inverse of long-term synaptic potentiation (LTP). Although LTD and LTP are expressed at synapses throughout the brain, they are particularly robust at the Schaffer collateral synapses in the CA1 region of hippocampus. The hippocampus is therefore an excellent preparation to dissect the molecular basis of bidirectional synaptic plasticity. Insights gained here cannot only be applied to synaptic modifications elsewhere in the brain, they are also relevant to understanding the basis of hippocampus-dependent memory storage and diseases of cognition.

In the course of studying LTD we made a discovery that has turned out to have major therapeutic significance for human developmental brain disorders that cause autism. One form of hippocampal LTD is triggered by activation of metabotropic glutamate receptor 5 (mGluR5) and requires immediate translation of mRNAs at synapses. In the course of studying this type of synaptic plasticity, we discovered that protein synthesis (and LTD) downstream of mGluR5 is exaggerated in the mouse model of fragile X (FX). Human FX is caused by the silencing of the FMR1 gene, and is the most common inherited form of intellectual disability and autism. Insight gained by the study of LTD suggested that exaggerated protein synthesis downstream of mGluR5 might be pathogenic, and contribute to many symptoms of the disease. Subsequent tests of the "mGluR theory" have shown that inhibition of mGluR5 can correct multiple mutant phenotypes in animal models of fragile X ranging from mouse to fruit fly. Human clinical trials were initiated based on the strength of this science, and results to date indicate that treatments can be developed to substantially benefit this patient population. The mGluR theory has contributed to a major paradigm shift that genetic diseases of brain development, historically viewed as untreatable, may be ameliorated or corrected with appropriate therapy.

Current work in the laboratory is focused on two related themes: (1) mechanisms and regulation of naturally occurring synaptic plasticity in visual cortex, and (2) pathophysiology of genetically defined developmental brain disorders. We primarily study mouse models, and we use a broad range of methods that include but are not limited to brain slice electrophysiology and biochemistry, in vivo electrophysiology and 2-photon functional and structural imaging, and behavioral analysis. Our lab is "question oriented" rather than "method oriented". We will apply any technology that is needed to address the questions of greatest interest.

Symposium A: Talk No. 1

Altered neuron morphology in Angelman Syndrome and Chromosome 15q Duplication Syndrome cell-derived neurons

Judy E. Bloom[1,2], Carissa L. Sirois[1,3], Noelle Germain[3], Stormy J. Chamberlain[3], Leslie M. Loew[2]
[1] Department of Neuroscience, UConn Health, Farmington, CT
[2] Richard D. Berlin Center for Cell Analysis and Modeling, UConn Health, Farmington, CT
[3] Department of Genetics and Genome Sciences, UConn Health, Farmington, CT

In mature neurons, *UBE3A*, which encodes for an E3 ubiquitin ligase, is only expressed by the maternal allele due to genomic imprinting. Loss of the maternal copy of *UBE3A* results in Angelman Syndrome (AS), a neurodevelopmental disorder characterized by absent speech, severe seizures, intellectual disability, motor dysfunction and a happy disposition. On the other hand, the duplication of the maternal region surrounding *UBE3A* results in Chromosome 15q Duplication Syndrome (Dup15q). Dup15q is the most common chromosome abnormality associate with autism spectrum disorder, but other common symptoms include developmental and motor skill delays and seizures. Mouse models of AS indicate that there is a reduction of dendritic spines, while a common characteristic of autism is an increase of dendritic spines in both human and mice. However, very little research has been done to understand how human neurons with these specific disease-causing mutations are affected. Here we use patient-specific induced pluripotent stem cells to examine the maturation of forebrain neurons in both of these disorders. We have developed fluorescent stem cell lines in order to examine the neurons as they mature during neuronal differentiation. High-quality images were acquired using confocal microscopy for multiple time points throughout development of AS, Dup15q, and control neurons. We found that by 12 weeks of age, AS neurons show a severe reduction of dendritic spines while Dup15q show an increase of spines compared to control. Additionally, both AS and Dup15q have smaller somas and reduced dendritic branching when compared to control. These deficits remain until at least 18 weeks in culture. These data provide a first glimpse of the development of human AS and Dup15q neurons. To further understand the role of UBE3A on the neuron morphology, we used an antisense oligonucleotide (ASO) to knock down levels of UBE3A in Dup15q and in control neurons. The control neurons treated with the ASO mimicked the phenotypes seen in AS neurons. While the Dup15q neurons treated with the ASO appeared to have a normal phenotype. These results suggest that UBE3A the regulation of UBE3A is critical for normal neuronal morphology. To further understand if these phenotypes are driven by intrinsic cellular defect or due to disease-related changes in the cellular environment, we mixed AS and control neurons. Fluorescent AS neurons grown on control cells showed a normal phenotype where the soma size, dendritic branching, and dendritic spine density was comparable to control neurons. However, the fluorescent control cells grown on AS cells showed the AS phenotype of reduction in soma size, branching, and dendritic spines. This suggests that the environment surrounding the neurons is the driving force behind these morphology changes.

Support: Connecticut Regenerative Medicine Fund & National Institute of General Medical Sciences (P41 GM103313).

Symposium A: Talk No. 2

Response of individual neurons in the hippocampus to novel events

Shang Lin (Tommy) Lee[1,2], Ryan Troha[1,2], Ian Stevenson, Ph.D.[1,2], Etan J. Markus, Ph.D.[1,2]

[1]Behavioral Neuroscience Division, Department of Psychological Sciences, University of Connecticut, Storrs, CT
[2]The Connecticut Institute for the Brain and Cognitive Sciences

Presenting author's e-mail address: Shang.Lee@uconn.edu

Abstract:
In both humans and rats, the hippocampus is important for memory and navigation. Individual hippocampal neurons are spatially tuned, and fire in specific physical locations. Together, these "place cells" provide a representation or map of an environment. However, if the environment or behavioral context is altered, a place cell can change its firing pattern or "remap". The hippocampus is a long structure with multiple sub-regions of diverse connections. Much is known regarding the place cells in the dorsal region of the hippocampus, since these are close to the surface of the brain and relatively accessible. Less is known about ventral hippocampal place cells, which are located much deeper. We have constructed a 64-channel hyperdrive, with 16 independently moveable tetrodes targeting both dorsal and ventral regions. Firing characteristics will be determined while recording cells simultaneously from both regions as freely-moving rats transverse a maze for a food reward. We are measuring how the spatial tuning of these neurons is modified when a rat experiences a novel event. By recording from neurons throughout the hippocampus, we can determine regional differences in the representation of change. Taken together, these results will lead to a better understanding of the neurobiological mechanisms of memory.

Support
The Crandall-Cordero Fellowship
CT Institute for the Brain and Cognitive Sciences (IBACS)

Symposium A: Talk No. 3

BACE1 Inhibition Effect on Astrocytic Amyloid Beta Clearance

John Zhou, Dr. Riqiang Yan

Body: Amyloid beta (Aβ) protein in the brain is regarded as the primary causative agent of Alzheimer's disease (AD). Increased production of Aβ or impaired clearance of Aβ results in the accumulation and aggregation of Aβ into insoluble, toxic plaques, which are surrounded by glia cells. Beta secretase 1 (BACE1) is the rate-limiting enzyme in the production of Aβ through its initial cleavage of amyloid precursor protein (APP). Because of this, inhibiting BACE1 as a means to reduce Aβ production has emerged as a major therapeutic goal for Alzheimer's disease. Recently, our lab has found that inhibiting BACE1 during adulthood reverses previously formed plaques and rescues AD-associated behavioral deficits. Since plaque reversal requires active glia clearance and reactive astrocytes surrounding plaques express BACE1, we hypothesize that BACE1 may have a previously unexplored role in astrocytic Aβ clearance. Using in vitro primary astrocyte culture, we found that BACE1 inhibition enhances both astrocytic Aβ uptake and degradation. Furthermore, using scRNA seq, we found that BACE1 knockout mice had an increased number of reactive astrocytes and these reactive astrocytes' from BACE1 knockout mice have transcriptomes distinct from reactive astrocytes from wild-type mice. Some of the gene signatures upregulated in BACE1 knockout astrocytes, such as apolipoprotein E (ApoE) and Clusterin (Clu), are known to play a role in clearing Aβ. A certain gene coding for a chemokine, C-X-C motif ligand 14 (CXCL14), was also upregulated in these BACE1-/-astrocytes, however CXCL14 function in the field of AD is unkown. Preliminary results show that CXCL14 is localized to the central core of Aβ plaques, which suggests that CXCL14 may act as an "eat me" signal for Aβ.

Support: This work is supported by NIH grants (AG025493, NS074256) and an award from Alzheimer's Association (NPSPAD-10-174543) to Dr Riqiang Yan.

