UCONN HEALTH

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

PROGRAM & AGENDA

Wednesday, May 10, 2023
8:00 am – 5:00 pm

Registration begins at 8:00 am

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

UCONN HEALTH

May 10, 2023

Dear Neuroscience Program Faculty, Postdocs, Students and Guests,

Welcome to the 23rd 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.
3. The facility is air-conditioned. You might want to bring a sweater or light jacket in case you find the temperature unpleasant.
4. **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.
5. Free Wi-Fi will be available courtesy of the Mark Twain House. The Wi-Fi 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.

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,

Byoung-Il Bae, Ph.D.
Professor

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UConn School of Medicine
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2023 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. Byoung-Il Bae	Auditorium
9:00 - 9:45 am	**Symposium A** *Moderator: Dr. Tuncdemir*	Auditorium
9:45 – 10:00 am	*Coffee Break*	Great Hall
10:00 – 10:45 am	**Symposium B** *Moderator: Dr. Spellman*	Auditorium
10:45 – 12:00 pm	**Poster Session 1**	Classroom
12:00 - 1:00 pm	*Lunch*	Great Hall
1:00 - 2:10 pm	**Poster Session 2**	Classroom
2:15 – 2:30 pm	**Vendor Recognition Presentation**	Auditorium
2:30 - 3:15 pm	**Symposium C** *Moderator: Dr. Grosmark*	Auditorium
3:15 – 3:25 pm	*Group picture*	Outside front entrance (staircase)
3:25 – 3:40 pm	*Coffee Break*	Great Hall
3:45 - 4:45 pm	**Keynote Address:** **Marina R. Picciotto, Ph.D.** Charles B.G. Murphy Professor in Psychiatry Deputy Chair for Basic Science Professor of Neuroscience and of Pharmacology Yale University	Auditorium
4:45 – 5:00 pm	**Presentation of Poster/Oral Awards & Closing Remarks**	

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.

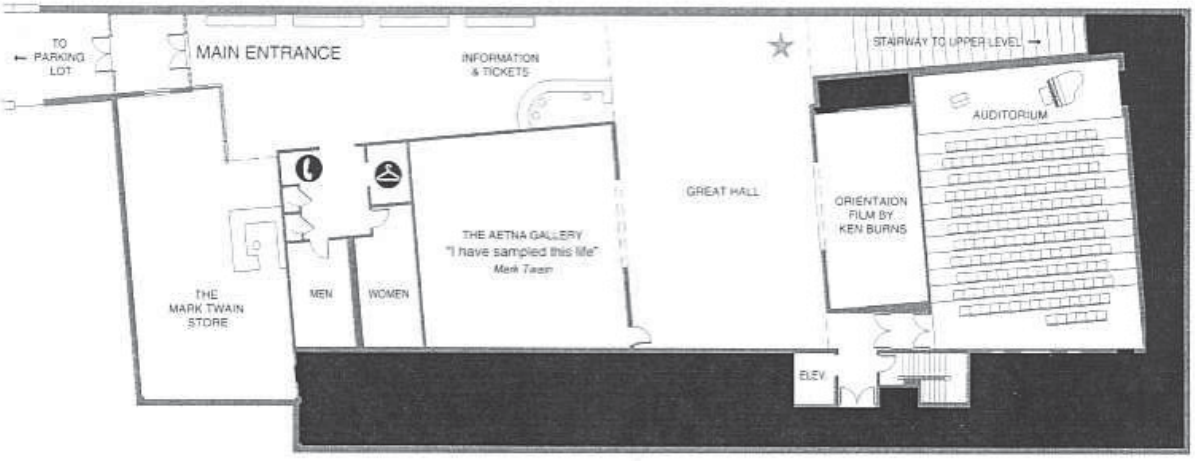
For Passengers with Limited Mobility or LOADING DOCK

(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.

