UCONN HEALTH

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

PROGRAM & AGENDA

Thursday, June 8, 2017
8:00 am – 6:00 pm

The Mark Twain House & Museum
351 FARMINGTON AVENUE
Hartford, CT 06105

2017 Meeting Program

Time	Event	Location
8:00 – 8:30 am	Registration Sign-in Continental Breakfast	Great Hall
8:30 - 8:45 am	Welcome Address Dr. Stephen Crocker	Auditorium
8:45 – 9:30 am	Symposium A: Talks	Auditorium
9:30 – 10:30am	Symposium B: Talks	Auditorium
10:30 - 11:45 am	Poster Session 1	Great Hall
11:45 – 1:00 pm	Lunch & Tours	Great Hall & Outdoor Terrace*
1:00 – 1:45 pm	Symposium C: Talks	Auditorium
1:45 – 2:30 pm	Symposium D: Talks	Auditorium
2:30 - 3:45 pm	Poster Session 2	Great Hall
3:45 - 4:00 pm	Vendor Recognition Presentation	Auditorium
4:00 – 5:00 pm	Keynote Address: Dr. Patrizia Casaccia, MD, Ph.D. Professor of Neuroscience Genetics and Genomic Sciences The Mount Sinai Hospital, New York, NY	Auditorium
5:00 - 5:15 pm	Presentation of Awards & Closing Remarks	Auditorium
5:15 - 6:00 pm	Reception: Featuring a special summer treat! "Make your own *gelato* sundae"	Outdoor Terrace*

*weather permitting

UCONN HEALTH

Stephen J. Crocker, Ph.D, Associate Professor
Associate Director, Neuroscience Graduate Program
Department of Neuroscience, UCONN Health
263 Farmington Ave, Farmington, CT 06030
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May 24, 2017

Dear Neuroscience Program Faculty, Postdocs, Students and Guests,

Welcome to the 16th annual Neuroscience Program Retreat! This year's event is being hosted at the Mark Twain House in Hartford. One of the best known American writers, Mark Twain was a remarkable man and the Mark Twain House is recognized as a National Historic Landmark. Complementary tours of the house have been made available to all participants - we hope you have taken advantage of this tremendous opportunity to visit one of the best places in Hartford!

There are a few items we would like to bring to your attention. **First, PLEASE BRING YOUR UNIVERSITY I.D.** This will allow others to know who you are, it will allow the Mark Twain House staff identify you as part of our event, and lastly it will also allow you 10% off in the Mark Twain gift shop! Second, the venue is air conditioned. This means that the temperature can vary quite a bit. If you have a tendency to get cold, please remember to bring a sweater or light jacket. Third, while parking is free, it is not unlimited. Please make every attempt to carpool so that we may minimize the total number of cars at the Mark Twain house. Driving instructions are provided in the pages following this letter. And, finally, the majority of the event will be held on the main floor of the Museum. If you are giving a poster, the poster number listed in the header on the page of your abstract in this program is the number of the poster board on which you should place your poster.

Please print or carry an electronic copy of this program with you to the meeting. Printed copies will not be provided for all participants at this year's event. Please also know that there will be free wireless internet access complements of the Mark Twain House. The wifi password will be provided at the meeting.

As we have in past years, presentations by students and postdocs will be judged for recognition. Judges may or may not identify themselves to you. Regardless, you will be judged on a variety of criteria including, but not limited to: knowledge, presentation and responses to questions. Winners of these awards will be announced at the end of the day.

The retreat this year will wrap-up with a special summer treat on the Terrace, weather permitting. If the temperature is too rainy, we will hold the reception in the Great Room.

If you have any suggestions for improving the event for next year, please don't hesitate to let members of the organizing committee know.

We hope you enjoy the day and find your experience helpful and rewarding.

On behalf of the Organizing Committee,

Sincerely,

Neuroscience Retreat Organizing Committee:
Stephen Crocker - Organizing Committee Chair & "Doctor" of Ceremonies
Zhao-Wen Wang – Abstract Organizing Chair
Jody Gridley - Commercial Liaison & Logistics Coordinator
Cory Willis, Judy Bloom & Alexandra Nicaise - Scientific Program Coordinators

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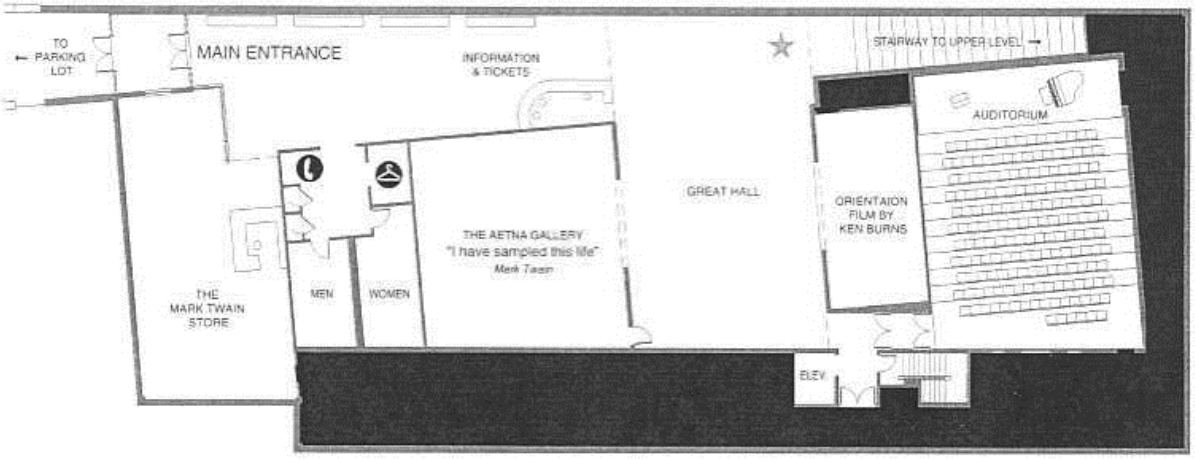
The Mark Twain House & Museum

Lower Level / Main Entrance



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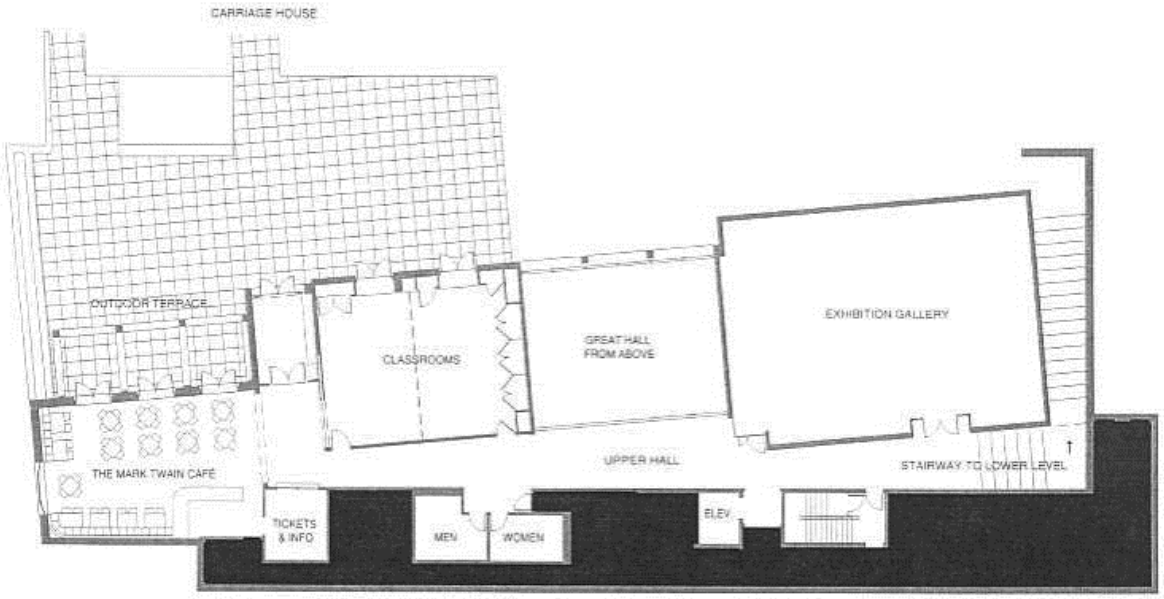
Upper Level

↑ TO THE MARK TWAIN HOUSE



3rd Floor–Research Library (By Appointment Only)

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 ACCESS TO THE 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.

Alphabetical Table of Contents by Presenter

KEYNOTE SPEAKER

"The Ins and Outs of the Myelinating Oligodendrocyte Cell Identity"

Patrizia Casaccia, MD, PhD

Professor of Neuroscience
Genetics and Genomic Sciences
The Mount Sinai Hospital, New York, NY

Dr. Patrizia Casaccia is Professor of Neuroscience, Genetics and Genomics; and Neurology. Dr. Casaccia is the Chief of the Center of Excellence for Myelin Repair at the Friedman Brain Institute at Mount Sinai School of Medicine. She received her medical degree with Honors from the University of Rome, and a PhD degree in Neurobiology from State University of New York (SUNY) Health and Science Center Brooklyn. She then trained at Cornell Weill Medical Center in New York and at the Skirball Institute for Molecular Medicine at NYU.

Dr. Casaccia's work adopts molecular and cellular techniques to find new therapies for multiple sclerosis. Her work includes translational research in regenerative and personalized medicine with a focus on myelin repair and a special emphasis on the effects of aging and gender differences. In addition, her research addresses the mechanism of neuronal damage in patients with MS leading to novel screening for the discovery of new therapies to protect the neurons and replace damaged myelin.

The new methodologies leading to personalized medicine include the generation of neural stem cells from patients' skin, and the analysis of DNA and RNA from the blood of multiple sclerosis patients. Her work is funded by grants from the National Institute for Neurological Disorders and Stroke and by the National Multiple Sclerosis Society.