Symposium B: Talk No. 4

Identifying alterations in satellite glial cell profile in response to cutaneous inflammation

Knight, BE.[1], Orozco, EE.[2], Young, EE.[3, 4, 5, 6], Baumbauer, KM.[1, 3, 4, 6]

Email: bknight@uchc.edu

[1]Department of Neuroscience, UConn Health,[2]Department of Skeletal Biology and Regeneration, UConn Health, [3]Center for Advancements in Managing Pain,[4]School of Nursing,University of Connecticut,[5]Genetics and Genome Sciences, UConn Health,[6]Institute for Systems Genomics

Within the dorsal root ganglia, the cell bodies of sensory neurons are ensheathed by satellite glial cells (SGCs). This close proximity between SGCs and sensory neurons suggests that SGCs may augment afferent activity, however the mechanism by which SGCs influence afferent function, and more specifically, nociceptive signaling is not well understood. As a first step in understanding the role of SGCs may play in the processing of noxious stimulation, we used flow cytometry (FC) to develop a cellular profile of SGC-related markers during naïve and inflamed states. Cutaneous inflammation was produced by injecting female C57BL/6 mice with complete Freund's adjuvant (CFA) or saline (s.c.) into the hairy skin of the right hind paw. Behavioral sensitivity to mechanical stimulation was assessed using von Frey filaments at baseline, 2, 7, 14, 21, and 28 days following inflammation. We found that CFA-treated mice developed hypersensitivity 7 days following, while mice injected with saline exhibited no changes in reactivity. When we isolated L1-L3 DRG 0, 2- and 7-days post-CFA for FC we found that, relative to naïve mice, inflamed mice showed increased expression of glial fibrillary acidic protein (GFAP), and the calcium sensing protein, S100, 2 days following inflammation. Seven days following inflammation we observed a decrease in GFAP, an increase in the glutamate converting enzyme, glutamine synthetase (GS), as well as a continued elevated level of S100. These data suggest that peripheral inflammation alters SGC-related markers in a temporally-specific manner that may contribute to the emergence of hypersensitivity.

Supported by NS096454 (KMB)

Symposium B: Talk No. 5

The Aging Bladder Phenotype: Evolution of an Adaptive System

Hardy, Cara C.[1,2,5], Keilich, Spencer R.[1,3], Harrison, Andrew G.[1], Knight, Britt E.[2], Baker, Dylan S.[1], Smith, Phillip P[1,4,5]*.

[1]Center on Aging, UConn Health, University of Connecticut SOM, Farmington, CT
[2]Department of Neuroscience, UConn Health, University of Connecticut SOM, Farmington, CT
[3]Department of Immunology, UConn Health, University of Connecticut SOM, Farmington, CT
[4]Department of Surgery, UConn Health, University of Connecticut SOM, Farmington, CT
[5]Institute for Brain and Cognitive Sciences, University of Connecticut, Storrs, CT

Abstract:
The prevalence of urinary dysfunction increases with age, yet current therapies are suboptimal. Incomplete understanding of the linkages between system, organ, and tissue domains across lifespan remains a knowledge gap. If tissue-level changes drive the aging bladder phenotype, consistent inter-domain correlations should be observed regardless of age. Few inter-domain correlations especially in younger animals would support the hypothesis that urinary performance is a measure of physiologic reserve derived from centrally-mediated adaptive mechanisms. Male and female mice across four age groups underwent sequential voiding spot assays, pressure/flow cystometry, bladder strip tension studies, histology, and qPCR analyses. The primary objective of this study was to test the impact of age on the cortical, autonomic, tissue functional and structural, and molecular domains, respectively, and identify inter-domain correlations among variables showing significant changes with age within these domains. Behavior revealed diminished peripheral voiding and spot size in aged females. Cystometry demonstrated increased micturition interval and post-void residual, and loss of volume sensitivity with preservation of voiding contraction strength, with almost half of oldest old mice failing under cystometric stress. Strip studies revealed no significant differences in adrenergic, cholinergic, or EFS sensitivity. Histology showed increased detrusor and lamina propria thickness, without change in collagen/muscle ratio. *Adrb2* gene expression decreased with age. No consistent inter-domain correlations were found across age groups. Our findings are consistent with a model in which centrally-mediated adaptive failures to aging stressors are more influential over urinary resilience outcomes than local tissue changes.

Funding:
K76 AG054777-01 Beeson Emerging Leaders Career Development Award in Aging (NIA)
The Connecticut Institute for the Brain and Cognitive Sciences, Seed Grant

Symposium B: Talk No. 6

PAD4: A Potential Target for Gliosis in Age Related Macular Degeneration

Sarah Palko; Nicholas Saba; Megan Rouillard; Paola Bargagna-Mohan; Royce Mohan
Neuroscience, University of Connecticut Health Center, Farmington, CT, United States

Introduction: Citrullination is the post-translational modification (PTM) involving the conversion of peptidyl arginine residues to citrullines. Citrullination is catalyzed by peptidyl arginine deiminases (PADs) and acts on several different proteins including glial fibrillary acidic protein (GFAP) and vimentin. This PTM has long been implicated in disease, and recent data suggests its involvement in armd(AMD). Citrullination has been shown to increase in the retinas of AMD patients, though PAD2 was not linked to the increased citrullinated protein. Previously, we showed that PAD4, specifically, was responsible for increased citrullination of GFAP filaments in an alkali injury model of retinal gliosis. In this study, we have tested whether PAD4 is expressed in the laser-injury model of retinal gliosis, emulating AMD. Methods: Four to six laser lesions targeted to the retinal pigment epithelium were created using the Meridian Merilas 532 nm laser coupled to the Micron III fundus imaging system (Pheonix Research Laboratories). Both male and female 4-6 month old C57B1/6 mice were used and sacrificed at various time points. Tissue cryosections of eyes were collected and stained with antibodies against PAD4, F95 (citrullinated protein), and GFAP, along with DAPI to stain nuclei. Fluorescent secondary antibodies were applied to stained sections and analyzed by epifluorescence microscopy. A low salt buffer was employed to extract soluble fraction (non-cytoskeletal) from control and 7-day post injury eyes, and both the soluble and insoluble cytoskeletal fractions were subjected to western blot analysis. Results: We found colocalized staining of GFAP filaments and citrullinated targets along the Muller cell processes in retinas 7 days post injury, which was abundant in the inner retinal layers. In addition, we also observed similar colocalized staining of GFAP and PAD4 in the Muller Glia 7 days post injury in the lesion site. Interestingly, PAD4 and citrullinated targets that co-localize with GFAP filaments occur in proximity to the nerve fiber layer despite injury the posterior end of the retina. This citrullination pattern follows the temporal induction of GFAP filament polymerization that starts at the Muller end feet and progresses towards the cell soma. Furthermore, western blotting revealed an increase in PAD4 and GFAP abundance in the insoluble cytoskeletal extracts from injured eyes, whereas, citrullinated protein of the size of vimentin increased in soluble protein extracts. Conclusions: Western blot and immunofluorescence staining results suggest that PAD4 is likely responsible for increased citrullination. Moreover, increased citrullination leads to changes in intracellular distribution of targeted proteins and potentially could cause dynamic changes in regulation of how intermediate filament polymerization is governed during gliosis. Because PAD4 was induced and a key player in the citrullination axis, this enzyme is a potential target for AMD, as it provides an important druggable target and biomarker of reactive gliosis in the retina.

Support: NIH R21EY028699; John A. and Florence Mattern Solomon Endowed Chair

Symposium C: Talk No. 7

Can we find signs of AD in the auditory system using stimulus evoked potentials?

A. L. Burghard, C. M. Lee, D. L. Oliver

The auditory system is our fastest and temporally most precise sense. It can decipher timing differences down to the µs scale. All the more puzzling is the existence of neurons in the auditory system, which – when stimulated strong enough – continue to fire for seconds to minutes even after the stimulus presentation has stopped. These neurons were discovered by Ono et al. (2016) in the inferior colliculus (IC), a major hub in the auditory system. The IC receives ascending inputs from lower brainstem nuclei and descending inputs from the cortex, and sends both excitatory and inhibitory projections to the thalamus. The long-duration after-discharge (AD) is present in both excitatory and inhibitory neurons. The study by Ono et al. used single-unit recordings and estimated that about 30% of the neurons in the IC exhibit AD behavior. Their role in auditory processing, however, is unknown.

The goal of the present study was to determine, if and in which manner the AD firing has an influence on successive sound-evoked responses. We used multi-channel single shank electrodes (16 channels/shank) to record multi-unit activity in the IC of anaesthetized mice to learn more about the AD phenomenon. This approach allowed us to record from several frequency laminae in the IC simultaneously. We recorded both spontaneous as well as stimulus driven activity before and after the presentation of a long duration sound (≥ 60s).

We found that AD firing altered both the spontaneous activity and the stimulus-evoked activity. The altered spontaneous activity is of special interest in the context of tinnitus, a disease that is defined as the perception of a sound in the absence of an external sound stimulus. We, therefore, plan to compare AD behavior in animals with tinnitus to control animals without tinnitus. The altered stimulus-evoked activity might provide further insight in basic sound and/or speech processing. Most of our daily life/communication happens in a noisy environment where long-duration sounds precede important auditory cues, with sometimes overlapping frequency content.

This study was supported by DM170509

Symposium C: Talk No. 8 - presentation cancelled

Release of GABA from Nigro-Striatal Dopaminergic Projections

Lu Li*, Nagaraj D. Halemani*, Eric. McKimm, Jinyoung Jang, M. Milosevic, Michal Ragan And SD Antic (* Li and Halemani contributed equally)

Dept. of Neuroscience, UConn Health. Farmington. CT.

Dopaminergic axons, originating in the substantia nigra (SNc) and terminating in the striatum, constitute the nigro-striatal pathway critically regulating motor and cognitive functions. Nigro-striatal fibers synapse on to GABAergic medium spiny neurons (MSN), and have been shown to release GABA from dopamine DA fibers (DA-GABA). In the present study, we have used DAT-ChR2 expressing mice, optogenetic stimulation and electro-physiology to study the properties of the DA-GABA synaptic transmission in striatum.