The Mark Twain House & Museum

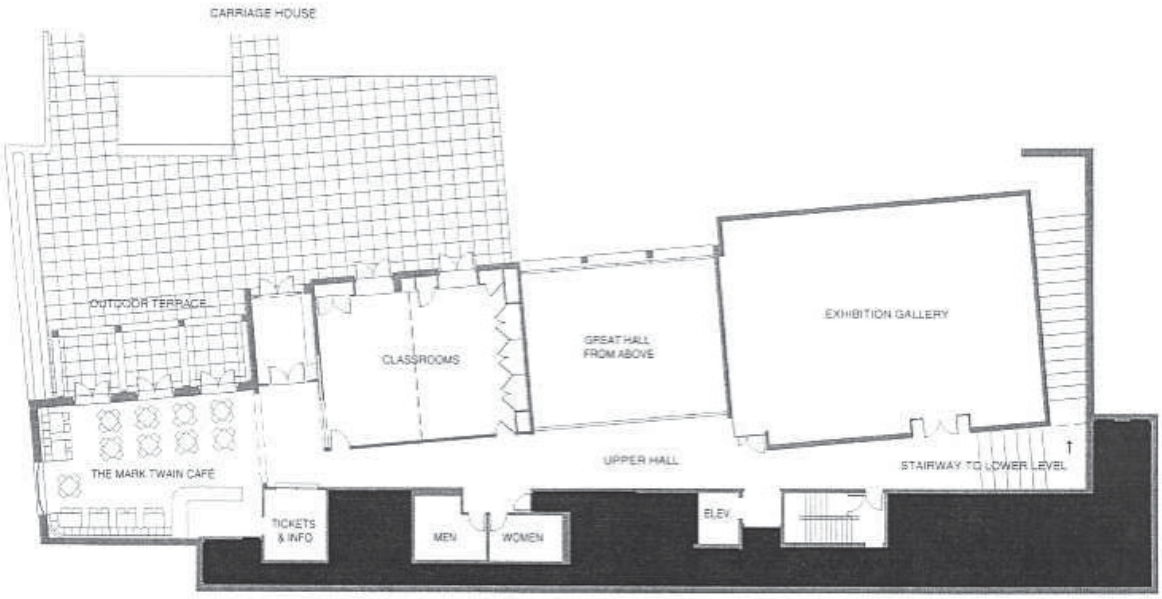
Lower Level / Main Entrance



★ TOURS START HERE

Upper Level

↑ TO THE MARK TWAIN HOUSE



3rd Floor–Research Library (By Appointment Only)

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KEYNOTE SPEAKER

Acetylcholine as a neuromodulator: a link between cognition and emotional behaviors?

Marina R. Picciotto, Ph.D.

Charles B. G. Murphy Professor in Psychiatry
Professor of Neuroscience and Pharmacology
Director Division of Molecular Psychiatry and
Deputy Chair for Basic Science Research, Dept. of Psychiatry
Deputy Director, Kavli Institute for Neuroscience

Yale University School of Medicine

Dr. Picciotto is a pioneer in the study of the neurotransmitter acetylcholine and its receptors (AChRs) in the brain, including research related to addiction, depression, learning and appetite. Her work spans molecular genetic, biochemical, cell biological, anatomical, electrophysiological, behavioral and human studies, highlighting her philosophy that strong basic neuroscience can be clinically relevant.

The goal of her laboratory is to understand the role of acetylcholine signaling in complex behaviors and mouse models relevant to psychiatric illness. This work involves *in vivo* imaging of acetylcholine dynamics and its consequences on the activity of networks of neurons involved in cognition-, reward- or stress-related behaviors, as well as molecular genetic studies of AChRs and their role in mediating the actions of acetylcholine during development and in adulthood. The laboratory uses molecular genetic tools (knockout and transgenic mice, viral vector-mediated gene transfer), coupled with use of genetically-encoded sensors (to measure neurotransmitter levels or calcium signaling), and biochemical, pharmacological and behavioral analyses. Her lab has demonstrated a critical role of specific nAChRs in nicotine reward, providing a defined molecular target for pharmacological intervention for smoking cessation as well as identifying the anatomical basis for other behaviors related to the effects of acetylcholine. Ongoing projects in the laboratory include studying the interaction between acetylcholine and circuits involved in stress response, appetitive learning or reward. Her lab also studies nicotine-induced neuroadaptations in intracellular signaling in dopamine neurons contributing to behaviors related to nicotine addiction and the role of nAChRs in neuronal morphology and function during development.

Dr. Picciotto has served as a member of NIDA's Scientific Council (2011-2014), as Treasurer of the Society for Neuroscience (2013) and as President of the Society for Research on Nicotine & Tobacco (2018-2019). Dr. Picciotto has been awarded the Presidential Early Career Award for Scientists and Engineers, The Human Frontiers Science Program Organization 10th Anniversary Award, the Waletzky Prize for Research on Substance Abuse and the Carnegie Prize in Mind and Brain Sciences. She was elected to the National Academy of Medicine in 2012 and as a Fellow of the American Association for the Advancement of Science in 2014, where she served as Chair of the Section on Neuroscience from 2019-2020. Dr. Picciotto has served on a number of editorial boards and was Editor-in-Chief of *the Journal of Neuroscience* until the end of 2022. She is currently President-elect of the Society for Neuroscience.

Symposium A: Talk No. 1

Dysfunctional sodium channel kinetics as a novel epilepsy mechanism in chromosome 15q11-q13 duplication syndrome

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

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Duplication of the maternal chromosome 15q11.2-q13.1 region causes Dup15q syndrome, a highly penetrant neurodevelopmental disorder characterized by severe autism and refractory seizures. Although *UBE3A*, the gene encoding the ubiquitin ligase E3A, is thought to be the main driver of disease phenotypes, the cellular and molecular mechanisms that contribute to the development of the syndrome are yet to be determined. We previously established the necessity of *UBE3A* overexpression to the development of cellular phenotypes in human Dup15q neurons, including increased action potential firing and increased inward current density, which prompted us to further investigate sodium channel kinetics. We use a Dup15q patient-derived induced pluripotent stem cell line that was CRISPR-edited to remove the supernumerary chromosome and create an isogenic control line.