Symposium A: Talk No.1

Effort-related decision making in mice: a genetic and pharmacological study using touchscreen operant methods

*****R. Presby[1], J.-H. Yang[1], S. Cayer[1], R. Rotolo[1], R. Fitch[1], M. Correa[2], J. D. Salamone[1]; [1]Psychological Sci., Univ. of Connecticut, Storrs, CT; [2]Psicobiologia. Univ. Jaume I, Castello, Spain

Funding: University of Connecticut Research Foundation Murine Behavioral Neurogenetics Facility (MBNF) / The Connecticut Institute for Brain and Cognitive Sciences (IBACS)

Motivated behaviors can involve a high degree of persistence and work output. In a complex environment choices based on cost/benefit analyses, such as effort-related decisions, must often be made. Effort-related motivational symptoms (e.g., anergia, low effort bias, and fatigue) have been identified in depression, schizophrenia and other disorders. Models of effort-related decision making in rodents have been developed that allow animals to choose between high-effort alternatives that lead to more highly valued rewards vs. low-effort alternatives that lead to less valued rewards. These models have been extensively used in rats, and are being developed in mice. Haloperidol is a dopamine (DA) D2 receptor competitive antagonist that is used as an antipsychotic in humans and has been shown to induce a low-effort bias in rodents. Studies conducted in rats using fixed ratio (FR)/choice or progressive ratio/choice operant tasks have shown that haloperidol suppresses ratio lever pressing (the high-effort choice) at low doses that do not suppress approach and intake of lab chow (the low effort/low reward option). In the present studies mice were tested in touch screen operant boxes using a choice procedure that offered the option of panel pressing on FR2 or FR4 schedules for a preferred liquid diet vs. approaching and consuming small food pellets that were concurrently and freely available in the chamber. Haloperidol (vehicle, 0.05, 0.1, and 0.15 mg/kg IP) was injected into 3 groups of male COMT variant mice with mixed S129 and C57BL6J backgrounds (WT, and transgenic mice with either humanized COMT Val or Met alleles; from JAX lab). In both the FR2/ and FR4/choice studies, haloperidol induced a dose-related decrease in panel pressing. There also was a significant overall group difference in panel pressing between the WT and Val groups at both the FR2 and FR4 schedules. Doses of haloperidol that reduced panel pressing had no significant effect on intake of the concurrently available food pellets, indicating that all types of food-related behaviors were not affected equally by DA antagonism. On completion of the haloperidol experiment, the 3 groups of mice were taken off food restriction and given ad libitum access to lab chow to devalue food reinforcement by reducing appetite. Similar to what has been reported in rats, there were significant decreases in both panel pressing and pellet intake across all 3 groups. Thus, DA antagonism induced effects that were different from those produced by reinforcer devaluation. These exploratory studies open up the possibility that mouse touch screen procedures can be used in pharmacological investigations of effort-based choice.

Symposium A: Talk No.2

Newly identified cellular subpopulations in the developing cerebellum

John Wizeman[1], Qiuxia Guo[1], and James Y.H. Li[1,2*]

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

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

The developing cerebellum is an excellent experimental paradigm for studying neurogenesis and morphogenesis. Despite recent progress, mechanisms underlying the generation of different subtypes of neurons and non-neuronal cells remain unclear. The goal of this study was to determine the molecular decisions underlying cell fate specification and to characterize unstudied cellular subpopulations in the developing cerebellum. To accomplish this, we performed single cell RNA sequencing on embryonic day (E) 13.5 mice. Transcriptomes from approximately 9,800 cells were analyzed through several bioinformatics tools coupled with immunohistochemistry, *in situ* hybridization, genetic fate mapping, and inspection of published expression data. Reiterative clustering of all cells and individual lineages identified the major cerebellar cell types and subpopulations of each lineage. Through pseudo-temporal ordering to elucidate developmental trajectories we identified novel transcriptional programs that control cell fate specification of multiple cerebellar populations. Our analysis was also able to reveal a previously uncharacterized rare population of cells (<0.25%) that are derived from the roofplate and persist at the cerebellar midline. Interestingly, this group of cells expresses several signalling molecules, including *Fgf17*. Deletion of *Ptpn11*, which is essential for FGF signaling, results in vermal hypoplasia in mice. Remarkably, despite the persistence of the roofplate-derived cells, *Fgf17* expression was missing and cell proliferation was significantly decreased in the surrounding cells of *Ptpn11* mutants. Our data suggests that this midline population might be a signaling center to organize the cerebellar midline and populate the vermis. Together, our data revealed useful markers for studying the cerebellum, important specification decisions, as well as a number of previously unknown subpopulations that may play an integral role in the formation and function of the adult cerebellum. Importantly, we identified a potential mechanism of vermis formation, which is affected by multiple congenital cerebellar defects like Dandy-Walker and Joubert syndromes.

Support: R01MH094914

Symposium A: Talk No.3

Hearing with an oversized inferior colliculus – is bigger better?

A. Burghard, N. Morel, D. Oliver

The inferior colliculus (IC) is a major hub of auditory processing. After different aspects of an acoustic signal are processed in the auditory brainstem nuclei, this information converges in the central nucleus of the IC (ICC) onto one tonotopic map. According to the synaptic domain theory, the inputs from different auditory brainstem nuclei to the ICC cluster in specific sub-regions and, thus, form functional zones superimposed on the single tonotopic map. These sub-regions may send this information via the thalamus to distinct areas of the core auditory cortex with different functions. One approach to the detailed study of function and anatomy in the ICC sub-regions is to genetically manipulate the IC during development.

In the present study, we used a mouse model with an oversized IC. Developed by Dee et al. (2016), MEK1 (also referred to as mitogen-activated protein 2 kinase 1, MAP2K1) is overexpressed in this mouse in the tectal stem cell zone from which the IC originates. Consequently, the stem cells remain in the proliferation stage longer, thus increasing the number of stem cells, but also delaying neurogenesis. This results in more, but later developed IC neurons. The gross anatomy shows that in all MEK1 mice the IC is massively enlarged compared to littermate controls.

We tested the hearing thresholds of MEK1 mice using the click-evoked auditory brainstem response (ABR) and the amplitude-modulated frequency following response (AMFR). To obtain the AMFR audiogram, we used narrow-band noise (0.3 octave) centered at frequencies 2-40 kHz and a modulation frequency of 42.9 Hz. We tested 5 and 13 week old MEK1 mice as well as age-matched littermate controls. Our preliminary electrophysiological evaluation of the MEK1 mice showed a diverse phenotype. In comparison to the audiograms of littermate controls, some MEK1 mice had an almost normal audiogram, while others showed elevated thresholds. We also measured the growth in the AMFR signal amplitude in response to increasing sound level intensity. In some MEK1 mice there was a reduced growth function especially close to threshold. Interestingly, the hearing threshold did not predict the amplitude growth function or vice-versa. We also measured the peak synchrony of the AMFR signal and found that synchrony was slightly degraded in most MEK1 mice in comparison to littermates. In summary, our preliminary observations of a mouse with a massively enlarged IC suggest that this structural change may result in more than one type of alteration in the circuitry of the auditory midbrain and more than one hearing phenotype. Bigger may not be better.

Supported by NIH R21DC013822 and UConn Spring 2017 Health Research Program (N.M.)

Symposium B: Talk No.4

Cellular Senescence Underlies Myelin Defect of Neural Progenitor Cells from PPMS Patients

Alexandra M. Nicaise[1], Cory M. Willis[1], Rosa M. Guzzo[1], Laura Haynes[2], Stephen J. Crocker[1]
[1]Department of Neuroscience, University of Connecticut School of Medicine, Farmington, Connecticut, United States
[2]Center on Aging and Department of Immunology, University of Connecticut School of Medicine, Farmington, Connecticut, United States

Primary progressive multiple sclerosis (PPMS) is a chronic progressive demyelinating disease of the central nervous system (CNS) without any effective treatment. To examine the potential for brain repair as a treatment strategy for PPMS we developed iPS cells from blood samples of PPMS patients and age matched non-disease controls. Neural progenitor cells (NPCs) from controls protected against cuprizone, a myelin injury model, whereas PPMS NPCs failed to provide neuroprotection. In examining the effect of NPCs on oligodendrocyte progenitor cell (OPC) maturation, we found that PPMS NPC conditioned media did not promote oligodendrocyte maturation *in vitro*. In analyzing the PPMS NPCs we found that they express key hallmarks of cellular senescence, a cell stress response that results in an adaptive cellular physiology. Cellular senescence can be caused by aging or disease, which has been linked to degenerative disorders of the CNS. The PPMS NPCs expressed hallmarks of senescence including elevated expression of p16, p53, senescence-associated β-galactosidase (SA-β-gal) activity, shortened telomeres, and a senescence-associated secretory phenotype (SASP). Using rapamycin we found that the expression of these hallmarks was reversed. Treatment of the PPMS NPCs with rapamycin recovered oligodendrocyte differentiation in OPCs when treated with PPMS NPC conditioned media. Based on these data, we hypothesize that cellular senescence is responsible for the impaired function of PPMS NPCs on OPC maturation. These data for the first time may reveal a mechanism by which NPCs via secretion of factors prevent endogenous remyelination in white matter lesions. While our data point to cellular senescence as a potentially important feature of NPCs in PPMS future studies will explore whether this process is active and important in remyelination failure in other forms of this disease.