MSNs in the ventral striatum were relatively more depolarized with higher input resistance and lower light response compared to MSNs in the dorsal striatum.

Optogenetically-induced DA-GABA transmission exhibited a relative refractory period of ~10 seconds. By measuring optogenetically induced depolarizations in DA neurons, we ruled out ChR fatique as a possible cause for refractorness of DA-GABA transmission.

GABA-A receptor antagonist (gabazine) and sodium channel antagonist (tetrodotoxin) completely blocked DA-GABA transmission in MSNs, whereas blocking AMPA-R & ACh-R (using DNQX or carbachol) or stimulating with exogenous BDNF did not affect the amplitude and duration of evoked DA-GABA potentials in MSN or the refractory period.

Our findings points to dominant effect of GABAergic input onto MSNs with a minimal modulatory influence of glutamate, BDNF and Ach.

This work is supported by MH109091 and NS 099573

Symposium C: Talk No. 9

Role of acute P2X4 blockade on long-term recovery in ischemic stroke

Pranay Srivastava[#], Chunxia G Cronin[*], Victoria L Scranton[#], Kenneth A. Jacobson[†], Bruce T Liang[*] and Rajkumar Verma[#]

#Department of Neuroscience, UConn Health, Farmington, CT 06032, USA
*Calhoun Cardiology Center, UConn Health, Farmington, CT 06032, USA
†National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20892-0810, United States

Abstract:
Ischemic stroke is one of the leading causes of death and disability in humans. Several approaches have been employed to minimize the damage and restore functional recovery; however, limited availability of treatment options warrants exploration of robustly validated pharmacological targets. P2X4, a purinergic receptor for adenosine triphosphate ATP, regulates both the activation of myeloid immune cells (infiltrating monocytes/macrophages and brain-resident microglia) and synthesis of cell supportive factors. Following stroke, there is an excessive ATP release from the dying or damaged neuronal cells leading to over-stimulation of P2X4R that further exacerbates ischemic injury. Hence we hypothesize that acute pharmacological blockade of P2X4R will restrict acute immune response of myeloid cells and contribute to stroke recovery both acutely and chronically. 8-12 weeks old C57BL/6 wild type mice of both sexes were subjected to a 60 min right middle cerebral artery occlusion (MCAo) followed by 3 or 30 days of reperfusion. Mice were randomly divided in two groups – Group I-Vehicle (0.5% methyl cellulose, PO x 3 days post 4 hr. of MCAo); Group II (P2X4R antagonist 5-BDBD (1mg/kg PO daily x 3 days post 4 hr. of MCAo). Neurobehavioral end points (Open field and Rota-rod) were performed at day 2, 7, 14, 21 and 30 while social interaction test and tail suspension test were performed at day 30. Neurochemical estimation (western blot and ELISA) was performed at day 3 and 30. Blood Brain Barrier (BBB) permeability and infiltration of peripheral immune cells were measured at day 3 with Evans blue dye extravasation and flow cytometry respectively. 5-BDBD treatment significantly (*P<0.05) reduced the infarct volume, neurological deficit (ND) score, blood brain barrier (BBB) permeability and total leukocyte and myeloid cell infiltration at 3 days after stroke and showed a progressive motor recovery (*P<0.05) and reduction in anxiety like behavior during long term recovery. We also observed a significant (*P<0.05) increase in the level of neurotrophic factor BDNF and anti-inflammatory protein annexin A1 at day 30 after 5-BDBD treatment suggesting its plausible mechanism in post stroke rehabilitation. Hence, acute P2X4R inhibition protects against ischemic injury at both acute and chronic time point after stroke by regulating the acute immune response and increasing the levels neurotropic factors to support neuroprotection and neurorehabilitation.

Support: This work was supported by AHA Career Development award 18CDA34110011 (to Rajkumar Verma). The authors declare no competing financial interests.

Poster Session 1 / No. 1

Chimera RNAi Knockdown of γ-Synuclein in Human Cortical Astrocytes Results in Cellular Arrest and Apoptosis.

*Timmy Le,[1] Fotis Andreo,[1] Brooke MacKinnon,[1] Melanie Shaffer,[1] Adam Silver, Ph.D.,[1] Evan Jellison, Ph.D.,[2] Andrew Koob, Ph.D.[1]

[1] Neuroscience Program, Biology Department, University of Hartford, West Hartford, CT
[2] Immunol., Univ. of Connecticut Hlth. Ctr., Farmington, CT.

*Corresponding Author, TLe@hartford.edu

Elevated levels of γ-Synuclein (γ-syn) expression have been noted in the progression of glioblastomas, and also in the cerebrospinal fluid of patients diagnosed with neurodegenerative disease. In the human cortex, γ-syn is expressed by astrocytes, and its function in relationship to these two opposing disease states is not well understood. Previous studies have shown that lentiviral-delivered γ-syn siRNA can effectively diminish cellular proliferation in breast cancer cell lines. Therefore, in order to gain a better understanding of the endogenous role of γ-syn, primary human cortical astrocytes were treated with chimera RNAi targeting γ-syn after release from cell synchronization. qPCR revealed increased mRNA levels of endogenously expressed γ-syn at 48hrs after release, which was effectively knocked down with the introduction of chimera RNAi. Additionally, γ-syn protein expression was also diminished after chimera RNAi treatment as demonstrated by western blot at 48hrs post release from cell synchronization. In conjunction with decreased γ-syn expression, 5-Bromodeoxyuridine (BrdU) and Ki67 analysis showed decreased astrocytic cellular proliferation in cells treated with RNAi. Coinciding with decreased cellular proliferation, an elevated expression of histone H3 pSer10 (pHH3), but not cyclin dependent kinase 2 pTyr15 (pCDK2) after RNAi treatment was observed, indicating M-phase, but not G1/S cellular arrest. To further investigate the effect of M-phase cellular arrest in astrocytes after γ-syn RNAi, cells treated with propidium iodide were analyzed via flow cytometry for phases of the cell cycle and apoptosis. RNAi treatment resulted in increased apoptotic cells 48 hours after release relative to 24hrs, while there were no significant differences in apoptotic cells in the control groups between 24 and 48hrs. Additionally, western blot confirmed increased cleaved caspase-3 in RNAi treated astrocytes when compared to the vehicle control at 48hrs. Therefore, γ-syn exerts its effect at M-phase to allow normal astrocytic progression through the cell cycle, as evidenced by decreased proliferation marker expression, increased pHH3, activated caspase-3, and apoptotic cells after RNAi knockdown. Since cortical astrocytes retain proliferative capabilities to maintain the cellular environment, aberrant γ-syn levels would likely contribute to or be a consequence of degeneration.

Financial Support: Funding provided by the Partnership in Innovation and Education Fellowship awarded to T. Le, the University of Hartford Neuroscience Program, and the College of Arts and Sciences Dean's Fund for Research and Creativity.

Poster Session 1 / No. 2

The Combined Treatment of α-Synuclein and γ-Synuclein on Human Cortical Astrocytes

Andromidas F, Le T, MacKinnon B, Silver AC and Koob AO
Neuroscience Program, Biology Department, University of Hartford

Abstract:
Exogenous γ-synuclein (γ-syn) treatment of human cortical astrocytes has previously been shown to cause cellular proliferation and subsequent protective brain derived neurotrophic factor (BDNF) release. Additionally, knockdown of endogenous γ-syn resulted in cell cycle arrest and apoptosis in human cortical astrocytes. In the same protein family, neuronally derived α-synuclein (α-syn) can be removed from the extracellular space and degraded by astrocytes, while aggregated α-syn induces neurodegeneration. Likewise, astrocytes in tissue culture exposed to monomeric and aggregated α-syn at certain concentrations undergo cellular stress and astrogliosis. This study tested the astroprotective effects of γ-syn with the neurodegenerative effects of α-syn simultaneously. To ensure the effects observed were due to extracellular treatment, qPCR analysis was performed for endogenous astrocytic γ-syn mRNA levels. qPCR revealed no significant differences in γ-syn mRNA levels after treatment of astrocytes with α-syn alone or α-syn/γ-syn combined after 24 hours. Immunocytochemistry studies then demonstrated that α-syn/γ-syn treatment increased overall cell numbers, while α-syn alone had no influence on cellular proliferation. Also, astrocytes treated with α-syn alone initiated astrogliosis as evidenced by the percent of cells expressing glial fibrillary acidic protein (GFAP), while α-syn/γ-syn reduced astrogliosis, with the result no different than controls. Further analysis of ALDH1L1, a marker not associated with injury or disease, demonstrated no differences across control and treatment groups. However, lactate dehydrogenase (LDH) analysis also exhibited no differences after 48 hours when comparing treatment groups and controls. These initial results suggest that γ-syn induced proliferation is likely not affected by α-syn, but that α-syn induced astrogliosis is diminished by γ-syn introduction. Future studies will further examine synuclein combination treatments to determine if the known effects of α-syn on autophagy and apoptosis pathways are also influenced by γ-syn.