We perform whole-cell patch clamp electrophysiology on Dup15q and corrected control neurons at two time points of *in vitro* development. Compared to corrected neurons, Dup15q neurons show increased sodium current density and a depolarizing shift in fast inactivation. Moreover, onset of slow inactivation is delayed, and a faster recovery from both fast and slow inactivation processes is observed in Dup15q neurons. A fraction of sodium current in Dup15q neurons (~ 15 -20%) appears to be resistant to slow inactivation. Not unexpectedly, a higher fraction of persistent sodium current is also observed in Dup15q neurons. These phenotypes were modulated by the anticonvulsant drug rufinamide. Sodium channels play a crucial role in the generation of action potentials, and sodium channelopathies have been uncovered in multiple forms of epilepsy. For the first time, our work identifies in Dup15q neurons dysfunctional inactivation kinetics, which have been previously linked to multiple forms of epilepsy. Our work can also guide therapeutic approaches to epileptic seizures in Dup15q patients and emphasize the role of drugs that modulate inactivation kinetics, such as rufinamide.

Symposium A: Talk No. 2

Therapeutic depletion of CD8+ T-cells prevents neuropathology in Globoid cell Leukodystrophy

Pearl A. Sutter[1,6], Antoine Ménoret[2], Evan R. Jellison[2], Alexandra M. Nicaise[1,3], Allison M. Bradbury[4], Anthony T. Vella[2], Ernesto R. Bongarzone[5], Stephen J. Crocker[1,2]

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

This work was supported in part by grants from National Institutes of Health National Institutes of Health (R56NS099359-01A1 and R01NS131327 to SJC) and the National Multiple Sclerosis Society (RG-1802-30211 to SJC). PAS was supported by an NIH F30 award (NS129238-01).

Symposium A: Talk No. 3

Effects of the Atypical Antipsychotic and D3/D2 Partial Agonist Cariprazine on Effort-based Choice

Alev Ecevitoglu[1], Gayle A. Edelstein[1], Katie Beard[1], Régulo Olivares[1,2], Ashley Kovach[1], Ryan Conrad[1], Merce Correa[1,2], John D. Salamone[1]

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Schizophrenia is characterized by positive symptoms (hallucinations and delusions), cognitive and behavioral disorganization, and negative symptoms (blunted affect, anhedonia, asociality, and avolition). Avolition can defined as a decrease in goal-directed behavior which can also be shown as a reduction in the selection of high-effort alternatives on effort-related tasks. To this date, there is no exact known cause for schizophrenia, yet, it has been demonstrated that dopamine (DA) transmission regulates the processes involved in the generation of various symptoms of schizophrenia. Initially developed medications, also known as “typical” or “first generation” antipsychotics, are DA receptor antagonists that primarily target D2 receptors. Although they are successful in diminishing positive symptoms, they remain ineffective at treating negative symptoms, and may actually worsen them. This need for better therapeutics led to the development of second- and third-generation antipsychotics that have multiple targets and exhibit fewer side effects. These newer “atypical” antipsychotic drugs include Cariprazine (trade name Vraylar) that is a D3 preferring D3/D2 receptor partial agonist. Both clinical and preclinical data suggest that cariprazine is effective in treating negative symptoms such as “anhedonia”. To test the influence of cariprazine on the goal-directed activity that is characteristic of another negative symptom, avolition, the current study focused on effort-related choice in male rats. Rats were trained on the fixed ratio (FR) 5/chow feeding choice task, in which they were given the option of lever pressing for a preferred reward (high carbohydrate pellets) vs. approaching and consuming a less preferred reward (lab chow). When administered alone, cariprazine was found to shift choice behavior, inducing low effort as measured by reduced lever pressing for pellets, but increasing intake of the concurrently available chow. This shift in choice behavior in rats was partially reversed by an A2A antagonist Istradefylline, as seen previously with reversal of DA antagonism on effort-based choice behavior. Given these results, it is possible that cariprazine could be acting similar to a D2 antagonist due to its weak partial agonism of DA receptors. Furthermore, avolition as measured by exertion of effort in goal-directed activity could be dissociable from the other negative symptoms. Taken together, these findings underline the importance of developing novel therapeutics, especially for the treatment of negative symptoms of schizophrenia, and support the idea that individual negative symptoms may have distinct neural mechanisms.

Symposium B: Talk No. 4

The Minor Cannabinoid Cannabinol Blocks Endotoxin-Induced Proinflammatory Cytokine Production in Mice

Vanegas, S. Olivia[1,2], Gamage, Thomas F.[3], Maturano, Jonathan[4], Sarlah, David[4], Kinsey, Steven G.[1,2]

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The analgesic and anti-inflammatory effects of cannabinoids are well established. For example, the effects of the major phytocannabinoid, Δ^9-tetrahydrocannabinol (THC), and to a lesser extent cannabidiol (CBD), have been studied for decades, and recent work has included other compounds produced by the plant, including minor cannabinoids and terpenoids. The goal of the present study was to determine the *in vivo* effects of three lesser-studied, minor phytocannabinoids: cannabinol (CBN), cannabichromene (CBC), and cannabicyclol (CBL). To measure acute effects of each compound, adult male and female C57BL/6J mice were administered CBN, CBC, CBL (10-200 mg/kg, ip), or vehicle and tested repeatedly in the tetrad test battery (i.e., bar test catalepsy, tail immersion, core body temperature, and spontaneous locomotor activity). Mice treated with CBN (≥25 mg/kg) displayed classic cannabinoid effects, including acute antinociception, that were only partially blocked by pretreatment with either rimonabant (CB_1 selective antagonist; 3 mg/kg, ip) or istradefyllene (adenosine A2A selective antagonist; 3.2 mg/kg, ip). CBL (≥50 mg/kg) induced hypothermia that was fully blocked by istradefyllene, suggesting a cannabinoid receptor-independent mechanism. Similarly, rimonabant pretreatment had no effect on CBC-induced immobility (200 mg/kg). To assess potential analgesic effects in a chronic pain state, a separate group of mice subjected to chronic neuropathic injury (CCI) displayed acetone-induced cold allodynia that was attenuated by either CBN (≥50 mg/kg) or CBL (100 mg/kg). In the LPS-induced inflammatory pain model, CBN (100 mg/kg) attenuated both paw edema and proinflammatory cytokine levels. Together, these findings suggest that the minor phytocannabinoids cannabinol and cannabicyclol reduce pain and inflammation, via cannabinoid receptor-dependent and -independent pathways.