Symposium B: Talk No.5

Signaling by FGF Receptor 2, not FGF Receptor 1, Regulates Myelin Thickness Through Activation of ERK1/2–MAPK, Which Promotes mTORC1 Activity in an Akt-Independent Manner

M. Furusho[1], A Ishii[1] and R. Bansal[1]
[1]Department of Neuroscience, University of Connecticut Medical School, Farmington, CT, USA,

FGF signaling has emerged as a significant “late-stage” regulator of myelin thickness in the CNS, independent of oligodendrocyte differentiation. Therefore, it is critically important to identify the specific FGF receptor type and its downstream signaling molecules in oligodendrocytes to obtain better insights into the regulatory mechanisms of myelin growth. Here, we show that FGF receptor type 2 (FGFR2) is highly enriched at the paranodal loops of myelin. Conditional ablation of this receptor-type, but not FGF receptor type 1 (FGFR1), resulted in attenuation of myelin growth, expression of major myelin genes, key transcription factor Myrf and extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) activity. This was rescued by upregulating ERK1/2 activity in these mice, strongly suggesting that ERK1/2 are key transducers of FGFR2 signals for myelin growth. However, given that the PI3K/Akt/mechanistic target of rapamycin (mTOR) pathway is also known to regulate myelin thickness, we examined FGFR2-deficient mice for the expression of key signaling molecules in this pathway. A significant downregulation of p-mTOR, p-Raptor, and p-S6RP was observed, which was restored to normal by elevating ERK1/2 activity in these mice. Similar downregulation of these molecules was observed in ERK1/2 knock-out mice. Interestingly, since p-Akt levels remained largely unchanged in these mice, it suggests a mechanism of mTORC1 activation by ERK1/2 in an Akt-independent manner in oligodendrocytes. Taken together, these data support a model in which FGFs, possibly from axons, activate FGFR2 in the oligodendrocyte/myelin compartment to increase ERK1/2 activation, which ultimately targets Myrf, as well as converges with the PI3K/Akt/mTOR pathway at the level of mTORC1, working together to drive the growth of the myelin sheath, thus increasing myelin thickness.

Supported: NIH grant NS38878

Symposium B: Talk No.6

Title: Role of Rab27 in regulated secretory vesicle trafficking

Prem K. Shrestha[1], Yi Wu[2], Richard E. Mains[1], and Betty A. Eipper[1]
[1]Department of Neuroscience and [2]Richard D. Berlin Center for Cell Analysis and Modeling
University of Connecticut Health Center, Farmington, CT (06030), USA

Rab proteins are Ras-like small GTPases that regulate intracellular vesicle trafficking in eukaryotic cells. They comprise the largest subgroup of the Ras superfamily, with >60 members in many mammals. Rab27A is unique among Rabs because it is the only example of a Rab specifically implicated in a human genetic disease. Loss-of-function mutations in *RAB27A* result in Griscelli syndrome, a rare autosomal disorder characterized by partial cutaneous albinism and immunodeficiency due to failure of cytotoxic T lymphocytes (CTLs) to secrete the content of their lytic granules. *RAB27B* encodes a homologous protein with distinct, but partially overlapping functions. In addition to being expressed in melanocytes and cytotoxic T lymphocytes, Rab27A is highly expressed in secretory granule-rich neuroendocrine, exocrine and immune cells. The discovery of three distinct types of mammalian Rab27A effectors [Synaptotagmin-like protein (Slp), Slp homologue lacking C2 domains (Slac2), and Munc13-4] that specifically bind the GTP-bound active form of Rab27A has greatly accelerated our understanding of the molecular mechanisms of Rab27A-mediated membrane traffic. To explore the roles of Rab27A and Rab27B in coordinating secretory granule exocytosis and the endocytosis and recycling of secretory granule membrane proteins, we turned to AtT-20 cells, a well characterized mouse corticotrope tumor cell line which utilizes two well characterized secretory pathways: the constitutive pathway for immediate secretion and the regulated pathway for storage and release in response to stimulation. We first established the specificity of commercially available Rab27A and Rab27B antibodies and used GFP-tagged Rab27A and Rab27B to determine that both proteins are expressed in AtT-20 cells, with levels of Rab27B approximately 3-fold higher than levels of Rab27A. Immunofluorescence microscopy demonstrated that both Rab27A and Rab27B were associated with vesicles; only a small fraction of these vesicles contained ACTH. To evaluate function, we designed lentiviral vectors containing shRNA constructs specific for Rab27A or Rab27B; knockdown lines for Rab27A, Rab27B, and both Rab27A/Rab27B were established. Western blot analysis demonstrated a 40-50% reduction in the single knockdown lines and a more than 80% reduction in the double knockdown line. Based on the crystal structure of the Rab27A-Slp2a complex, we designed a FRET-based Rab27 biosensor that will allow us to relate Rab27 activation to the events involved in secretory granule exocytosis and the internalization of granule membrane proteins.

Grant support: DK032948 and GM117061.

Symposium B: Talk No.7

Aging in Primary Astrocyte Cultures Impacts Inflammation-induced Extracellular Vesicle Functions as Part of the Senescence-associated Secretory Phenotype

Cory Willis, Antoine Menoret, Alexandra Nicaise, Evan Jellison, Anthony T. Vella, Stephen J. Crocker
Departments of Neuroscience[1] and Immunology[2],
University of Connecticut School of Medicine, Farmington, CT

Astrocytes regulate key homeostatic functions in the central nervous system (CNS) and robustly respond to injury and inflammation. To better understand how astrocytes may influence their local environment within the CNS in response to inflammation, we have examined how extracellular vesicles (EVs) released by astrocytes in response to exposure to the proinflammatory cytokine interleukin-1β (IL-1β) related to the reaction of astrocytes to mechanical injury. We find that IL-1β-treated astrocytes released EVs, that when isolated, potently recapitulated the inhibitory effect of this cytokine on astrocyte responses in a scratch wound assay. It is well recognized that increased age is a risk factor for developing neurological disease and limiting potential for regeneration and recovery. To determine whether the age of astrocytes in culture was a factor that influenced the response of astrocytes to IL-1β and EV function, we next maintained astrocytes for several months *in vitro* and established that these cultures developed markers of cellular aging (as indicated by cellular senescence markers). In contrast to young astrocyte cultures, IL-1β-treated aged astrocytes was found to enhancedscratch wound recovery. Moreover, EVs collected from IL-1β-treated aged cultures were found to evoke an “aged” wound recovery response when applied to naive young astrocytes. Similarly, the EVs collected from IL-1β-treated young astrocyte cultures were also found to evoke a “young” scratch wound response in the aged cultures. These findings provide novel evidence for a potentially important function of astrocyte EVs as a active process involved in an astrocyte response to inflammation and mechanical injury that is also influenced by aging.

Support: This work was supported by funding from the National Institutes of Health (NIH R21NS087578, to SJC and ATV) and the National MS Society (RG-5001-A-3 to SJC).

Poster Session 1 / No.1

Interactions between ethanol and BDNF modulate presynaptic glutamate release at cortical synapses

Mason L. Yeh & Eric S. Levine

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

Acute ethanol (EtOH) exposure produces intoxication through its actions on synaptic neurotransmission in the central nervous system. However, the precise pre- and/or postsynaptic targets of EtOH in modulating glutamatergic transmission remain to be fully elucidated. Our group and others have shown that EtOH can modulate the activity of postsynaptic NMDA receptors in CA1 of mouse hippocampus and layers 2/3 of the overlying cortex. In particular, we have found that low concentrations of EtOH (10 mM) can prevent the increase in NMDA receptor activity triggered by exposure to the neurotrophin BDNF. In the present studies, we extended this work to investigate the modulation of presynaptic glutamate release by EtOH, and potential interactions with BDNF. In initial experiments using whole-cell patch clamp electrophysiology, we monitored miniature excitatory postsynaptic currents (mEPSCs) in the presence of EtOH (10 mM) and found no changes in frequency or amplitude of mEPSCs in pyramidal cells in layer 5 of visual cortex or CA1 of the hippocampus. In a separate study, we established that BDNF acts at the presynaptic terminal to rapidly potentiate glutamate release in these brain regions, an effect which is dependent on presynaptic NMDA receptors. We adapted this paradigm to probe for the role of EtOH in modulating presynaptic release of glutamate. Consistent with previous results, we observed an increase in mEPSC frequency, but not amplitude, in layer 5 of mouse visual cortex and CA1 of the hippocampus after bath-application of BDNF. Interestingly, the effect of BDNF on mEPSC frequency was blocked in the presence of EtOH, suggesting that EtOH may affect presynaptic release properties. Furthermore, our data suggests that presynaptic NMDA receptors are a target of EtOH. In addition, we will probe for other presynaptic targets by examining different concentrations of EtOH, as this may determine the modulatory role of EtOH on glutamate release. These studies may provide valuable insight to the mechanisms underlying the proper coding of information and learning and memory, which may be compromised due to EtOH consumption.

This work was supported by NIH/NIMH R01 MH094896.

Poster Session 1 / No.2

Applying Rule-Based Modeling to understand UBE3A's role in dendritic spine morphogenesis.

Judy E. Bloom[1,2], Carissa Sirois[1,3], Noelle Germain[3], Fabian Offensperger[4], Martin Scheffner[4], Stormy J. Chamberlain[3], Michael L. Blinov[2], and Leslie M. Loew[2]

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UBE3A encodes for the ubiquitin ligase UBE3A which targets substrates to be degraded by the 26S proteasome. In neurons, only the maternal copy of *UBE3A* is expressed due to genomic imprinting. The loss of the maternal copy of *UBE3A* results in Angelman Syndrome (AS), a neurodevelopmental disorder characterized by absent speech, motor dysfunction, severe seizures, intellectual disability, and a happy demeanor. Despite the cause of AS being well established, the mechanism behind UBE3A is still unknown. Multiple targets of UBE3A have been proposed, but many have failed to be found in neurons. The lack of ubiquitination and consequently degradation of UBE3A substrates is predicted to increase the substrate protein levels in AS.