Funding Sources: University of Hartford Dean's Research and Teaching Funds, and the Neuroscience Program

Poster Session 1 / No. 3

Vesicle-associated membrane proteins in NG2 glia are necessary for proper myelination of the spinal cord

Robert Z. Horning, Akiko Nishiyama

Email: robert.horning@uconn.edu

NG2 glia, or oligodendrocyte precursor cells (OPCs), are vital for the myelination of axons in the brain and spinal cord. Many NG2 receive synaptic inputs from neurons through glutamate and GABA (1,2,3), but whether these cells are capable of bidirectional communication remains unknown. NG2 cells express voltage-dependent Ca^{2+} channels and transcripts encoding SNARE proteins that are necessary for vesicle docking in neurons. We therefore hypothesized that NG2 cells respond to neuronal synaptic inputs by releasing bioactive molecules in a Ca^{2+}-dependent manner. To test this, we generated transgenic mice in which BoNT/B-IRES-EGFP is expressed in NG2 cells. We crossed IBOT mice (4), which express a cre-inducible botulinum toxin, with NG2cre mice (5). In these mice, BoNT/B cleaves vesicle-associated membrane proteins (VAMPs), thereby inhibiting Ca^{2+}-dependent exocytosis or any other VAMP-dependent processes in NG2 cells and their progeny. IBOT:NG2cre double transgenic mice exhibited a robust phenotype, with severe motor defects, hypomyelination of the spinal cord, and a significant deficit in oligodendrocyte population density, suggesting an essential role of VAMPs and potentially exocytotic activity in NG2 cells in myelination.

Source of financial support: NMSS Pilot Award PP-1809-32554, VAMP2-mediated exocytosis in NG2 cells in needed for myelination

References:
(1) Bergles, DE, Roberts, JD, Somogyi, P Jahr, CE. 2000. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405, 187-91.
(2) Ge, WP, Yang, XJ, Zhang, Z, Wang, HK, Shen, W, Deng, QD Duan, S. 2006. Long-term potentiation of neuron-glia synapses mediated by Ca^{2+} -permeable AMPA receptors. Science 312, 1533-7.
(3) Lin, SC Bergles, DE. 2004. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat Neurosci 7, 24-32.
(4) Slezak, M, Grosche, A, Niemiec, A, Tanimoto, N, Pannicke, T, Munch, TA, Crocker, B, Isope, P, Hartig, W, Beck, SC, Huber, G, Ferracci, G, Perraut, M, Reber, M, Miehe, M, Demais, V, Leveque, C, Metzger, D, Szklarczyk, K, Przewlocki, R, Seeliger, MW, Sage-Ciocca, D, Hirrlinger, J, Reichenbach, A, Reibel, S Pfrieger, FW. 2012. Relevance of exocytotic glutamate release from retinal glia. Neuron 74, 504-16.
(5) Zhu, X, Bergles, DE Nishiyama, A. 2008. NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135, 145-57.

Poster Session 1 / No. 4

Modulation Effects of Anti-aging Protein Klotho in Brain Bioenergetic Response

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

Background: Brain cells are highly energy demand for maintaining ion and synapse homeostasis. Impairment of brain metabolism and elevation of oxidative stress is a significant cause of cognitive abnormalities of Alzheimer disease (AD). Targeting both oxidative stress and mitochondrial energy metabolism may be therapeutically efficacious for the treatment of AD. Klotho (KL) is identified as an anti aging factor during normal and disease state. In the brain, KL is expressed in the choroid plexus and hippocampus and other regions of neurons but its function remains to be fully understood. As a type 1 transmembrane protein, full length KL1 is located in the cell membrane and binds FGFR1 to form a co-receptor complex for mediating FGF signal transduction. Full length KL can be cleaved by α-secretases at two ecotdomain sites: one close to the juxtamembrane region to release its secreted ectodomain (~130 kDa) called KL^{ECTO}, which lacks both transmembrane and intracellular domains; another between the KL1 and KL2 domains, releases KL1, KL2 two fragments (~65 kDa each). KL has a naturally spliced isoform which contains the entire KL1 domain and a distinct C-terminus coded from the alternative axon. This spliced form, named sKL, has approximately the same molecular weight (~65 kDa), similar to KL1.

Our hypothesis is that different KL variants differentially influence brain energy metabolism in the cell specific manner. We intend to accomplish two objectives:1) evaluate the modulation effects of KL on Neuro-2a, neuron and astrocytes bioenergetic response. 2) elucidate the roles of each KL variants and how they would affect the metabolism.

Methods: Neuro-2a and Astrocytes were exposed to recombinant KL for 24hrs, cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in a Seahorse XF-96 extracellular flux analyzer (Seahorse Biosciences-Agilent). Plasmids DNA expressing KL, sKL, KL1, KL2, KL^{ECTO} were tested in cultured Neuro-2a cells for signaling studies.

Results: 1) For cellular metabolism studies, we found that Neuro-2a cell exhibits elevation of OCR upon recombinant KL treatment. KL appears to be rerounting pyruvate through Krebs, which would increase mitochondria respiration and produce more ATP. For astrocytes, KL treatment lead to reduce OCR but elevate ECAR, suggesting that KL shifts the astrocytes to be glycolytic, which produce more lactate to nourish neurons. 2) For signaling studies, we first analyzed the effect of KL variants on AMPK, a master of energy sensor and its down-stream effector acetyl CoA carboxylase (ACC) in Neuro-2a cell. Overexpression of KL variants, AMPK and ACC phosphorylation were elevated. And its upstream effector LKB1 is activated. We next analyzed the effects of klotho on mitochondrial bioenergetics signaling PI3K-AKT-GSK3β axis, we observed that phospho-PI3K, phospho-Akt, phospho-GSK3β were activated, indicating KL would regulate the mitochondria bioenergetics. We also found that phospho-Erk1/2, phospho-CREB,phospho-p38a were activated, whereas phospho-PDK1 were unchanged upon overexpression KL variants.

Conclusion: This study indicated multifacet roles of KL: 1) the modulation effect of KL is cell type dependent. 2) distinctive forms of KL variants would elicit different signal event. Our future study will determine whether KL will impact AD metabolism.

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

Poster Session 1 / No. 5

Regulation of SLO-2 potassium channel function by a molecular pathway of RNA-editing in *C. elegans* nervous system

Long-Gang Niu*, Ping Liu, Zhao-Wen Wang, Bojun Chen
Department of Neuroscience, UConn Health, Farmington, CT 06030

*Presenter email: niu@uchc.edu

Slo2 channels are a family of large-conductance potassium channels existing in both vertebrates and invertebrates. They are widely expressed in the nervous system and play important roles in shaping neuronal action potential firing pattern and regulating neurotransmitter release. However, very little is known how their function and expression are regulated *in vivo*. In this study, we performed a forward genetic screen for mutants that suppress a sluggish phenotype of a *C. elegans* strain expressing an engineered hyperactive SLO-2 channel. Mutants of the *adr-1* gene were thus isolated. *adr-1* encodes a catalytically inactive *a*denosine *d*eaminase *a*cting on *R*NA (ADAR) protein, and plays a regulatory role in RNA editing through working together with the catalytically active ADR-2 to cause changes in coding potential or expression levels of specific genes. Our electrophysiological recordings show that SLO-2-mediated whole-cell currents of motor neurons are significantly reduced in *adr-1 loss-of-function* (*lf*) mutants. Intriguingly, no editing events were found in *slo-2* transcripts, and *adr-1* mutants did not alter expression level of *slo-2* transcripts, suggesting that ADR-1 may regulate SLO-2 function by acting on a different molecule. To search for this putative molecule, we compared RNA-Seq data between wild type and an *adr-1* knockout mutant that was made by using the CRISPR/Cas9 approach. We found that mRNA level of the gene *W07G4.3*, which encodes an ortholog of human SCY1-like pseudokinase 1 (SCYL1), was significantly reduced in *adr*-1 mutants compared with wild type. Furthermore, we found that a specific nucleotide in the 3'UTR of *W07G4.3* RNA was edited much more frequently in wild type than in *adr-1* mutants. Importantly, our electrophysiological data show that *W07G4.*3 is required for normal SLO-2 function in neurons. Taken together, this study uncovers a novel molecular pathway that regulates SLO-2 function through RNA editing.

Supported by NIH GM113004 (BC), NIH R01MN085927 (ZWW).

Poster Session 1 / No. 6

Nociceptor Transcriptional Changes and Chronic Pain following Spinal Cord Injury

Jessica R. Yasko[1], Richard E. Mains[1]

[1] *Department of Neuroscience, UConn Health, Farmington, CT*

Email: jyasko@uchc.edu

Traumatic spinal cord injury (SCI) has devastating implications for patients, including a high prevalence for chronic pain. It is likely that pain develops within weeks to months after injury, or perhaps even earlier, and that many patients are being treated after the development of pain has already begun. Nociceptors, sensory neurons that transduce painful stimuli into neuronal signals, are located within the dorsal root ganglia (DRG) and are thought to play an important role in chronic pain. However, the diversity of this population has made it challenging to better understand mechanisms and to attribute pain modalities to specific cell populations. The net result following SCI is an increase in neuronal activity, which leads to changes in gene expression. To begin to address how peripheral sensory neurons near the site of injury may contribute to persistent pain, we examined SCI-induced changes in gene expression in DRG distal to the site of injury. To identify the targeted population of nociceptors, we retrograde labeled sensory neurons that project to the hairy hindpaw skin with wheat germ agglutinin (WGA) conjugated to an AF-488 dye. The neurons were labeled 2 days prior to SCI by injecting the sural, common peroneal, and saphenous nerve skin territories. For SCI, a laminectomy was performed at the T10 vertebral level, and injury was produced by a vessel clip with a closing pressure of 15g for 1 minute. Lumbar DRG L2-L6 were collected 4 days post-SCI, dissociated, and WGA-488 labeled cells were purified by fluorescence-activated cell sorting. RNA was extracted from the sorted cell populations of naïve, sham, or SCI treated mice for RNA sequencing. FPKM values validate that we successfully isolated the desired specific population of nociceptors. DESeq2 analysis shows significant gene changes in SCI vs. sham or naïve cell populations that may contribute to the onset of chronic pain.