Symposium B: Talk No. 5

Differential roles of 2-AG and anandamide in hippocampal long-term depression

Fouad Lemtiri-Chlieh and Eric Levine

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It is widely accepted that exogenous cannabinoids can impair short-term memory and cognition in humans and other animals. This is likely related to the disruption of synaptic plasticity, including long-term potentiation and long-term depression (LTD), by the global and sustained activation of CB1 cannabinoid receptors by exogenous agonists. Conversely, the temporally and spatially-restricted release of endogenous cannabinoid ligands may mediate or enhance synaptic plasticity in a synapse-specific manner. In particular, endocannabinoids (eCBs) act as powerful modulators of synaptic function by suppressing presynaptic transmitter release. We examined the role of eCB signaling in LTD by stimulating the axons emanating from the CA3 region (Schaffer collateral pathway) and recording field excitatory postsynaptic potentials (fEPSPs) from the CA1 stratum radiatum (SR) in hippocampal slices from juvenile mice. Two forms of LTD were studied: LTD was induced electrically with low frequency stimulation (LFS; 1Hz for 15 minutes), or pharmacologically, with a 10 min exposure to (S)-3,5-Dihydroxyphenylglycine (DHPG), a selective agonist of group I metabotropic glutamate receptors (mGluRs). A stable, long-lasting (>60 minutes) decrease in fEPSP magnitude (~40% decrease from baseline) was observed following either LFS or DHPG. Both forms of LTD required CB1 cannabinoid receptor activation, as the magnitude of suppression was significantly reduced in the presence of the CB1 receptor antagonist NESS 0327. In order to identify the endogenous cannabinoid ligand(s) involved, we used the DAG-lipase inhibitor DO34 to block synthesis of the eCB 2-AG, and the NAPE-PLD inhibitor LEI-401 to block the synthesis of anandamide. Interestingly, LFS-LTD was not significantly affected by DO34, but was almost completely blocked in the presence of LEI-401. DHPG-LTD, however, was significantly reduced by either DO34 or LEI-401. These results suggest that both forms of LTD are mediated by cannabinoid receptor activation, but LFS-LTD is selectively mediated by anandamide release, whereas DHPG-LTD involves both anandamide and 2-AG. These results may shed light on the differential mobilization of 2-AG and anandamide signaling at hippocampal synapses.

Funded by the National Institute of Health (Grant no. NS111986)

Symposium B: Talk No. 6

Targeting of *p21*^{Cip1}-highly-expressing cells alleviates neuropsychiatric disorders with obesity

Lichao Wang[a,b], Binsheng Wang[a,b], Nathan S. Gasek[a,b], Rachel L. Cohn[a,b], Taewan Kim[a,c], Ming Xu[a,b]

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Obesity remains a major factor for diabetes associated with insulin resistance and increases the risk of neuropsychiatric disorders, such as anxiety, depression, and cognitive deficits, resulting in a shortened in lifespan and healthspan. Cellular senescence, a state of permanent cell-cycle arrest, has been recognized as an important biological process underlying obesity and obesity-associated neurological disorders. My project aims to alleviate metabolic and neuropsychiatric dysfunction in obesity by targeting of *p21*^{Cip1}-highly-expressing (*p21*^{high}) cells, a rarely examined senescent cell population. By leveraging a novel transgene containing a *p21* promoter driving *Cre*, we demonstrate the accumulation of *p21*^{high} cells in brain with obesity, whereas, intermittently clearing *p21*^{high} cells alleviates anxiety- and depression-related behavior and improve the cognitive function for obese mice. Of note, clearance of *p21*^{high} cells decreased key factors of the senescence transcriptional program in hippocampus and amygdala. A survival study shows the intermittent clearance of *p21*^{high} cells extends the lifespan of obese mice fed with high-fat diet. Our findings indicate *p21*^{high} cells serve as a potential therapeutic target for neuropsychiatric disorders.

Symposium C: Talk No. 7

Bace1 deletion in the adult reverses epileptiform activity and sleep-wake disturbances in AD mice

Annie Yao, Patrick J. Halloran, Yingying Ge, Neeraj Singh, John Zhou, James Galske, Wanxia He, Riqiang Yan, Xiangyou Hu.