We have developed a rule-based model to investigate UBE3A's role in protein degradation and possible relation to dendritic spine morphogenesis. Our model encompasses the ubiquitin proteasome pathway (UPP), co-activators of UBE3A, possible UBE3A substrates, and upstream signaling of the UPP. We have modeled an AS patient's point mutation in UBE3A's HECT domain which diminishes ubiquitin ligase activity. *In vitro* assays have shown that the ubiquitin ligase activity of this mutant can be rescued with E6, a co-activator of UBE3A. However, our model does not show the same robust results in the presence of the entire UPP. When we simplify our model to only have the same molecules as the *in vitro* assays we successfully recapitulate the results. This mimics a current issue in the field with confirming UBE3A substrates *in vivo.* Our goal is to use model to understand the differences seen between *in vivo* and *in vitro* experiments.

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

Poster Session 1 / No.3

Axotomized adult retinal ganglion cells stimulated by extrinsic cues in a permissive environment survive and regenerate axons

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Retinal ganglion cells (RGCs) are projection neurons in the eye which, like other projection neurons in mammalian central nervous system (CNS), do not regenerate axons disrupted by an injury. The failure of axons to regenerate and restore long-distance connections between neurons limits recovery from ischemic or traumatic injury in the CNS. For example, blindness caused by trauma or stroke to the optic nerve disrupts the axons through which the RGCs pass information from the eye into the brain, resulting in irreversible blindness. Manipulation of various cell-autonomous and extrinsic factors identified to date stimulate only modest axon regeneration in the CNS, and no clinical treatments exist that could help patients with axonal injuries. Thus, the failure of RGC and other CNS axons to regenerate after injury remains a major unmet problem. Here, we used bioinformatic analysis of RNA-seq transcriptome profiles of RGCs to predict extracellular matrix (ECM) molecules with which embryonic RGCs (that grow axons robustly) could interact, and then tested their effect in culture on RGCs isolated by immunopanning for Thy1.1. We found that not only did one of these ECM molecules (identity masked due to proprietary information) enabled axotomized adult rodent RGCs to survive for a long period, but also stimulated long-distance axon growth in a permissive culture environment. Thus, the identified ECM molecule holds potential for promoting axon regeneration after injury to the optic nerve, and perhaps other parts of the CNS as well.

Poster Session 1 / No.4

Posture and Neuroendocrine Release: “Power Pose” Findings Tested with Novel Poses

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This experiment examined previous findings relating to posture and neuroendocrine release using novel postures. Widely reported previous finding found changes in cortisol and testosterone linked to specific body poses. Culturally and psychologically neutral postures using the same metrics (open/expansiveness) were implemented to see if any hormonal findings could be replicated. A measure of the changes to the levels of estrogen within the subject were examined along with cortisol and testosterone which have been studied previously. 17B-estradiol, testosterone, and cortisol levels were of interest due to their significant effects on the body, not just during maturation but throughout the life cycle. If physiology can be altered by simple bodily positioning, the phenomena could be used to improve the quality of peoples' lives in a minimally invasive way by influencing both physical and mental health.

Measurements were taken via passive drool salivary sampling at two time points: baseline and ~10 minutes later. Enzyme-linked immunosorbent assays were used to quantify neuroendocrine release for human subjects (12 female, 7 male). The hypothesis was testosterone and estradiol levels would increase in more expansive/obtuse postures while cortisol would decrease, and the opposite trends would be seen in more confined/acute poses. A questionnaire was used both to collect demographic information on the subject and on their experience. This attempt to identify and control for confounding variables doubled as a uniform way to occupy the subject before the second saliva sample was collected, allowing for changes in blood hormonal levels to be reflected in saliva samples. While the exact strength of correlation between plasma and salivary steroid levels vary by hormone and subject, the correlations from plasma hormone leakage into saliva are generally found to be strong enough for research purposes.

The results from this experiment had mixed results tending toward all postures lowering cortisol, and increasing testosterone and 17β-estradiol to a lesser degree. Acute and obtuse postures did not have any statistically significant differences. Further study comparing conscious or mindful body positioning compared to some unstructured control could help determine the significance of the results obtained.

Support: All research funded by the University of Hartford Neuroscience Program, MS.
200 Bloomfield Ave West Hartford.

Poster Session 1 / No.5

Effects of the ketogenic diet on behavioral responses to cocaine in rats

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The ketogenic diet (KD) is a high fat, low carbohydrate/protein formulation that has traditionally been used as a treatment for epilepsy; however, there is growing evidence that this diet may have much broader therapeutic potential in areas such as pain, learning and memory, and social behavior. Although the mechanisms of action of the KD are complex, one downstream consequence of the increase in blood ketones associated with the KD is an increase in adenosine availability within the brain. Of the multiple subtypes of adenosine receptors, the adenosine A_{2A} receptor subtype is found almost exclusively within the striatum, an area of the brain important for reward and reinforcement. The A_{2A} receptor colocalizes with the dopamine D_2 receptor in this brain area, and these two receptors act antagonistically to modulate dopamine-dependent behaviors. Recent studies have shown that pharmacological activation of the A_{2A} receptor reduces locomotor activity in rats induced by both acute and repeated cocaine injections, similar to the effects of a D_2 receptor antagonist. Hence, we predicted that a ketogenic diet would similarly decrease cocaine-induced locomotor responses in rats. Male and female Sprague-Dawley rats were placed on a strict 6.6:1 (fat:[carbohydrates+protein], by weight) KD or control diet at 5 weeks of age and then maintained on those diets for 3 weeks prior to behavioral testing. During testing, rats received daily i.p. injections of cocaine (15 mg/kg/ml) or saline vehicle for one week, were abstinent for a subsequent week, and then all animals received a final challenge injection of 15 mg/kg/ml cocaine. Our preliminary results suggest that the enhanced rearing (a form of stereotypy) induced by the challenge cocaine injection in animals previously injected with cocaine (vs. saline) was eliminated in KD females. This effect was not observed in KD males. These findings provide the first evidence that the KD can reduce behavioral responses induced by repeated exposure to drugs of abuse. Future studies will more specifically examine how sex steroid hormones contribute to the observed sex differences in effects of KD, as well as explore the neural mechanisms underlying the behavioral effects of this diet.

Poster Session 1 / No.6

Molecular basis of antidromic rectification of gap junctions between AVA interneurons and motor neurons in *C. elegans* escape circuit

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C. elegans AVA command interneurons play important roles in escape behavior, and contact A-type cholinergic motor neurons (A-MNs) through both electrical and chemical synapses. Our recent study shows that the gap junctions (GJs) between AVA and A-MNs only allow antidromic currents (from A-MNs into AVA), and that the function of these GJs depends on UNC-7 innexin in AVA and UNC-9 innexin in A-MNs (Liu et al., *Nat Commun* 2017). However, molecular basis of the antidromic rectification is unknown. To address this question, we began by expressing UNC-7 and UNC-9 in *Xenopus* oocytes, and analyzing biophysical properties of homotypic and heterotypic GJs formed by them. While UNC-9 has only one isoform, UNC-7 has at least three different isoforms (UNC-7a, UNC-7b and UNC-7c), which differ in the length of the amino terminal. UNC-7c has a short amino terminal (24 residues before the 1st membrane-spanning domain/TM1) like UNC-9 (26 residues before TM1) whereas UNC-7a and UNC-7c have additional 120 and 52 residues, respectively, before the first amino acid of UNC-7c. We recorded junctional currents (I_j) from paired oocytes by holding one oocyte at a constant voltage (-30 mV) while applying voltage steps of -150 mV to +50 mV (at 10-mV intervals) to the other oocyte. The voltage difference between the two oocytes is the junctional voltage (V_j). We measured the steady-state I_j at all the V_j steps (-120 to +120 mV), plotted the G_j – V_j relationship, and fitted the G_j – V_j relationship to a Boltzmann function. We found that homotypic GJs of UNC-9 and UNC-7c are similar in the G_j – V_j relationship but are very different from those of UNC-7a and UNC-7b. Although all the UNC-7 isoforms may form heterotypic GJs with UNC-9, only one of them can form heterotypic GJs that allow unidirectional current flow (from UNC-9 oocyte to UNC-7 oocyte). Expression of this specific UNC-7 isoform in AVA interneurons in an *unc-7* mutant significantly restored the antidromic junctional currents between AVA and A-MNs whereas the other two UNC-7 isoforms had either no effect or a much weaker effect. Taken together, our results suggest that 1) the amino terminal domain of UNC-7 plays important roles in GJ gating; 2) GJs between AVA and A-MNs probably consist of UNC-9 in A-MNs and a specific UNC-7 isoform in AVA; and 3) interactions between UNC-7 and UNC-9 hemichannels can reciprocally influence their gating properties.

Support: R01GM113004 (B.C.) and 2R01MH085927 (Z.-W.W.)

Poster Session 1 / No.7

Studying the Physiology of Thin Dendrites by Voltage-sensitive dye and Calcium-sensitive dye Imaging

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Thin dendritic branches of cortical pyramidal neurons receive and process information from other neurons in the form of electrical signals. The integration of electrical signals from a large number of synapses onto a single neuron is a complex process, underlying information processing in the mammalian brain, sensory perception, cognition and motor output. In order to understand synaptic integration and overall rules of information processing in individual cortical neurons, it is necessary to understand dendritic membrane properties and rules of dendritic integration (summation of synaptic inputs). The gap in the understanding of these mechanisms and processes is caused by technical limitations related to studying the physiology of thin dendrites. Thin dendrites do not tolerate standard microelectrode recordings. To overcome this problem here we utilize optical imaging of physiological signals occurring in distal dendrites. Layer 5 pyramidal cells in brain slices were patched (whole-cell) injected with voltage-sensitive dye JPW-3028, or calcium sensitive dye OGB1, and the dye was allowed to diffuse into the dendritic tree for at least 60 min. Fluorescent images of dendritic contours were then projected onto the fast CCD camera and sampled at 1,000 – 2,000 frames per second. Brief pulses of depolarizing current were injected into the cell body to evoke action potentials. Backpropagating action potentials were recorded optically in basal dendrites. Synaptic stimulations were used to evoke excitatory synaptic potentials and dendritic NMDA spikes, while recording optically at the site of input, in basal dendrites. Our goal is to characterize both voltage and calcium waveforms underlying local dendritic voltage transients (bAPs, EPSPs and NMDA spikes) and correlate them with the somatic voltage transients recorded via standard patch microelectrode. We plan to deduct dendritic membrane properties and the overall functional organization of the basilar dendritic tree by comparing the aforementioned voltage waveforms in subsequent sweeps; each sweep producing data from the same dendritic segment.