Funding:
DK032948 (Mains and Eipper)
UConn Graduate School

Poster Session 1 / No. 7

Exploring the Role of Rationally Designed Synthetic Peptides from CX3CL1 in Adult Neurogenesis

Manoshi Gayen[1], Riqiang Yan[1]

Department of Neurosciences, University of Connecticut Health Center, Farmington, CT 06030.

Email:gayen@uchc.edu

Abstract:
Extensive neuronal and synaptic degeneration contribute to the memory impairment, cognitive dysfunction, and behavioral deficits in Alzheimer disease (AD). Adult neuronal progenitor stem cell (NSPC) niche in the hippocampal brain region undergo differentiation and proliferate into new neurons under the influence of various intrinsic signaling factors. Fracktalkine, CX3CL1 is a chemokine predominantly expressed on the neuronal cell surface while its only receptor CX3CR1 is expressed by microglia. The crosstalk mediated by CX3CL1/CX3CR1 axis was suggested to regulate adult neurogenesis. However, our recent studies have shown that the intracellular C terminal domain of CX3CL1 (CX3CL1-ct), that does not interact with the receptor, has an inherent ability to induce various signaling pathways that led to enhanced adult neurogenesis. In this study, we explore the therapeutic potential of synthetic peptides designed from CX3CL1-ct sequence to trigger neurogenesis and compensate for the neuronal loss in AD.

Support:
This work is supported by funding from NIH grant awarded to Dr. Riqiang Yan.

Poster Session 1 / No. 8

Peptidergic signaling in *Chlamydomonas*

Raj Luxmi[1], Dhivya Kumar[2], Crysten E. Blaby-Haas[3], Richard E. Mains[1], Stephen M. King[2] and Betty A. Eipper[1,2]

Departments of [1]Neuroscience and [2]Molecular Biology and Biophysics, University of Connecticut Health Center, Farmington, CT, USA and [2]Brookhaven National Laboratory, Upton, NY, USA.

Email: luxmi@uchc.edu

Peptide-based communication is an ancient system that regulates a wide range of physiological and behavioral processes. Bioactive peptides are typically stored in secretory granules and released in response to a stimulus. Even the genomes of animals that lack neurons and muscles encode proteins that resemble preproneuropeptides and the post-translational processing enzymes needed to convert these precursors into typical bioactive products. We previously identified putative prepropeptides and these same processing enzymes in a unicellular green alga, *Chlamydomonas reinhardtii*. Although the secretory pathway in *C. reinhardtii* shares much in common with other eukaryotes, secretory granules are not present. Studies in animals revealed a role for peptide processing enzymes like peptidylglycine α-amidating monooxygenase (PAM) in secretory granules. PAM converts peptidylglycine substrates produced by subtilisin-like prohormone convertases and carboxypeptidases into the amidated products stored in granules. Our identification of a very similar, catalytically active PAM protein (CrPAM) in *C. reinhardtii* revealed the presence of CrPAM in the ciliary membrane and its key role in ciliogenesis. Knockdown approaches in planaria, mice and zebrafish demonstrated a conserved role for PAM in ciliogenesis. Cilia are sensory and secretory organelles that can respond to environmental signals by releasing bioactive ectosomes (also referred to as extracellular vesicles or EVs). With a cell wall covering its non-ciliary surface, we hypothesized that CrPAM might be released in ciliary ectosomes. Our analysis of ectosomes produced by the cilia of mating gametes revealed the presence of active CrPAM. Strikingly, CrPAM was excluded from ectosomes released by vegetative cells. PAM was never detected in the soluble secretome. Thus, the release of PAM from the cilium is a tightly regulated process. We also used mass spectrometry to search for the presence of amidated products in mating ectosomes. Following in-gel trypsin digestion and LC-MS/MS analysis, 1889 proteins were identified. A screen for amidated products identified three signal peptide containing proteins with an amidated C-terminus. An amidated peptide derived from one of these proteins acts as a chemoattractant specifically for minus, not plus, mating-type gametes. Ciliary ectosomes provide a previously unappreciated route for the secretion of amidated bioactive products that may also be utilized by motile and primary cilia in vertebrates.

Funded by DK32949 and GM051293 from the National Institutes of Health.

Poster Session 1 / No. 9

Novel subtypes of retinal ganglion cells identified by single cell RNA-seq analysis

Bruce A. Rheaume, Amyeo Jereen, Mohan Bolisetty, Muhammad S. Sajid, Yue Yang, Kathleen Renna, Lili Sun, Paul Robson, and Ephraim F. Trakhtenberg.

Email: rheaume@uchc.edu

Abstract: Retinal ganglion cells (RGC) are one of the broad classes of retinal cells that pre-process and pass to the brain visual information collected in the eye. Thirty subtypes of RGCs have been identified to date. Here, we have analyzed 6,225 purified, postnatal day 5 RGCs (with, on average, 5,000 genes per cell) separately from the right and left eyes by single cell RNA-seq, and classified them into 40 subtypes using clustering algorithms. The cells were similarly enriched for pan-RGC markers such as RBPMS, Tubb3, and Thy1. We have identified novel subtypes and markers, as well as the transcription factors predicted to cooperate in specifying RGC subtypes. Markers of 5 previously identified subtypes, Jam2, NPY, Pde1a, Trhr, and Gna14, were amongst the markers predicted to be unique to 5 out of the 40 RGC subtypes. We validated two novel RGC subtypes by fluorescent *in situ* hybridization (FISH) and immunostaining for markers we predicted to be uniquely enriched in these subtypes (subtype marker-positive RGCs were quantified as fraction of all RBPMS-positive RGCs). We also identified the right eye enriched subtype, which we validated by immunostaining *in situ*, using another novel marker predicted to be unique for this RGC subtype. In total, out of the 40 predicted subtypes, 5 were validated by markers in previous studies and 3 more were validated here using novel markers. A number of other established RGC markers were enriched in more than one subtype, suggesting that they label larger subpopulations of RGCs, that are further subdivided into subtypes based on the molecular differences in their transcriptomes. For example, Cartpt, Cdh6, and Col25a1 markers of the ON-OFF direction-selective RGCs were enriched in one group of subtypes, whereas Opn4, Eomes, and Igf1 markers of the intrinsically photosensitive RGCs were enriched in a different group of subtypes. These findings contribute to our understanding of the retinal component of the visual system through characterization of the molecular differences between RGC subtypes.

Acknowledgements: This work was supported by the University of Connecticut, School of Medicine, Start-Up Funds (to E.F.T.). Portions of this research were conducted at the High Performance Computing Facility, University of Connecticut. We are grateful to Sophan Iv (Research IT Services, University of Connecticut), Yi Zhang (Web Development IT Services, University of Connecticut), and Stephen King (High Performance Computing Facility, University of Connecticut) for assistance with the development of the website and online tools.

Poster Session 1 / No. 10

Molecular basis of junctional current rectification at electrical synapses

Yuan Shui, Ping Liu, Haiying Zhan, Bojun Chen, and Zhao-Wen Wang

Email: yshui@uchc.edu

Department of Neuroscience, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030-3401

Rectifying electrical synapses have been observed in many vertebrate and invertebrate species, and may impact neural circuit operation in diverse ways. However, little is known about the molecular mechanism of rectification. We investigated why junctional currents (I_j) are conducted almost exclusively in the antidromic direction at an electrical synapse between a pair of premotor interneurons (AVA) and postsynaptic A-type cholinergic motor neurons (A-MNs) in *C. elegans* escape neural circuit. These electrical synapses consist of UNC-7 innexin in AVA and UNC-9 innexin in A-MNs. UNC-7 has multiple isoforms differing in the length and sequence of the N-terminus. In the *Xenopus* oocyte heterologous expression system, all UNC-7 isoforms could form heterotypic gap junctions (GJs) with UNC-9 but only one of them, UNC-7b, resulted in GJs that strongly favored I_j in the UNC-9 to UNC-7 direction. Knockout of *unc-7b* alone in worms almost eliminated the I_j, and AVA-specific expression of wild-type UNC-7b substantially rescued the coupling defect of an *unc-7* null mutant. Neutralization of either one of two triplets of oppositely charged residues in UNC-7b N-terminus abolished the rectification property of UNC-7b/UNC-9 GJs. Based on these observations, we propose a model in which electrostatic interactions between charged residues allow UNC-7b N-terminus to block the channel when AVA is more depolarized than A-MNs, thus conferring the rectification property of AVA/A-MN GJs.

Supported by NIH R01NS109388, NIH R01MN085927, and NIH GM113004.