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Alzheimer's disease (AD) increases the risk for seizures and sleep disorders. We show that the deletion of Bace1 in adult 5xFAD mice was able to reverse epileptiform activity this AD mouse model. However, treating 5xFAD and APPNL-G-F/NL-G-F (APP KI) mice of either sex with the BACE1 inhibitor Lanabecestat (AZD3293) dramatically increased epileptiform spiking. Since adult Bace1 deletion in 5xFAD mice reduced epileptiform spiking, while AZD3293 treatment in adult AD mice increased spiking, it raises the possibility that AZD3293 has an off-target effect other than its inhibition of BACE1. Indeed, AZD3293 inhibits BACE2 near equal potency (Eketjäll et al., 2016). To interrogate this off-target possibility leading to increased neuronal excitability and enhanced synchronous firing, we performed *ex vivo* extracellular field recordings in acute mouse brain slices containing the hippocampus, in which we induced epileptiform activity with the potassium channel blocker 4-AP. AZD3293 treatment significantly increased synchronous burst firing frequency in Bace1-null mice beyond what is seen in global Bace1 inhibition, which suggests that chronic treatment of mice with AZD3293 will influence targets other than BACE1. In addition, we monitored sleep-wake pathologies in 5xFAD and APP KI mice and showed that increased wakefulness, decreased non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep in both 5xFAD and APP KI mice; BACE1 inhibition in the adult 5xFAD mice reversed plaque load and sleep disturbances, but not in APP KI mice. Further studies showed that APP KI mice had morphologically altered plaque-associated microgliosis compared to 5xFAD mice. Our findings suggest that differences in microglia-mediated Aβ sequestration and clearance between 5xFAD and APP KI mice may contribute to more severe sleep disturbances in APP KI mice. Together, BACE1 inhibition should be developed to avoid off-target effect for achieving benefits in reducing epileptic activity and sleep disturbance in Alzheimer's patients.

Support. AG059124-01A1 to X.H., RF1AG058261 to R.Y., AG025493 to R.Y., NS074256 to R.Y., and AG046929 to R.Y. X.H. was funded by R21 AG061609-01. A.Y. is funded by 1F30AG081134-01.

Symposium C: Talk No. 8

Regulation of LRRK2 mRNA stability by ATIC and its substrate AICAR through ARE-mediated mRNA decay in Parkinson's disease

Qinfang Liu[1], Dong Zhu[1], Naren Li[2], Shifan Chen[1], Liang Hu[2], Jianzhong Yu[2], Yulan Xiong[1]

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Parkinson's disease (PD) is a common neurodegenerative disorder that affects more than 10 million people worldwide. *LRRK2* (leucine rich repeat kinase 2) mutations are the most common causes for familial PD and major risk factors for idiopathic PD. Increase of LRRK2 protein levels has been found in certain PD patient tissues, which implies a significant role of LRRK2 expression in PD pathogenesis. However, it remains unclear how LRRK2 levels are regulated. Here, we demonstrated a novel ATIC-AICAR-AUF1 axis in the regulation of LRRK2 expression levels. Through an unbiased genome-wide genetic screen, we identified 5-aminoimidazole-4-carboxamide (AICA) ribonucleotide (AICAR) transformylase/ inosine monophosphate (IMP) cyclohydrolase (ATIC) as a key modifier of LRRK2 expression and toxicity. We further deciphered that AICAR, the enzymatic substrate of ATIC, modulates LRRK2 protein levels in vitro through a cell-type specific regulation and in vivo in mouse brains. Mechanistically, AICAR regulates LRRK2 expression specifically at mRNA levels through recruiting AU-rich element RNA-binding protein 1 (AUF1) to AU-rich elements (ARE) in a specific region of LRRK2 mRNA, and AUF1 in turn recruits the decapping enzyme complex DCP1/2 (decapping protein 1/2) to LRRK2 mRNA, leading to removal of the 5' cap of LRRK2 mRNA. In addition, we discovered that AICAR mediated-LRRK2 reduction rescues LRRK2-associated dopaminergic neurodegeneration and neuroinflammation in PD animal models. Taken together, this study provides the first and novel regulatory mechanism of LRRK2 function through LRRK2 mRNA decay and establishes the modulation of LRRK2 mRNA decay as a potential novel therapeutic strategy for PD, which is distinct from targeting enzymatic functions of LRRK2.

This work was supported, in part, by grants from NIH/NINDS R01 NS112506, NIH/NIA K01 AG046366 award, Parkinson's Foundation Stanley Fahn Junior Faculty award PF-JFA-1934, UConn Startup fund to Y.X. J.Y. was supported by grants from NIH/NIGMS R01 GM136904, the National Science Foundation 2115690.