Poster Session 1 / No.8

Identification of Novel Remyelinating Agents for Primary Progressive Multiple Sclerosis

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Primary progressive multiple sclerosis (PPMS) is a chronic demyelinating disease of the central nervous system (CNS) without any effective treatment. Patients with PPMS generally do not benefit from currently available immunomodulatory therapies which can be effective for relapsing-remitting MS (RRMS) patients. Hence, promoting endogenous brain repair by promoting the differentiation of myelin-forming oligodendrocytes from resident oligodendrocyte progenitor cells (OPCs) is viewed as a therapeutic goal by which to halt and possibly restore neurologic function in PPMS patients. We have recently developed and characterized induced pluripotent stem (iPS) cell lines from PPMS patients blood samples. From this work we concluded that there is an inherent defect in the ability of neural progenitor cells (NPCs) from PPMS lines to promote or support OPC differentiation (Nicaise et al. 2017 Exp Neurol.). As part of this work, we had shown that conditioned media (CM) from PPMS iPS-derived NPCs negatively impacted the response of OPCs to differentiate *in vitro.* This finding suggested that modeling PPMS using iPS-derived NPCs differentially effected the efficacy of recently reported remyelinating therapies to promote OPC differentiation. Based on these results we hypothesize that by using patient-derived iPS lines we may model the disease microenvironment in PPMS and therein develop a disease-emulating cell culture system. Using a candidate-based approach, our preliminary data indicate that we have identified compounds that can reverse the deficit in OPC differentiation using this cell culture disease modeling system. Given that this drug was not identified in previously published remyelinating drug screening studies, we suggest that rescreening drug candidate libraries using our disease modeling cell culture system may identify candidate compounds with therapeutic potential that would otherwise be overlooked. This finding also suggests that traditional screening approaches using non-disease modeling settings may have a high false negative discovery rate. This presentation will outline the design for development of a novel drug screening assay and the candidate validation approaches to be used to complement compound identification. Together, are expected to identify compounds with novel potential to promote brain regeneration in PPMS patients

Poster Session 1 / No.9

Engineering of near-infrared genetically encoded voltage indicators.

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Simultaneous optical modulation and readout of neuronal circuit activities is a promising neurotechnology, which could help us decipher how the brain's electrical signals relate to perceptual, cognitive, emotional and motor functions. During recent years, the use of genetically encoded (optogenetic) actuators such as channelrhodopsin, became overwhelmingly successful. On the other hand, genetically encoded voltage indicators (GEVIs) have not yet been satisfactorily optimized and their combination with optogenetic modulation has been difficult to achieve in practice. One major obstacle is the overlap of the spectral bands of light used to activate opsin-based actuators and at the same time excite and image available GEVIs. We propose to use novel bacteriophytochrome-based fluorescent proteins (FPs) to generate a new class of GEVIs that are excited and emit fluorescence in the near-infrared (NIR) spectrum (e.g. 720 nm). For this, we have engineered and characterized a set of spectrally diverse monomeric NIR FPs, termed miRFPs, and produced an effective FRET pair consisting of miRFP670 and miRFP720 proteins. Using this FRET pair we then designed several ratiometric FRET-based NIR GEVI constructs. We have also engineered several intensiometric NIR GEVI variants based on single NIR FPs, either miRFP703 or miRFP720. Both types of the NIR GEVI constructs demonstrated good plasma membrane localization and exhibited up to 4% ΔF/F in a response to a 100 mV depolarizing step in membrane voltage in patch-clamped HEK293 cells. To further optimize these NIR GEVI constructs, we apply an all-optical screening platform consisting of imaging hardware and mammalian cells stably expressing optogenetic actuators.

Supported by NIH Grant 1U01NS099573-01.

Poster Session 1 / No.10

Distinct mechanisms of hyperexcitability in chromosome 15q-associated neurodevelopmental disorders

Jeremy D. Schreiner, James J. Fink, Tiwanna M. Robinson, Eric S. Levine

The duplications and deletions of maternal chromosome 15q11.2-13 results in two clinically distinct neurodevelopmental disorders named Duplication 15q syndrome (Dup15q) and Angelman syndrome, respectively. Despite their opposite genetic causes, Dup15q and Angelman syndrome present with similar phenotypic traits, such as cognitive impairment, autism, and seizures. This may be in part due to a shared molecular pathway, since the chromosomal region implicated in Dup15q and Angelman syndrome encodes for E3 ubiquitin ligase (Ube3a), the known causative gene of AS. Using patient-specific induced pluripotent stem cell- (iPSC) derived neurons, we are able to directly record electrophysiological voltage and current characteristics from Dup15q and Angelman patient cells. We have previously shown that Angelman neurons have a unique phenotype that results in a more depolarized resting membrane potential (RMP). In this study, we have found that Dup15q neurons do not show this particular hyperexcitable phenotype, but instead show an increased rate of spontaneous action potential firing. Such a finding is an attractive mechanism for the high prevalence of seizures in Dup15q. In order to find out which specific channels may be responsible, we used pharmacological activators and blockers of a variety of channels/receptors associated with spontaneous action potential firing. We found that both Dup15q and control neurons respond similarly to activation and/or blockade of SK2, T-type calcium, BK, and HCN channels as well as GABA-A receptors. However, controls respond more strongly to general SK blockade and KCNQ block. Interestingly, Dup15q and Angelman neurons respond oppositely to blockade of KCNQ2, with Dup15q neurons having a diminished response compared to controls and AS neurons have a more dramatic response. We find this to also be the case when monitoring population firing using activators/blockers of KCNQ2. Finally, we find decreased neuronal expression of KCNQ using immunocytochemistry and flow cytometry. Overall, these distinct cellular phenotypes may prove useful for identifying novel targets for drug discovery and for screening potential therapeutics aimed at reversing the seizures and other symptoms associated with Angelman syndrome and Dup15q.

Symposium C: Talk No.8

Title: Deletion of the P2X4 receptor is neuroprotective acutely, but induces a depressive phenotype during recovery from ischemic stroke

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Abstract

Acute ischemic injury leads to severe neuronal loss by inducing inflammatory cascade, mediated mostly by activation of resident microglia and infiltrating monocyte/macrophages. P2X4 receptors (P2X4Rs), present on these immune cells fine-tune the inflammatory responses after acute injury. Excessive release of ATP during acute ischemic stroke triggers P2X4Rs, which leads to myeloid cell activation and proliferation to further increase post- ischemic inflammation. During recovery, however, P2X4Rs activation leads to the release of microglial brain-derived neurotrophic factor, which is an important factor in the maintenance of synaptic plasticity, cognition, and post-stroke behavioral recovery. We, therefore, hypothesized that deletion of the P2X4R specifically on myeloid cells would reduce infarct damage acutely while inducing depressive behavior during chronic recovery. Global or myeloid-specific (MS) P2X4R knockout (KO) mice and wild-type littermates of both sexes were subjected to right middle cerebral artery occlusion (60 min) followed by three or thirty days survival. Histological studies and behavioral analyses testing sensorimotor, memory, and cognitive function were conducted to determine the acute and chronic effects of receptor deletion.

Global P2X4R deletion led to reduced infarct size in both sexes. In MS P2X4R deletion, only female mice showed reduced infarct size, an effect which did not change with ovariectomy. MS P2X4R KO of both sexes showed swift recovery from sensorimotor deficits during acute recovery but exhibited a more pronounced post-stroke depressive phenotype that was independent of infarct size. Quantitative PCR analysis of whole cell lysate, as well as flow, sorted myeloid cells from the perilesional cortex showed increased cellular IL-1β, IL-6, and TNF-α mRNA levels but reduced plasma levels of these cytokines in MS P2X4R KO mice after stroke. We also found reduced BDNF gene expression in MS P2X4R KO. In conclusion, P2X4R deletion protects against stroke acutely but predisposes to depression-like behavior chronically after stroke. A time-sensitive approach should be considered when targeting P2X4Rs after stroke.

Key Words: P2X4R, Stroke, myeloid-specific, depression, neuroprotection

Funding: This work was supported by an institutional startup grant and AHA grant (14POST20380612) (R.Verma), The endowed Ray Neag Distinguished Professorship (B Liang),

Symposium C: Talk No.9

Establishing a molecular phenotype for Angelman Syndrome induced pluripotent stem cell-derived neurons

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Angelman Syndrome (AS) is a neurodevelopmental disorder occurring approximately once in every 15,000 live births, characterized by severe seizures, absent speech, motor dysfunction, profound intellectual disability, and happy demeanor. Loss of expression of the maternal copy of *UBE3A*, a gene regulated by tissue-specific genomic imprinting, causes AS. *UBE3A* encodes an E3 ubiquitin ligase that may also act as a transcriptional co-activator. We have derived induced pluripotent stem cells (iPSCs) from an AS patient with a missense mutation in *UBE3A* (F583S). The mutation is located in the HECT domain of the protein, which confers ubiquitin ligase activity. This mutation does not affect UBE3A RNA or protein levels, but causes a reduction in the protein's ubiquitin ligase activity, as demonstrated by *in vitro* ubiquitination assays. Using CRISPR/Cas9-mediated genome editing, we have corrected this point mutation in the AS F583S iPSCs, generating an isogenic control iPSC line (F583S-CTRL). Both F583S and F583S-CTRL iPSCs have been successfully differentiated into 12-week forebrain neurons. We present here preliminary electrophysiological data from these neurons. Whole-cell patch-clamp recordings were performed on 12-week F583S and F583S-CTRL neurons to determine the extent to which these AS iPSC-derived neurons recapitulate the electrophysiological phenotypes seen in other AS iPSC-derived neuron lines. Strand-specific mRNAseq was also performed on each neuron line to establish a molecular transcriptome phenotype for AS iPSC-derived neurons. We found that there were 742 significantly differentially expressed transcripts in the AS neurons as compared to the isogenic controls (padj. < 0.001). qRT-PCR was used to validate a handful of transcripts from the mRNAseq data. Together, these data provide us with a robust phenotype that can be used to assess the efficacy of pharmacological treatments for AS in human neurons.