Poster Session 1 / No. 11

RNA-Seq analysis reveals extracellular matrix pathway proteins as a potential mechanism of neuroprotection in P2X4R KO mice after ischemic stroke

Victoria Scranton*, Pranay Srivastava*, Chunxia Cronin#, Bruce Liang# and Rajkumar Verma*

Department of Neurosciences UConn Health, Farmington CT 06030
Calhoun Cardiology Center, UConn Health, Farmington CT 06030

Email: vscranton@uchc.edu

Stroke is the leading cause of disability in the United States. Despite recent advances, interventions to reduce damage and increase recovery after stroke are limited. This prompts us to identify new potential drug target proteins and their plausible mechanism for therapeutic exploitation. Our recent work suggests that genetic deletion of P2X4, a purinergic receptor for adenosine triphosphate ATP, provide acute neuroprotection and thus can be potential drug targets for the treatment of ischemic stroke however, potential mechanism remains unknown. Therefore, in this study, we employed RNA-seq technology to identify the gene expression profiles and their possible role in certain biological processes responsible for P2X4R dependent neuroprotection after stroke.

We subjected 8-12 weeks old male Global P2X4 KO and littermate WT mice to right middle cerebral artery occlusion MCAo for 60 min followed by 3 day reperfusion. After 3 days mice were sacrificed and prefrontal cortex tissue was isolated to extract total RNA using Trizol. A total of 500ng RNA from each sample was used for RNA-seq sample preparations at Yale Center for Genome Analysis using Illumina Hi Seq. DESeq2 and R technology was used to identify mRNA transcript expression profiles. Gene expression levels were measured as fragments per kilobase per million reads using the Cuff diff program. Only genes that exhibited changes in expression > 1.5-fold and had *P*- adjusted values using the Benjamini–Hochberg procedure lower than 0.05 (*Padj* < 0.05) were considered. A separate cohort was also used for Evans blue dye extravasation study. We found a total of 2246 differentially expressed genes (DEGs) in P2X4R KO vs WT tissue after stroke. Out of these DEGs 1920 gene were downregulated and 325 genes were upregulated in KO group after stroke. Gene ontology analysis to perform enrichment on the sets of top 10% up or down regulated gene in P2X4R KO suggest enrichment of genes related to ion channel transport system and extracellular matrix component respectively. QPCR and western blot studies for confirmation are undergoing. Evans blue dye extravasation study suggests reduced blood brain barrier permeability in KO mice. This data suggest that plausible involvement of extracellular matrix component pathways for neuroprotection in P2X4R KO mice after stroke.

Support: This work was supported by AHA Career Development award 18CDA34110011 (to Rajkumar Verma). All the declare no competing financial interests.

Poster Session 1 / No. 12

Search of novel purinergic P2X4 receptor antagonists for the treatment of ischemic stroke.

Pranay Srivastava[a], Viswanatha K Rao[a], Bruce T Liang[a], Terrence E. O'Brien[b], Rajkumar Verma[a]

[a]School of Medicine, UConn Health, 263 Farmington Ave, Farmington, CT 06030
[b]Atomwise, 717 Market Street, Suite 800, San Francisco, CA 94105

Email: raverma@uchc.edu

Abstract:
Stroke remains a leading cause of disability in the United States. Despite recent advances, interventions to reduce damage and enhance recovery after stroke are lacking. As both resident and the peripheral immune response (specifically cells from myeloid origin) contributes to secondary tissue damage and poor recovery even after several hours of stroke onset, targeted inhibition of myeloid cell response has become an area of major therapeutic interest. We have recently shown that P2X4R, a purinergic receptor for adenosine triphosphate (ATP), regulates activation of myeloid immune cells (infiltrating monocytes/macrophages and brain-resident microglia) after ischemic injury (Verma et al., 2017). Hyper-activation of P2X4Rs, due to ATP released from dying or damaged neuronal cells, contributes to ischemic injury. Motivated by this work, we hypothesized that acute inhibition of P2X4R would be beneficial after stroke. As commercially available P2X4R inhibitor has several limitations in their in vivo use e.g. insoluble in biological vehicle and limited blood brain barrier (BBB) permeability. Using cutting edge artificial intelligence technique available through the AIMS program at Atomwise, a high throughput virtual screen was used to identify novel small molecular inhibitors of P2X4R. The best scoring compounds from the screen were then clustered for scaffold diversity and then filtered for drug-like properties to arrive at a final subset of 72 potential P2X4R inhibitors. These 72 compounds and the standard P2X4R inhibitor, 5-BDBD were screened using Ca^{2+} influx assay in HEK293 cells expressing exogenous P2X4R. We found 6 active compounds with inhibitory activity in the μM range. Out of these six, one compound was comparable to 5-BDBD in its P2X4R inhibitory activity.

In summary, we identified a total of six potential small molecule inhibitors of P2X4R, which are presumably soluble in biological solvents and are BBB permeable. In next series of experiments, we will confirm their P2X4R inhibitory activity in primary microglial culture as well as in *in-vivo* model of ischemic stroke in rodents.

Acknowledgment: AIMS award from Atomwise, AHA Career Development award 18CDA34110011 and START PPOC grant to Rajkumar Verma.

Poster Session 2 / No. 13

Bichromophoric voltage sensors based on voltage-dependent translocation of a tethered quencher

Corey Acker<acker@uchc.edu>;* Ping Yan <pyan@uchc.edu>; Les Loew <les@uchc.edu>
*presenting author

Body of Abstract
Voltage sensitive dyes (VSDs) are used for *in vitro* drug screening and for imaging of patterns of electrical activity in tissue. Wide application of this technology depends on the availability of sensors with high sensitivity (percent change of fluorescence per 100mV), high fluorescence quantum yield, and fast response kinetics. A promising approach uses a 2-component system consisting of anionic membrane permeable quenchers with fluorophores labeling one side of the membrane; this produces voltage-dependent fluorescence quenching. However, the quencher must be kept at low concentrations, to minimize pharmacological effects, thus limiting sensitivity. By developing tethered bichromophoric fluorophore quencher (TBFQ) dyes, where the fluorophore and quencher are covalently connected by a long hydrophobic chain, the sensitivity is maximized and is independent of VSD concentration. A series of 13 TBFQ dyes based on the AminoNaphthylEthenylPyridinium (ANEP) fluorophore and the dipicrylamine anion (DPA) quencher have been synthesized and tested in an artificial lipid bilayer apparatus. The best of these, **TBFQ1**, shows a 2.5 fold change in fluorescence per 100mV change in membrane potential, and the response kinetics is in 10-20 ms range. This sensitivity is an order of magnitude better than commonly used VSDs. However the fluorescence quantum yield is only 1.6 %, which may make this first generation of TBFQ VSDs impractical for *in vivo* electrical imaging. Nevertheless, the design principles established here can serve as foundation for improved TBFQ VSDs. We believe this approach promises to greatly enhance our ability to monitor electrical activity in cells and tissues.

Support
This study was supported by the National Institutes of Health via grant Nos. EB001963.

The authors declare the following competing financial interest(s): The subject matter described in this article is included in patent applications filed by the University of Connecticut. LML, AC, and PY are founders and owners of Potentiometric Probes LLC, which sells voltage sensitive dyes.

Poster Session 2 / No. 14

Elucidating the role of *C1ql1* in the maturation of Oligodendrocyte Precursor Cells

Andrew Tang[1,2], Joyshree Biswas[2], Brianna Thompson[2], Robert Pijewski[2], Madeleine Youngstrom[2], Akiko Nishiyama[3], Stephen Crocker[2], David C. Martinelli[2]

[1] Department of Biology, University of Hartford, 200 Bloomfield Ave, West Hartford, CT, 06117 (Electronic Address: atang@uchc.edu)
[2] Department of Neuroscience, University of Connecticut School of Medicine, 263 Farmington Ave, Farmington, CT, 06030
[3] Department of Physiology and Neurobiology, University of Connecticut, 75 North Eagleville Road, Unit 3156, Storrs, CT, 06268

During central nervous system (CNS) development, the axons of neurons are ensheathed in a substance known as myelin. Multiple sclerosis is a disease in the CNS characterized by progressive demyelination of axons and results in disruption of intracellular communication and neuron death. Five percent of an adult's brain cells are oligodendrocyte progenitor cells (OPCs). OPCs are the immature and undifferentiated form of an oligodendrocyte, the cell that produces myelin. In addition to producing myelin during development, mature oligodendrocytes have the potential to remyelinate and repair damage caused by demyelinating diseases. Based on preliminary *in vitro* results, a gene of interest, complement component 1 q subcomponent-like 1 (*C1ql1*) is expressed by OPCs, which is not yet appreciated in the literature. In this study, we aim to characterize *C1ql1* and its developmental expression using our *in vivo* mouse model. We first analyzed *C1ql1 e*xpressing cells at key points of mouse developmental stages. We found that *C1ql1* is expressed as early as post-natal day three and expression persists throughout adulthood. Next, we analyzed *C1ql1* expression and compared it to the expression of known markers for OPCs and mature oligodendrocytes. These markers are platelet derived growth factor receptor alpha (PDGFRα), which marks OPCs, and anti-adenomatous polyposis coli clone 1 (CC1) which marks oligodendrocytes. Compared to the expression profiles of PDGFRα and CC1, *C1ql1* expression overlaps entirely with the expression profile of PDGFRα and 22% with CC1. This discovery leads us to conclude that *C1ql1* expression persists deeper into the maturation of an oligodendrocyte than PDGFRα does. We then aimed to generate a conditional knockout of *C1ql1* specifically in OPCs. This was done by breeding our mice containing floxed *C1ql1* alleles with conditional knockout potential to a Cre driver mouse that expresses Cre selectively in OPCs. To check for off target Cre expression, we verified that *C1ql1* expression is not expressed in OPCs, but persists in the inferior olivary nucleus, where *C1ql1* is highly expressed in climbing fibers. We hypothesize that C1QL1 is a ligand that binds to a receptor on astrocytes known as brain specific angiogenesis inhibitor 3 (BAI3), which causes the release of tissue inhibitor of metalloproteinases 1 (TIMP1). TIMP1 is known to cause OPCs to mature into oligodendrocytes. Our research plans to elucidate and validate this stepwise differentiation pathway utilizing RNA interference to knock down and overexpress proteins in our proposed pathway in order to elucidate the role of each individual aspect of the pathway. Overall, this project aims to investigate the signaling pathway which results in maturation of OPCs in the CNS. Future research on manipulation of this pathway can potentially lead to a therapeutic option for those suffering from demyelinating diseases such as multiple sclerosis.