Symposium C: Talk No. 9

Sound-Evoked Potentiation as a Test for Tinnitus

Emily Fabrizio-Stover*, Alice Burghard*, Chris Lee*, Douglas Oliver*

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Tinnitus, the perception of sound with no external stimulus, affects over 50 million people in the United States alone. Behavioral tests are used to assess tinnitus in laboratory animals for research, but this process is expensive, time consuming, and subjective because the testing paradigms differ between research groups. An objective, non-invasive, electrophysiological test is needed to bypass these difficulties and allow for more efficient testing of tinnitus. The novel auditory stimulus paradigm (NSP) (patent pending) developed in the Oliver lab has been shown to alter sound-driven activity in the inferior colliculus. We believe that recording auditory brainstem responses (ABRs) after the application of the NSP will show tinnitus-specific differences. Tinnitus CBA/CaJ mice were generated using an awake, unilateral sound exposure. ABR responses to tone pips, narrow-band noise, and chirps at three or more frequencies were collected from tinnitus, non-tinnitus, and unexposed control mice. We measured the peak-trough amplitudes for each waveform to calculate a tinnitus score to quantify the effect of the NSP. When comparing tone-pip evoked ABRs in the exposed ear for tinnitus and non-tinnitus mice, non-tinnitus mice had significantly lower scores than non-tinnitus mice, suggesting that the NSP suppresses sound-driven responses. This effect is more significant for higher frequencies and later waves. However, there is no significant difference between tinnitus and the control. Furthermore, using a correlation analysis, tone-driven ABRs from non-tinnitus mice were significantly more affected by the NSP than tinnitus mice. A differential time frequency analysis analyzing the effect of the NSP also shows tinnitus specific ‘hotspots’ at tinnitus frequencies, but not at non-tinnitus frequencies. When comparing the tinnitus scores from tone, narrow-band noise, and chirp ABRs, tone-driven responses had the most change. However, this change was seen only in non-tinnitus animals. Together, these data suggest that our NSP can generate measurable tinnitus specific changes in the ABR and may lead to an individualized, non-invasive, electrophysiological test for tinnitus.

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Poster Session 1 / No. 1

Elucidating the role of non-imprinted genes in Dup15q syndrome neuronal phenotypes

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Dup15q syndrome, which is caused by maternal duplication of the chromosome 15q11-q13 region, is characterized by developmental delay, motor deficits, seizures, and autism. Of the duplicated genes in this region, *UBE3A,* which encodes a ubiquitin ligase, is exclusively expressed from the maternal allele in neurons due to genomic imprinting. Because it is the only maternally imprinted gene in this region, it is believed to be the major driver for Dup15q syndrome phenotypes. However, overexpression of *UBE3A* alone in mouse models fails to fully recapitulate behavioral phenotypes, suggesting a contributing role of other genes in the region. To gain a better understanding of this syndrome, we use patient-specific neurons derived from induced pluripotent stem cell lines as well as isogenic CRISPR-corrected lines. Using patch clamp electrophysiology, we have shown that human Dup15q neurons exhibit a hyperexcitability phenotype characterized by increased action potential firing and increased excitatory synaptic activity. We have also found that that overexpression of *UBE3A* alone is insufficient to mimic all cellular phenotypes, indicating a role for other non-imprinted genes in the duplicated region. Non-imprinted genes in this region include a cluster of $GABA_A$ receptor genes (*GABRB3, GARBA5,* and *GABRG3),* and *HERC2*, another ubiquitin ligase, all of which are associated with neurodevelopmental disorders. To evaluate the role of these genes, we have normalized the expression of *GABRB3* and *HERC2* in Dup15q neurons using antisense oligonucleotides and performed electrophysiological recordings at various developmental time points. Preliminary results suggest that normalizing *GABRB3* reverses some of the hyperexcitability phenotypes observed in Dup15q neurons, indicating a potential role for this gene in Dup15q. Ongoing experiments will determine if *HERC2* overexpression also contributes to neuronal phenotypes in this syndrome. Identifying the role played by non-imprinted genes in this region is imperative for developing more effective therapies for Dup15q and for generating improved mouse models.

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Poster Session 1 / No. 2

Investigating excitatory synaptic proteins in hypothalamic arousal neurons

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Hypocretin/Orexin (H/OX) neurons in the lateral hypothalamic area (LHA) are critical regulators of the wake state, and their disruption results in aspects of the sleep disorder narcolepsy. Despite much research into the circuitry and behavioral role of H/OX neurons, little is known about the molecular building blocks of their wake-promoting synapses. Through single-cell RNA sequencing, our lab identified two transcripts uniquely expressed in H/OX neurons within the LHA that encode putative synaptic proteins, *Nptx2* and *C1ql3*. After confirming mRNA expression with fluorescence *in situ* hybridization (FISH), we demonstrated NPTX2 and C1QL3 protein co-localization in H/OX soma and terminals in a novel *C1ql3*-HA mouse. Furthermore, we conditionally knocked out (cKO) *C1ql3* from H/OX neurons by expressing Cre recombinase in the LHA of *C1ql3*$^{flox/flox}$-mVenus mice. We found that compared to uninjected littermate controls, *C1ql3* H/OX-cKO mice had decreased H/OX-IR in the LC, an important projection target of H/OX neurons that promotes wakefulness. These results support our hypothesis that NPTX2 and C1QL3 may be important synaptic proteins that regulate H/OX synapses and their effect on wakefulness.