Support: NIH/NICDH; Connecticut Regenerative Medicine Fund; Angelman Syndrome Foundation; Fighting Angels Foundation

Symposium C: Talk No.10

Effect of Lead on NGF Regulated Embryonic Axonal Development: Novel understanding from chicken embryonic Dorsal Root Ganglion Cultures

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Lead consumption continues to be a severe problem, even in developed countries, despite the fact that its effects, including cognitive impairments, mobility issues, and organ damage, are common knowledge. This problem is especially pronounced when lead is present during fetal development, however the effects of lead on fetal neuronal development are still understudied. Nerve growth factor is essential for regulating neuronal growth, proliferating and differentiating axons, and sensory nerve survival; it is also incredibly vulnerable as it is holistically affected by lead toxicity. In this study, we investigated the growth and differentiation of dorsal root ganglion (DRG) cells, cultured from 8-10 day old chicken embryos and exposed to nerve growth factor (NGF) (200ng/ml). Four primary cultures (NGF only, DRG only, Lead only, and Lead & NGF; 12 DRG cells per culture) were observed and compared for six days, using ZEN and QCapture to document axonal growth daily. Repeated experiments and different concentrations of lead served as further levels of treatment. Preliminary results suggested growth and differentiation differences in axonal development. Overall, DRG cells were less dense and have less overall neurite outgrowth when treated with lead, with or without the presence of NGF. Digital phase contrast live images of these cultures were analyzed by Image J and continued analysis by the Sholl method along with immunostaining, using neuron markers with nucleus florescent counterstaining. Our findings showed stunted axonal growth when DRG developed in growth medium concentrated with Lead Acetate Trihydrate comparing to those that have not been exposed to lead during their developmental process.

Support for this work was provided through the Dorothy Goodwin Scholarship to KV by The Women's Advancement Initiative, advancing each woman's potential in the HCW tradition at the University of Hartford, and the Dean's research fund to YL from University of Hartford.

Symposium D: Talk No.11

Ketogenic Diet Produces Rapid, Region-Specific Alterations in Brain NAD^+ Impacting Downstream Effectors

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The ketogenic diet's (KD) anti-epileptic effects have long been documented, and recent research highlights its therapeutic potential in various brain-related disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and traumatic brain injury. Although fundamental mechanisms underlying its beneficial effects across diverse neurological disorders are not well understood, the KD's high fat, low carbohydrate, and moderate protein composition lowers glucose availability, increases ketone utilization as an energy source, and improves mitochondrial function. Cellular energy production depends on the availability of nicotinamide adenine dinucleotide (NAD), an essential metabolic coenzyme and a signaling molecule that exists in oxidized and reduced forms, NAD^+ and NADH, respectively. The oxidized form, NAD^+, is a marker for mitochondrial and cellular health and serves as a substrate for Sirtuins, a deacetylating group of enzymes that modulate gene expression, inhibit inflammation and increase longevity. NAD^+ also serves as a substrate for PARP-1, an ADP-ribosyltransferase that plays a major role in DNA damage repair and serves as a marker for oxidative DNA damage. Based on the differential utilization of NAD during glucose-based versus ketone-based ATP generation, we hypothesized that the primary mechanism behind the beneficial effects of the KD is altered cellular levels of NAD. Normal rats were fed ad libitum regular chow or KD for 2 days or 3 weeks followed by ketones, NAD, Sirtuins, and PARP quantifications. Significant increases in ketones, NAD^+ levels, and NAD^+/NADH ratio were detected as early as 2 days and remained elevated at 3 weeks, indicating a KD-induced early and persistent metabolic shift in specific brain regions. Analysis of important NAD^+ downstream targets showed significant changes in enzymes' levels and activities that could impact mitochondrial and cellular health. The results of these alterations in downstream pathways are being currently evaluated. Increasing NAD^+ is a coveted therapeutic endpoint for health and longevity. Our studies show that a KD induces a rapid and region-specific increase in NAD^+ levels in the brain which results in significant alterations of downstream pathways important for limiting cellular and DNA damage. These changes may be at the core of the molecular mechanisms of the diet and significantly contribute to the beneficial effects of this metabolic therapy in diverse brain disorders.

Support: Women Advancement Inititive, University of Hartford, College of Art and Science, University of Hartford

Symposium D: Talk No.12

A Novel Vimentin Probe Illuminates Mitochondrial Dynamics

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Purpose: WFA-Verde is the *first-in-class* vimentin-binding imaging probe. As vimentin is known to bind to mitochondria, we have employed WFA-Verde as a probe to investigate vimentin-dependent mitochondrial activity.

Methods: We employed baby hamster kidney (BHK-21) cells and primary rabbit corneal fibroblasts (RbCFibros). Cells were cultured in glass bottom dishes for 18 h in complete DMEM, prior to experiments. BHK-21 cells were treated for 30 min with MitoTracker Red CMXRos (50 nM) in the presence or absence of nocodazole (10 μM), and then pulsed with WFA-Verde (250 nM) for 5 min. WFA-Verde labeled filaments and mitochondria were recorded live using epifluorescence. In other experiments, BHK-21 cells were treated only with nocodazole, and WFA-Verde labeled cells were fixed/permeabilized, and stained with β-tubulin antibody. To follow mitochondrial dynamics, BHK-21 cells were starved for 2 h with glucose-free DMEM medium, treated with MitoTracker, washed, pulsed with WFA-Verde and then treated with sodium azide (NaN3, 0.05%) and 2-deoxy-glucose (2-DG, 50 mM). Mitochondrial fission was followed live as described above. Mitochondria-vimentin interactions were also tested in RbCFibros. Cells were treated with MitoTracker Red (50 nM) for 30 min, pulsed with WFA-Verde (250 nM) for 5 min, and followed live as described above.

Results: WFA-labeled vimentin co-localizes with mitochondria in both BHK-21 cells and RbCFibros. Disruption of the microtubules (MTs) with nocodazole causes collapse of both WFA-Verde structures and mitochondria in BHK-21 cells. Interestingly, WFA-Verde-labeled vimentin did not overlap with β-tubulin staining, revealing a specific correlation with mitocochondria rather than with MTs. Glucose deprivation forces mitochondria to elongate, and MitoTracker labeled the tubular structures. WFA-Verde co-stained these long mitochondria showing overlap with MitoTracker labeling. The induction of reactive oxygen species by treatment with 2-DG and NaN3 caused WFA- Verde-labelled vimentin filaments to became fragmented, following mitochondria fission.

Conclusions: WFA-Verde labels vimentin structures that co-localize with mitochondria and faithfully reflects mitochondrial dynamics after perturbation of MTs. This probe also affords the monitoring of mitochondrial reorganization, and fragmentation after induction of fission. WFA-Verde can be a useful diagnostic tool to study vimentin- mitochondria interactions in ocular injury and disease.

Commercial Relationships: Paola Bargagna-Mohan, University of Kentucky; US patent 8,735,178 (P), University of Connecticut; Application No. 62/374,376 (P); Santosh Keshipeddy, University of Connecticut; Application No. 62/374,376 (P); Dennis Wright, University of Connecticut; Application No. 62/374,376 (P); Royce Mohan, University of Kentucky; US patent 8,735,178 (P), University of Connecticut; Application No. 62/374,376 (P)

Support: R01EY016782; John A. and Florence Mattern Solomon Endowed Chair

Symposium D: Talk No.13

Reversing Effort-Related Motivational Impairments with the Adenosine A_{2A} Receptor Antagonist Preladenant

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Adenosine plays an important role in regulating sleep, cognition, motor control, and motivation. While there are four adenosine receptor subtypes, A_1 and A_{2A} are the primary subtypes in the brain. A_1 receptors have a broad distribution, but postsynaptic A_{2A} receptors are highly concentrated in the striatal complex, where they are largely located on GABAegic enkephalin-positive medium spiny neurons that also express dopamine (DA) D2 receptors. Co-localized A_{2A} and D2 receptors are capable of forming heteromers, converging onto the same metabotropic signal transduction pathway. Because DA is known to play a central role in effort-based aspects of motivation, the interactions between drugs acting on DA and adenosine have been investigated. Studies of effort-related choice allow animals the option of selecting a high-effort activity for a preferred reward vs. a low-effort low reward option. DA antagonism and accumbens DA depletions induce a low-effort bias in rodents, which can be reversed by inhibiting adenosine A_{2A} receptors, while A_1 receptor antagonists are ineffective at reversing the effects of D1 or D2 antagonists. This highlights the particular importance of understanding the role of A_{2A} receptors in effort-related aspects of motivation. Tetrabenazine (TBZ) is a VMAT-2 inhibitor that blocks DA storage, depletes accumbens DA, and reduces D1 and D2 signaling. TBZ induces depressive symptoms and motivational impairments in humans, such as fatigue and anergia. In rodents, TBZ produces a low-effort bias in effort-based choice studies. At baseline, rats tested on the fixed ratio (FR) 5/chow feeding choice task demonstrate a preference for lever pressing for the more palatable food choice and eat little concurrently available chow (low effort/low reward option). TBZ-treated rats shift away from lever pressing and increase chow consumption. Previous research reported that the adenosine A_{2A} receptor antagonist MSX-3 could reverse the effects of TBZ. More recent research has focused on preladenant, a highly selective A_{2A} receptor antagonist that is being studied preclinically and clinically for its potential use in the treatment of Parkinsonism and depression. Preladenant reversed the effort-related effects of TBZ in rats tested on the FR5/chow feeding choice task and increased high-effort progressive ratio lever pressing when administered alone. The ability of preladenant to enhance effort-related motivational functions underscores the potential utility of selective A_{2A} receptor antagonists for the treatment of motivational dysfunctions associated with psychiatric or neurological disorders such as depression, Parkinsonism and schizophrenia.