Support: National Multiple Sclerosis Society Pilot Grant

Poster Session 2 / No. 15

Biochemical Analysis of the Synaptic Regulator C1QL3 and its Binding Partners

Matthew Sticco, Brianna Thompson, Susanne Ressl, and David Martinelli

Email: sticco@uchc.edu

Synapses are specialized neuronal cell-cell junctions, which allow for the formation of neural networks by promoting fast, efficient, and stable communication between neurons. C1QL3 is a secreted protein that is expressed in a small subset of brain regions, mostly in the limbic system of the adult brain. C1QL3 is a known high affinity ligand of BAI3, an adhesion-class GPCR that is located post-synaptically when expressed in the central nervous system. It has been shown that C1QL3 regulates synapse formation and/or maintenance by conditional C1QL3 knockout mice. This knockout also results in abnormalities in fear memory likely due to the reduction in synapse number after C1QL3 knockout. The mechanism by which C1QL3 influences synapse density is currently unknown but could involve a trans-synaptic adhesion complex between C1QL3, BAI3, and an identified, but yet-to-be-validated, presynaptic binding partner, NPTX1. Another protein, Spock2, is thought to be a negative regulator of this complex. Through a series of site-directed mutagenesis experiments, we hope to reveal the binding surfaces of C1QL3 and validate the identity of its pre-synaptic binding partner. In addition, we hope to determine binding constants for each C1QL3 binding partner. We also aim to show this complex in vivo using three-color immunofluorescence. This may provide insights about how C1QL3 influences the formation and/or maintenance of synapses.

Funding Source: UCHC Start-up funds to D.M.

Poster Session 2 / No. 16

Aging and the Effect of Senolytic Treatments on the Aged Central Nervous System

Robert Pijewski[1,*], Iman Al-Naggar[2], Connor Merinder[1], Cory Willis[1], Alexandra Nicaise[1], Phillip P. Smith[2], Stephen J. Crocker[1]

Email: pijewski@uchc.edu

Department of Neuroscience[1], and UConn Center on Aging[2], UConn Health, Farmington, CT

The molecular aspects of physiological aging have been implicated as a major contributor to development of neurological diseases. Chronological aging is associated with alterations in stress response pathways, cell cycle gene expression and pro-inflammatory factors collectively called cellular senescence. Experimental evidence has demonstrated that elimination of senescent cells (senolysis) promotes tissue rejuvenation in peripheral organs such as the pancreas and heart. Cellular senescence has recently been implicated in degenerative diseases of the central nervous system (CNS) including Alzheimer's disease and multiple sclerosis. In the context of neurological diseases, cellular senescence may contribute to neuroinflammation, reduced regenerative potential, diminished cognition, and impaired neurological functions. While experimental studies support application of senolytic agents to treat disease, prophylactic treatment in younger populations and treatment of aged individuals without neurological disease are currently being explored. However, the effectiveness and impact of senolytics on the CNS is not known. In this project, we have investigated the efficacy and impact of senolytic treatment on cellular senescence and CNS myelination in the aged CNS. In the first set of experiments we quantified the expression of cellular senescence genes in the young (12 week) and aged CNS (24 month) to establish what are the appropriate markers to assess aging and the effects of senolytic treatments on the CNS. In a second set of experiments we determined the effect of a senolytic treatment currently in clinical trials on the aged CNS which is a combination of dasatinib and quercetin (DQ). Results from this study have identified and validated senescence biomarkers in the aged CNS and we have identified an unexpected but profound negative impact of senolytic treatment on CNS myelination that results in pathology. Based on the increased reported use of this treatment approach, and accumulating studies that implicate aging in neurological diseases, caution may be warranted on the use of senolytics to target cellular senescence to treat neurological diseases.

This work was supported in part through a seed grant from the Connecticut Institute for the Brain and Cognitive Sciences and an operating grant from the National Multiple Sclerosis Society.

Poster Session 2 / No. 17

Regulation of synaptic plasticity by BDNF-endocannabinoid interactions

Fouad-Lemtiri Chlieh, Tiwanna M. Robinson and Eric S. Levine

Dept. of Neuroscience, University of Connecticut School of Medicine, Farmington CT

Email: flemtirichlieh@uchc.edu

The goal of these studies is to explore the functional interactions between brain-derived neurotrophic factor (BDNF) and endogenous cannabinoids (eCBs) in regulating activity-dependent synaptic plasticity in the neocortex. Both BDNF and eCBs have been implicated in a diverse range of physiological processes, including sensory perception, motor coordination, memory, and cognitive abilities. Disruption of BDNF and/or eCB signaling may play a role in several neurologic and psychiatric disorders, including anxiety, depression, schizophrenia, and seizure disorders, and these neuromodulatory systems are currently major targets for the development of novel therapeutics. Although there is evidence for crosstalk between BDNF and eCB signaling, little is known regarding potential synaptic interactions. We have recently shown that BDNF suppresses presynaptic GABA release at cortical inhibitory synapses and this effect is mediated by the BDNF-induced release of eCBs from postsynaptic pyramidal cells that act as retrograde signals. We have also found that BDNF induces eCB release at cortical excitatory synapses, and the suppressive effects of eCBs on glutamate release can mitigate the direct enhancing effects of BDNF at these synapses. We are now poised to explore the functional relevance of these synergistic as well as antagonistic interactions in regulating activity-dependent synaptic plasticity. In the present studies, we examined the interactions between BDNF and eCB signaling at cortical layer 5 excitatory synapses, using a pharmacologically-induced long-term potentiation (LTP) protocol that increases intracellular cAMP and enhances NMDA receptor activation. We found that this chemical-LTP requires endogenous BDNF and TrkB signaling because it was prevented by the TrkB antagonist ANA-12 or the Trk tyrosine kinase inhibitor K-252a. The response to this induction protocol was also impaired in mice engineered to express a common human variant in the BDNF gene (Val66Met) that reduces activity-dependent BDNF release. The role of eCB signaling will be explored through the use of CB1 receptor antagonists and as well as inhibitors of eCB synthesis and metabolism. Release of eCBs at these excitatory synapses may diminish plasticity whereas eCB suppression at inhibitory synapses could enhance excitatory plasticity. Our overarching hypothesis is that the balance of BDNF and eCB signaling in specific circuits regulates the direction and magnitude of synaptic plasticity.

Supported by a grant from the National Institutes of Health.

Poster Session 2 / No. 18

Electrophysiological profiling of human iPSC-derived neurons with an autism-linked duplication in Chromosome 15

Marwa Elamin, Tiwanna M. Robinson & Eric S. Levine

Dept. of Neuroscience, University of Connecticut School of Medicine, Farmington CT

Email: elamin@uchc.edu

Abstract:
Chromosome 15q11-q13 duplication syndrome (Dup15q) is a neurodevelopmental disorder caused by duplication of the maternal copy of this region of the long arm of chromosome 15. Affected children typically present with autistic behavior, epileptic seizures, and a wide range of intellectual and motor disabilities. The most common (and most severe) type of Dup15q syndrome involves an extra piece of chromosome 15 that contains an isodicentric duplication with two extra copies of the 15q11-q13 region (Idic15q). A less common (and a milder) type involves an interstitial duplication within chromosome 15 that contains one extra copy of the same region (Int15q). We are using neurons derived from induced pluripotent stem cell (iPSC) lines from Dup15q patients and unaffected controls to identify cellular phenotypes related to the underlying pathophysiology. We have recently identified differences in excitability and synaptic transmission between Idic15q neurons and unaffected controls over the course of *in vitro* development. The present study compares the electrophysiological profiles of two cell lines with either an Idic15q or Int15q duplication. Patch clamp recordings performed at 7-8 weeks *in vitro* revealed that both cell lines display an “accelerated maturation” phenotype characterized by increased sodium and potassium currents compared to controls. Action potential characteristics (larger amplitude; decreased width) also point to an increased functional maturity of both Dup15q lines compared to controls at that age. An increase in spontaneous synaptic frequency is observed only in the line with an isodicentric duplication. In mature neurons (16-19 weeks), the differences in the current recordings and action potential characteristics dissipate, but the increase in synaptic frequency in the Idic15q line persists. These differences are likely due to increased expression of *UBE3A*, which is the only maternally expressed gene in the region. Understanding the cellular deficits in these two types of duplications will aid in identifying the detrimental effects of increasing *UBE3A* gene dosage. Furthermore, pinpointing the primary deficits in these neurons could aid in understanding the pathophysiology of autism or seizures at a cellular level. Future directions include investigating the hyperexcitability phenotype and plasticity deficits in the Int15q line and examining the effect of normalizing *UBE3A* levels at different time points during development.