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Poster Session 1 / No. 3

The Molecular Role of C1QL3 and its Binding Partners in Synaptic Regulation

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Neuropsychiatric disorders often cause lifelong disruptions of patients' lives and lack satisfactory pharmacological interventions. Symptoms in these disorders are likely associated with disruption in synapses resulting from dysfunctional synaptic adhesion molecules (SAM), but the mechanisms by which SAMs may contribute to synaptic dysfunction have not been fully elucidated. Complement C1q like 3 (C1QL3), is a promising SAM for potential therapeutics for neuropsychiatric disorders because it selectively regulates excitatory synapses and may therefore constitute a target for correction of the excitatory/inhibitory synaptic imbalances associated with several neuropsychiatric disorders. To contribute to the future development of novel therapeutics, I will elucidate the molecular mechanisms contributing to C1QL3's roles in regulating excitatory synapses. My preliminary data suggest that C1QL3 associates with the adhesion G protein-coupled receptor B3 and neuronal pentraxin 1 at synapses in mouse primary neuron cultures, consistent with our previous data suggesting that these proteins promote cell-cell adhesion in heterologous cells in a C1QL3-dependent manner. We have also identified a novel binding partner of C1QL3, SPOCK2, that preliminary data suggest promotes excitatory synaptic density in a C1QL3 dependent manner. These findings may suggest that C1QL3 regulates synaptic maintenance by tethering the pre- and post-synaptic membranes. We will use mutant structure function analysis to validate that C1QL3 complex formation promotes synapse maintenance and elucidate the C1QL3 dependent activity of SPOCK2 in promoting synaptic density.

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Poster Session 1 / No. 4

Evaluation of Different Classes of Monoamine Uptake Inhibitors: Effects on Effort-based choice

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Current antidepressant development efforts include the evaluation of triple reuptake inhibitors (TRIs). TRIs exert their effects by blocking uptake of the monoamines (dopamine (DA), serotonin (5-HT), norepinephrine (NE)) and increasing extracellular concentration. It is hypothesized that TRIs could have superior effects due to enhancing neurotransmission of all three monoamines, which could result in improved efficacy and quicker onset of antidepressant response in comparison to commonly prescribed antidepressants. Previous testing of the TRI NOE-115, failed to increase selection of the high effort option on our effort-based choice task. It is hypothesized that this was due to the compound not having a high enough potency at the DA transporter (DAT). Previous evidence suggests that compounds that facilitate DA transmission, but not 5-HT or NE, increase the selection of the high effort behavior or reverse the behavioral effects of the VMAT-2 inhibitor tetrabenazine (TBZ) on effort-based choice tasks. It is hypothesized that for a TRI to be able to reverse the effects of TBZ on the fixed ratio (FR) 5 chow feeding choice task, the compound must have its greatest potency at DAT. To deconstruct the role of different monoamines and test this systematically, three different monoamine transport inhibitors (NET inhibitor Atomoxetine; serotonin-norepinephrine uptake inhibitor (SNRI) Duloxetine; norepinephrine-dopamine uptake inhibitor (NDRI) Nomifensine) are being tested for their ability to attenuate TBZ-induced shifts in behavior on the FR5 chow feeding choice task. Ultimately, the TRIs diclofensine and centenafidine will also be tested for their ability to attenuate TBZ-induced shifts in behavior on the FR5 chow feeding choice task. Additionally, microdialysis with high-performance liquid chromatography methods and techniques are being developed in order to measure extracellular concentration of DA, NE, and 5-HT in awake and behaving animals after administration of the compounds listed above.

Poster Session 1 / No. 5

A role of the locus coeruleus to gustatory cortex pathway in novel taste processing.

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Norepinephrine (NE)-producing neurons in the locus coeruleus are well-known to modulate sensory processing in an arousal-dependent manner. Although a large literature has examined how LC activity influences vision, audition, somatosensation, and olfaction, the role of LC in taste processing remains to be explored. Several studies suggest the involvement of NE in signaling taste novelty and regulating animals' response to novel tastes. Based on this evidence, we asked the following questions: (1) How does taste familiarity affect the dynamics of LC-NE neurons, and (2) how does the activation of LC efferents in the primary gustatory cortex (GC) affect response to a novel taste? Our fiber photometry calcium imaging results of the LC show that LC activity is suppressed during licking behavior in mice, regardless of taste novelty. Our behavioral experiments demonstrate that optogenetic activation of the LC-GC pathway increases the preference for a novel sweet substance (saccharine). Further, the history of LC-GC pathway activation during the initial exposure increased saccharine preference in a subsequent encounter. These results suggest that perturbation of the LC-GC pathway can induce immediate and lasting changes in taste perception. Collectively, our findings demonstrate that natural LC dynamics influence taste-guided behavior.

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Poster Session 1 / No. 6

Can different training regimens modulate the dopaminergic system and affect preference for active versus sedentary sources of reinforcement?