Poster Session 2 / No.11

Transcriptional and physiological profiling of individual sensory neurons and peripheral tissue following spinal contusion injury

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Persistent pain following SCI is an adverse consequence of neural injury and is a debilitating concern for most patients. Extensive work has characterized spinally-mediated alterations that contribute to ongoing pain processing, but it is now evident that primary afferents also play a role in the generation and persistence of SCI pain. Here we examine SCI-induced changes in gene expression and the physiological properties of sensory neurons, as well as the skin and muscle in which they innervate. Mice received a spinal contusion injury at T10-11, which resulted in paralysis of the hind limbs. Mice were then sacrificed 1 or 7 days following SCI to examine alterations in gene expression in hind paw hairy skin, quadriceps femoris, as well as spinal cord and dorsal root ganglia collected from above, at, and below the level of injury. Analysis of real-time RT-PCR data revealed increased expression of multiple pain-related genes in whole tissue, such as ASIC1, ASIC3, Calc-α, and TRPA1. We also performed single cell real-time RT-PCR to elucidate changes in gene expression within individual sensory neurons. Cutaneous afferents were backlabeled via pressure injection of WGA and IB-4 conjugated fluorescent dyes into the saphenous nerve to identify peptidergic and nonpeptidergic afferents, respectively. Individual fluorescently labeled neurons were collected and subjected to real-time RT-PCR. Analysis of WGA-labeled neurons revealed significant increases in the expression of ASIC3, GFRα1-3, P2X3, TRPA1, and TRPV1 mRNA, while IB-4-labeled exhibited significant increases in the expression of ASIC3, GFR α1-3, P2X3, P2Y1, and TrkA, as early as 1 day following injury. Whole cell patch clamp recordings were also performed on dissociated back-labeled small diameter cutaneous neurons from L2 and L3 DRG 1 day following SCI, and afferents collected from injured mice exhibited increased rates of spontaneous firing when compared to naïve neurons. Our data suggest that SCI results in a rapid sensitization of afferent pathways, and that sensory neurons significantly contribute to the development of SCI-induced pain. Increases in ASIC3 expression in both peptidergic and nonpeptidergic nociceptors as early as 24hr after SCI may indicate that changes in the expression of ASIC3 drive the differential responses of nociceptors that occur following SCI.

Poster Session 2 / No.12

BKIP-1, an auxiliary subunit critical to SLO-1 function, inhibits SLO-2 potassium channel *in vivo*

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Auxiliary subunits are often needed to tailor K^+ channel functional properties and expression levels to specific cellular requirements. A number of auxiliary subunits have been identified for mammalian Slo1, a high-conductance K^+ channel gated by membrane voltage and cytosolic Ca^{2+}. Experiments with heterologous expression systems show that some putative Slo1 auxiliary subunits can also regulate other K^+ channels of the Slo family. However, it remains to be proved that a single auxiliary subunit may regulate more than one Slo channel within the same cell in native tissues. *C. elegans* has two channels of the Slo family: SLO-1 and SLO-2. The latter is also a high-conductance K^+ channel gated by membrane voltage and cytosolic Ca^{2+}. In addition, it is sensitive to cytosolic Cl^-, which acts synergistically with cytosolic Ca^{2+} in activating the channel. BKIP-1 was initially identified as an auxiliary subunit of SLO-1. It facilitates SLO-1 surface expression, regulates SLO-1 biophysical properties in a Ca^{2+}-dependent manner, and is indispensable for SLO-1 physiological function in both neurons and muscle cells (Chen et al., *J Neurosci* 2010). A recent study shows that BKIP-1 genetically interacts with both SLO-1 and SLO-2 to control terminal differentiation of AWC olfactory neurons (Alqadah et al., *PLoS Genet* 2016). However, it remains to be determined how BKIP-1 regulates SLO-2 function. In this study, we recorded SLO-2 whole-cell currents and single-channel activities of motor neurons and muscle cells in dissected worms and compared them between strains with and without BKIP-1. Surprisingly, we found that the BKIP-1 strain displayed smaller SLO-2 whole-cell currents and single-channel open probability (P_o) compared with the control strain. In addition, we found that BKIP-1 reduced SLO-2 Cl^- sensitivity and activation rate without altering SLO-2 Ca^{2+} sensitivity, and that BKIP-1 shortened the mean open time of SLO-2 single channel events. In contrast, BKIP-1 did not alter whole-cell currents mediated by SHK-1, a *Shaker*/K_V1-type K^+ channel, suggesting that BKIP-1 does not regulate K^+ channels indiscriminately. These results suggest that BKIP-1 may serve as an auxiliary subunit to inhibit SLO-2. To our knowledge, BKIP-1 is the first example showing that a single auxiliary subunit may exert opposite effects on two evolutionarily related channels in the same cells.

Support: R01GM113004 (B.C.) and 2R01MH085927 (Z.-W.W.)

Poster Session 2 / No.13

Post-traumatic Stress Disorder: Constructing and Comparing Predator Odor and Electric Footshock as Valid Animal Models

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Post-traumatic stress disorder (PTSD) is a disorder of long-term alterations in cognitive, emotional and physiological systems following the exposure to a traumatic or life-threatening event. While PTSD animal research is still in its infancy, present models and the highly traumatic methods being used call to question the validity and ethics of current approaches. In this study, male Wistar rats were exposed to either a physical stressor of electric footshock or a psychological stressor of predator odor (red fox urine) using a three-day tiered stress exposure model in conjunction with situational reminders. Behavioral data was collected using the elevated plus maze and acoustic startle response. Chronic changes in behavior were assessed at three behavioral assessment time points of 24h, 1 week and 2 weeks post-exposure. The magnitude of behavioral disruption was systematically graded using inclusion/exclusion cut-off behavioral criteria (CBC). Animals were classified as having an extreme (EBR), partial (PBR) or minimal behavioral response (MBR). Predator odor 2-Day and 3-Day rats displayed significantly disrupted behavior on the EPM and the ASR when compared to electric footshock rats. Three-Day stress exposure produced chronic disruption in behavior under both stressor conditions. All rates of EBR were induced by predator odor 3-Day exposure at all three behavioral assessment time points. These findings provide insightful information on the efficacy repetitive psychological stress over physical stress in PTSD animal research.

Poster Session 2 / No.14

Consequences of spinal cord injury on expression of pain-relevant genes in the viscera

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Patients with spinal cord injury (SCI) often report experiencing pain originating in a variety of tissues below the injury level. This pain can often transition from acute to a chronic condition and, once chronic, the pain can be difficult to treat with traditional analgesics. Post-SCI pain can significantly affect quality of life and may also impair recovery of function following SCI. Approximately one third of SCI patients report abdominal pain as a primary symptom (Ebert, 2012). Further, visceral pain, although not experienced by all SCI patients, is frequently reported as one of the most severe types of pain (Finnerup et al., 2008). The etiology of this intense pain phenotype is unclear given that visceral organs are left uninjured in SCI. However, recent evidence from somatic pain models (i.e. originating in the tissues of the limbs) suggests that SCI may alter peripheral pain processes resulting in increased pain transmission from the periphery, even in the absence of peripheral injury. The present studies were designed to evaluate changes in pain-related gene expression in the visceral organs following SCI to potentially identify candidates important for the induction of visceral pain. To induce SCI, mice received a contusion injury (70kD impact, 10 seconds dwell time) at level T10-11 using an impactor (Infinite Horizons, Precision Systems and Instrumentation; Fairfax Station, VA) (Hook et al., 2012). The injury resulted in complete paralysis below the level of the forelimbs. The mice were sacrificed and perfused with cold saline either 24 hours or 7 days after SCI and visceral organs (bladder and colon) were collected for RNA extraction. RNA was then made into cDNA for qPCR to examine expression of a panel of pain-related genes. Dorsal root ganglion (DRG) corresponding to lumbar splanchnic nerve (LSN) and pelvic nerve (PN) at T12/13-L1 and L6-S1, respectively, were also collected and cultured for immunocytochemistry (ICC) to look at protein of interest expression as identified with qPCR. We found that we were able to detect changes in the profile of expression for these genes, providing insight into potential molecular mechanisms of visceral pain induction.