Supported by grants from the National Institutes of Health and the CT Regenerative Medicine Research Fund.

Poster Session 2 / No. 19

Cellular composition of iPSC-derived cultures from patients with Dup15q syndrome

Jadin James, Tiwanna M. Robinson, Marwa Elamin, Fouad Lemtiri-Chlieh & Eric S. Levine

Dept. of Neuroscience, University of Connecticut School of Medicine, Farmington CT

Email: jjames2@uchc.edu

Chromosome 15q duplication syndrome (Dup15q) is a neurogenetic disorder caused by a maternal interstitial duplication or a supernumerary isodicentric triplication (idic15) of chromosome 15q11.2-13.1. It is a clinically distinct neurodevelopmental disorder that is estimated to occur in 1 in 15,000 live births, with male and females affected equally. It is characterized by hypotonia, developmental delay, intellectual disability, electroencephalogram (EEG) abnormalities, epilepsy, and has frequently been associated with autistic behavior or autism spectrum disorder. EEG abnormalities and epilepsy of all varieties occur in 60% of individuals with Dup15q, which is typically difficult to control with current medications and may result in sudden unexpected death. The mechanisms underlying this increased seizure susceptibility are unknown. Using patient-specific induced pluripotent stem cell (iPSC) lines, we have found that neurons derived from Dup15q patients show enhanced excitatory synaptic currents and increased spontaneous firing, which could make them more prone to seizure-like activity. Because changes in excitatory-inhibitory balance can contribute to seizure susceptibility, we used immunocytochemistry to determine whether the cellular composition of iPSC-derived cultures was affected by the Dup15q genotype. We used a differentiation protocol that, in the absence of specific morphogens, leads to a default generation of forebrain neurons. Cultures derived from a single Dup15q patient and a single control subject were fixed at 15 weeks *in vitro* and stained for β-tubulin (TUJ1), a neuron-specific microtubule protein, TBR1, a transcription factor specific for glutamatergic neurons, and GAD67, a marker for GABAergic neurons. There were no significant differences between control and Dup15q cultures in the proportion of glutamatergic neurons or GABAergic neurons. We also determined the percentage of astrocytes present in these cultures by staining for the nuclear marker DAPI and the astrocyte markers GFAP and S100b. At 15 weeks *in vitro*, the proportion of DAPI-positive cells that stained positive for S100b or GFAP was not significantly different for Dup15q and control cultures. Because endocannabinoid signaling has been strongly implicated in seizure susceptibility, we have also carried out preliminary experiments characterizing the expression of CB1 cannabinoid receptors and the physiological responses to cannabinoid agonists in control iPSC-derived neurons.

Supported by grants from the National Institutes of Health and the CT Regenerative Medicine Research Fund.

Poster Session 2 / No. 20

Voltage Imaging in Brain Slices Using Several Variants of Genetically Encoded Voltage Indicators

Milena M. Milosevic, Jinyoung Y. Jang, and Srdjan D. Antic

UConn Health, Farmington, CT, USA

Email: milosevic@uchc.edu

Genetically encoded voltage indicators (GEVI) enable optical monitoring of important features of the neuronal circuit dynamics, to which calcium imaging technique is completely blind. In the current study, we evaluate the feasibility of new voltage imaging methods for studying population signals in cortical networks. GEVI variants from several laboratories were tested in HEK293 cells and cultured neurons. In the in vitro experiments, optical signals were successfully obtained using ArcLight, ASAP1, ASAP2s, FlicR1, Bongwoori-Pos6, and NIR-GEVI1. In addition, voltage sensors were applied to mouse neurons in situ by: [1] classical voltage-sensitive dye (VSD) labeling (using dye JPW-3028); [2] intracerebroventricular injections of adeno-associated viruses (AAV) with GEVI cargo (ArcLightD, ASAP2s, Bongwoori-R3, and Archon1); or [3] transgenic GEVI animal breeding (VSFP Butterfly 1.2). Synaptic stimulations in layer 2/3 or layer 5 were used to evoke cortical population signals. Single-photon wide-field imaging was used to sample signals from many locations simultaneously. Signal-to-noise ratio (SNR), optical signal rise-time and decay-time were compared between several GEVI variants. In one series of experiments, VSD imaging and GEVI imaging were performed on the same brain slice in sequential trials. Synaptic stimulus location, intensity and duration were kept fixed between trials. VSD and GEVI recording trials were only seconds apart, thus allowing the most direct comparisons of the VSD and GEVI optical signals. In addition to comparison of features of voltage indicators available today, we discuss the GEVI brain slice imaging as an experimental tool for dissection of brain circuits.

Transgenic animals and reagents were kindly provided by C. Song & T. Knopfel (VSFP Butterfly 1.2 transgenic animal); M.Z. Lin Lab (AAV-ASAP2s); J. Platisa & V. Pieribone (AAV-ArcLightD); B.J. Baker Lab (AAV-Bongwoori); and K.D. Piatkevich & E.S. Boyden (AAV-Archon1).

Support: U01MH109091

Poster Session 2 / No. 21

Voltage and calcium signals in dendrites of medium spiny neurons.

Jinyoung Jang and Srdjan D. Antic

Email: jang@uchc.edu

The medium spiny neuron (MSN) is a key neuron in the basal ganglia circuit, involved in several major neurological disorders including Parkinson's disease, Huntington's disease and attention deficit hyperactivity disorder (ADHD). MSNs exhibit two neural states. The "Down state" (hyperpolarized membrane potential) alternates with the "UP state" (depolarized membrane potential) in accordance to the temporal availability of glutamatergic synaptic inputs impinging onto their spiny dendrites. To understand the functional roles of MSNs, it is necessary to study dendritic physiological properties. Intrinsic membrane properties of MSN dendrites are poorly understood because glass electrode recordings are not tolerated well by thin dendritic branches. Here, we performed simultaneous somatic whole-cell recording with dendritic imaging to focus on dendritic properties. We injected voltage sensitive dyes (JPW-3028 and JPW-4008) or Ca^{2+} indicators (OGB1 and Fluo-5N) into MSNs. Dendritic membrane potential changes were induced by backpropagating action potentials (bAPs) or by local application of glutamate on dendrite. Our results reveal an increasing dendritic AP peak latency depending on the distance from the soma. The amplitudes of action potential-induced dendritic Ca^{2+} influxes decrease with distance from the soma suggesting a distance dependent attenuation of bAPs in dendrites of MSNs. Next, we characterized the voltage waveforms of glutamate evoked plateau potentials in dendrite and cell body simultaneously. Invariably, the dendritic plateaus preceded somatic plateau potentials and the initiation phase of the dendritic UP state (plateau) was steeper than that in the soma, consistent with a local initiation of the dendritic plateau. Detailed characterization of voltage and Ca^{2+} signals is expected to reveal the intrinsic dendritic properties and bring us closer to the functional roles of MSNs in the basal ganglia circuits.

Supported by NIH Grant: NIH MH109091

Poster Session 2 / No. 22

UDERSTANDING 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

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

BACE-1 regulate Aβ (1-42) induced neuro-inflammation by modulating microglial function.

Neeraj Singh, Xiangyou Hu and Riqiang Yan.

Department of Neuroscience, UCONN Health, School of Medicine, University of Connecticut, Farmington, CT

Alzheimer's (AD) is a late-onset, progressive neurological disorder, characterized by the extracellular deposition of Amyloid beta (Aβ) / senile plaques and intracellular accumulation of neurofibrillary tangles (NFT) in the neuronal cells. Inefficient processing/clearance of amyloid plaques by the activated glial cells often leads to persistent oxidative stress and neuro-inflammation, causing irreversible loss of neurons in the hippocampus and cortex, often resulting in cognitive deficits. Identifying signaling molecules regulating Amyloid beta processing or clearance offers potential target in developing therapies for the treatment of AD. β-site amyloid precursor protein cleaving enzyme 1 (BACE-1), an aspartyl proteases β-secretase enzyme, cleaves amyloid precursor protein (APP) at the β-site to release a soluble N-terminal fragment and a membrane-anchored C-terminal fragment. Previously, our lab had demonstrated that Bace-1 deficiency inhibits Amyloid plaque formation/deposition and reverse AD associated pathology in AD mice model. Intriguingly, BACE-1 deletion post Aβ plaque deposition also leads to significant clearance of Aβ in 5FAD mice model. In the present study, we hypothesize that besides regulating neuronal Amyloid processing, BACE1 plays a major role in regulating microglial function. Using primary microglia culture derived from WT and BACE1 deficient mice, we demonstrated that Bace-1 regulate microglial mediated clearance of Aβ(1-42) by modulating phagocytic activity as well as upregulating cellular degradation machinary. Our results demonstrate that BACE-1 deficiency or pretreatment with pharmacological inhibitors of BACE-1 significantly enhanced uptake β-Amyloid (1-42) in both microglial and BV-2 cell line. Interestingly, our RNA-Seq data suggest that microglial BACE-1 regulate expression of numerous gene regulating pro inflammatory cytokines, chemokines, autophagy and phagocytosis 12 hr post Aβ1-42 treatment. Furthermore, as compared to WT, Aβ treatment significantly upregulated signaling pathways associated with phagocytosis as well as lysosomal degradation machinery necessary for efficient clearance of Aβ in BACE-1 KO microglial. Currently, our invitro results are further being validated in WT and conditional Bace-1 KO 5xFAD mice models.

Funding Source- NS074256, AG046929

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 2020