Poster Session 2 / No.15

TIMP-1 regulates the emergence of cutaneous inflammatory hypersensitivity

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Chronic pain is a significant health concern that affects millions of people worldwide. Inflammatory tone is known to predict the onset of clinical pain, and determining the requisite molecules that contribute to its emergence is critical for the development of novel treatments. Peripheral injury and inflammation result in the dynamic release of molecules that aid in tissue repair as well as result in nociception. Research has shown that enzymes important for tissue remodeling, matrix metalloproteinases (MMPs), contribute to the development of neuropathic and inflammatory pain. However, the specific role of TIMP-1 in pain development is not well understood. To examine how TIMP-1 regulates inflammatory hypersensitivity, we injected the proinflammatory substance, complete Freund's adjuvant (CFA), intradermally in C57/Bl6 mice. We observed an increase in the release of TIMP-1 in the skin 24 hours following inflammation. Interestingly, this increase in TIMP-1 release was negatively correlated with the onset of behavioral hypersensitivity. To identify the cell type responsible for the release of TIMP-1 in the skin, we used the protein transport inhibitor, brefeldin A, to prevent the release of TIMP-1 in skin explants prior to *in vitro* inflammation using inflammatory soup. Immunohistochemical analysis confirmed that inflammation increased the presence of TIMP-1 in keratinocytes. These data suggest that keratinocyte derived TIMP-1 attenuates the development of inflammatory pain. To test this, we examined the impact of cutaneous inflammation in TIMP-1 KO (T1KO) mice, and found that T1KO mice develop early onset mechanical hypersensitivity that persists for at least 7 days following inflammation. Moreover, cutaneous inflammation resulted in elevated levels of circulating MMP-9, as well as increased expression of the neurotrophic factor receptors, GFRα1-3, in the skin, suggesting increased peripheral nociceptive signaling following inflammation. Finally, to test the ability of TIMP-1 to attenuate the development of behavioral sensitivity, T1KO mice received 10uL intradermal injections of recombinant mouse TIMP-1 at concentrations of 25ng/uL, 50ng/uL, or 100ng/uL at the time of 10uL CFA injection. We observed a dose-dependent attenuation of inflammatory hypersensitivity. Collectively, these results suggest that TIMP-1 acts as a gating mechanism to prevent the development of inflammatory pain, and therefore presents a novel candidate for potential therapeutic agent.

Funding Sources: R03NS096454, UCONN/UCHC InCHIP Grant, UCHC Convergence Grant

Poster Session 2 / No.16

Light-induced activation of dopamine, glutamate and GABA release on striatal medium spiny neurons in brain slices

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The nigrostriatal pathway, originating in the substantia nigra (SN) and terminating in the striatum, plays a critical role on cognitive and motor functions relevant to a variety of disorders including Parkinson's disease, addiction, and schizophrenia. Striatal medium spiny neurons (MSNs) bear dopaminergic receptors whose activation influences behavior and cognition. A transgenic mouse with light-sensitive channels in dopaminergic neurons (DAT-ChR2) is routinely used to evaluate the physiology of dopamine (DA) neurotransmission. The present study explores the simultaneous transmission of DA, glutamate and GABA, upon blue light (475 nm) activation of DAergic axons in the striatum (brain slices) by whole-cell recordings of the postsynaptic responses in MSNs. Repetitive light activation of striatal DAergic fibers revealed a strong depression of the amino acid neurotransmitter output from these fibers, with a relative refractory period of approximately 10,000 ms. However, successive light pulses on the cell bodies of DAergic neurons in SN showed a 100-fold shorter refractory period (100 ms) for firing somatic action potentials. These differences in recovery time suggest inefficient transmission of information from DA axons to MSNs, or likely the result of severing the axons in slice preparations. A pharmacological block of DA receptors did not produce any effects on light-induced synaptic currents in MSNs. In other words, DA release appeared not to have any effect on the postsynaptic glutamate and GABA currents, although both glutamate, GABA and DA were stimulated by the same pulse of light. Further exploration of light- and synaptically-induced DA-glutamate co-release will allow for a more thorough understanding of the complex relation between glutamate and DA on neuropsychiatric disorders influenced by the nigrostriatal pathway.

Supported by NIH Grant: U01MH109091

Poster Session 2 / No.17

Prospective Memory and Judgments of Learning: Examining the Effects of Traumatic and Mild Traumatic Brain Injury

Marissa Lee Stein
Faculty Advisor: Sarah Raskin, PhD.

This study examined the effects of traumatic brain injury/mild traumatic brain injury (TBI, mTBI) on prospective memory (PM) and judgments of learning (JOL). Research that has assessed the effects of TBI on JOL, and PM in particular, has shown that prospective memory failures are the most frequently reported memory deficit post- brain injury (Raskin, Buckheit & Waxman, 2011), and that those who have sustained TBI/mTBI displayed tendency to overestimate their memorization ability (JOL) while healthy adults underestimated their ability (Knight et al., 2005). In this study 15 students with TBI and 18 healthy students were presented with a computerized measure of PM JOL. Subjects were required to study and memorize word pairs to measure retrospective memory (RM), learn and memorize color cues (PM), and make predictions about how accurately they would remember them (JOL). They were then tested on their word pair recall accuracy while simultaneously being tested on color cue response. Data from this study showed a significant difference in scores between groups in word recall accuracy in the delayed recall condition only; however, contrary to what has previously been shown, it was the post-TBI participant group that scored higher. In both groups, PM JOL correlated positively with RM JOL only in the delayed condition.

Research of this nature may help to increase understanding of post brain injury memory and learning deficits and inform treatment strategies.

Poster Session 2 / No.18

Identification of a distinctive miRNA target 'miR-141-3p' in Post-Stroke Socially isolated aged mice

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MicroRNAs (miRNAs) are a class of short non-coding RNAs that have been identified as a potentially powerful interventional tool for many diseases, including stroke. Very recent studies have found that microRNAs also mediate many aspects of social interaction. Social environments can directly influence miRNA expression, which then triggers a plethora of downstream gene changes. This led us to hypothesize that miRNA regulation is involved in the detrimental effects of post-stroke social isolation (SI). Eighteen-month-old male C57BL/6 mice were pair-housed (PH) for two weeks prior to stroke and randomly assigned to various housing conditions (ST-ISO or ST-PH) immediately after stroke (ST). Mice were sacrificed either at 3, 7 or 14 days after 60-minute right MCAO or sham surgery (n=4-6/group) and perilesional frontal cortex were isolated for miRNA analysis. Total RNA was isolated using either Qiagen miRNeasy® Mini Kit/ miRVANA miRNA isolation kit (Ambion, Life Technologies) using the supplier's protocol. Whole miRNOme analysis of total RNA isolated from brain tissue was performed using miRCURY LNA™ Universal RT microRNA. Post-treatment with an 'in vivo ready' antagomir of miR-141-3p (7mg/kg i.v/day x 3 days; n=7/group) was given through lateral tail veins. Using whole miRNOme analysis of approx. 800 miRNA, we found miR-141-3p as a unique miRNA whose expression significantly upregulated in a time-dependent manner up to day 14 after stroke. The post treatment with 'in vivo ready' antagomir of miR-141-3p reduced the isolation-induced increase in miR-141-3p to levels almost equal to that of ST-PH controls. Post-treatment significantly reduced mortality (by 21% as compared to –ve control) after stroke. mRNA targets analysis study using qPCR confirmed the significant ($p<0.05$ vs. Neg Control) upregulation of target several genes like arg-1, Ccl22, and TGFbr1, markers of M2 type microglial activation, after antagomir treatment.

The present data suggests the important role of miR-141-3p in post-stroke isolation. Temporal expression profiling studies suggest its validation as potential targets. Post-treatment data confirms *in vivo* feasibility of miRNA modulation after stroke.

Funding: This work was supported by National Institutes of Health grants R01 NSO77769 and NS055215 (to Louise McCullough) and AHA postdoctoral fellowship 14POST20380612 (to Rajkumar Verma)

Poster Session 2 / No.19

Unbiased classification of retinal ganglion cell subtypes using massively parallel digital transcriptome profiling of single cells

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Retinal ganglion cells (RGCs) are projection neurons in the eye which pre-process and pass to the brain visual information collected in the eye. Thirty types of RGCs, differing in morphology, localization, or function, have been described in the mammalian retina. A number of subsets of these RGC subtypes have been labeled in transgenic mouse lines and several subtype-specific markers have been identified. However, the molecular differences between, and the markers unique to, the large majority of RGC subtypes are unknown. Here, we have purified mouse RGCs in large numbers and analyzed over 2,500 RGCs from each eye by single cell massively parallel digital transcriptome profiling using the 10x Genomics Chromium platform. We then bioinformatically classified RGCs into subpopulations based on their transcriptome signatures using CellRanger and CellView pipeline that includes tSNE and DBSCAN clustering algorithms. Not only has the clustering analysis revealed subpopulations enriched for the markers of known RGC subtypes, but it has also predicted novel subtypes and markers. Further characterization of the novel RGC subtypes is underway.

Poster Session 2 / No.20

The Hygiene Hypothesis in Multiple Sclerosis: Inducing TLR2 Tolerance Treats the Myelin Repair Component as well as the Inflammatory Component of MS

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Multiple sclerosis (MS) is a central nervous system autoimmune disease characterized by both an inflammatory/demyelinating component and a defective myelin repair (re-myelinating) component. Re-myelination defects, which may underlie progressive forms of MS, remain poorly understood. Recent studies implicate toll-like receptor 2 (TLR2) signaling as contributing to both the inflammatory component and defective re-myelination of MS. We previously reported evidence that the "Hygiene Hypothesis," as represented by a systemic deficiency in microbiome-derived "tolerizing" TLR2 ligands, may be involved in MS. In proof-of-concept studies, we reported that inducing TLR2 tolerance with low doses of microbiome-derived TLR2 ligands significantly attenuates adoptively transferred EAE. Here we ask if TLR2 tolerance also enhances the re-myelinating component of MS.

Utilizing the cuprizone-induced murine model of demyelination, we report that inducing TLR2 tolerance significantly improves myelin thickness during the re-myelination phase of the disease. Systemic TLR2 tolerance was induced by administering low dose TLR2 ligands for 16 days after halting cuprizone administration. Evaluation of myelin integrity via Black-Gold staining and electron microscopy (g-ratios) revealed significantly enhanced myelin recovery in mice tolerized after cuprizone treatment.

These results indicate that TLR2 tolerance enhances myelin repair via mechanisms independent of modulating systemic immune function. Given that defective re-myelination may represent an important component of the pathophysiology in progressive MS, our results suggest that inducing TLR2 tolerance may represent a two-pronged approach for treating both the inflammatory and myelin repair components of MS.

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