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Physical exercise (PE) has been shown to have positive effects on some mental disorders such as depression or Parkinson's disease (PD). Exercise modulates certain components of the dopaminergic (DA) systems. The mesolimbic DA pathway (Nucleus Acumbens, NAcb) plays an important role in regulating the vigor and effort required in motivated behaviors. In experiments with rodents, PE regimens have demonstrated to exert therapeutic effects in models of PD and also in paradigms that evaluate depressive measures. DA antagonism/depletion induces anergia in effort-based-decision-making tasks. However, little is known about the neural mechanisms underlying decision-making processes that establish preferences for sedentary versus activity-based reinforcers. The present study assessed the impact of previous experience with different exercise regimes on reinforcer preference as well as the impact of DA depletion via tetrabenazine (TBZ), a catecholamine depleting agent and vesicular transport inhibitor (VMAT-2), on choice behavior. CD1 male mice were trained daily either in a programmed-automatic running wheel (RW) that forced animals to move, or on a cage with an activity wheel to allow the animals to run voluntarily. The control groups had locked-RWs. After 9 weeks of training, animals were tested in a T-maze-3-choice task developed for the assessment of preference between physical activity (RW) vs. more sedentary reinforcers (sucrose pellets or a non-social odor). All groups preferred to spend more time interacting with the RW. TBZ produced a relative change in preference in the control group; it reduced the time they spent running, while increasing the time they spent eating. However, both the forced-exercise group, and the voluntary exercise group were insensitive to the effects of TBZ. These results suggest that physical exercise could be a preventive strategy against the appearance of anergic symptoms common in several mental diseases, in addition to an effective treatment to reduce them.

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Poster Session 1 / No. 7

Differential effects of synthetic cannabinoid and terpenoid administration on experimentally induced pruritus

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Introduction: Pruritus is the sensation that invokes a desire to scratch. Treatments for clinical presentations of pruritus are usually resistant to pharmaceutical intervention, such as antihistamines. The endocannabinoid system is a potential target for pruritus treatments. For example, WIN 55,212-2 (WIN) reduces scratching via CB_2. β-caryophyllene (BCP) is a naturally available sesquiterpene found in *cannabis sativa* previously shown to be a CB_2 and PPAR-γ agonist, which have both been shown to reduce scratching upon activation. The potential antipruritic activity of BCP was investigated using an established 5-HT model of pruritus. We hypothesized that BCP would reduce scratching via CB_2 or PPAR-γ receptors.

Methods: Adult male and female C57BL/6J mice were administered 5-HT or Compound 48/80 to induce scratching. WIN 55,212-2 (0.1-0.3-1-3mg/kg, i.p.) or BCP (12.5-25-50-100-200mg/kg, s.c.) was administered to reduce scratching. In a separate group of mice, rimonabant (3 mg/kg, i.p.) or SR144528 (3mg/kg, i.p.) were administered to probe receptor mechanism of WIN. After injection with the pruritic agent, mice were immediately placed in sound-attenuating chambers, video recorded for 30 min, and hind paw scratching was quantified by a blinded observer. Pruritic activity of BCP alone (25-50-100mg/kg, s.c.) was also assessed. All analyses were performed using one-way ANOVA with Dunnett's post-hoc.

Results: WIN 55,212-2 (≥0.3mg/kg, i.p.) reduced Compound 48/80-induced scratching and was blocked by both the CB_1 and CB_2 antagonists. BCP did not reduce 5-HT induced scratching in mice. Surprisingly, BCP (100mg/kg, s.c.) administered alone induced scratching.

Conclusions: BCP induces scratching, independent of 5-HT administration. The receptor mechanism through which this pruritic activity occurs is unknown, though future studies will attempt to block both CB_2 and PPAR-γ.

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Poster Session 1 / No. 8

INHIBITING ENDOCANNABINOID AND CYCLOOXYGENASE ENZYMES REDUCES POSTSURGICAL PAIN IN MICE

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Introduction –Inhibition of either monoacylglycerol lipase (MAGL), the primary enzyme that catabolizes the endocannabinoid 2-arachidonoyglycerol (2-AG) or cyclooxygenase (COX), the enzymes that synthesize prostaglandins, reduces pain and inflammation in various rodent models. We hypothesized that a combination of MAGL and COX inhibitors (i.e., JZL184 and diclofenac sodium), attenuates allodynia caused by hindpaw incision (HPI) a model of post-surgical pain in mice.

Methods – Under isoflurane anesthesia, equal numbers of adult male and female C57Bl/6J mice were subjected to HPI, in which a small incision was made and sutured in the plantar surface of one hind paw. Approximately 24 hours post-surgery, JZL184 (1-40 mg/kg, ip), MJN110 (0.55 – 5 mg/kg), the NSAID diclofenac sodium (1.85-50 mg/kg, ip), the CB_2 agonist, LY2828360 (3 mg/kg, i.p) or vehicle (5% ethanol, 5%, kolliphor EL, 90% saline, ip) was administered. A separate cohort was co-administered JZL184 (1, 40 mg/kg, i.p), diclofenac sodium (1.85, 50 mg/kg, i.p), or both compounds. A separate cohort of mice was administered rimonabant (3 mg/kg, i.p) or SR144528 (3 mg/kg, i.p) prior to JZL184 (40 mg/kg, ip). Mice were also injected repeatedly with JZL184 (8 mg/kg, s.c), cannabidiol (25 mg/kg, s.c), or vehicle before surgery and once daily post-surgery until recovery. Mechanical allodynia was quantified using von Frey filaments.

Results – Approximately, 24 hours post-surgery, acute MAGL inhibition via JZL184 (≥4 mg/kg) or MJN110 (≥5 mg/kg), the CB_2 agonist, LY2828360 (3 mg/kg) or the NSAID diclofenac (≥5.56 mg/kg) each attenuated HPI-induced mechanical allodynia. The combination of subthreshold doses of both diclofenac and JZL184 attenuated HPI-induced allodynia. The CB_2 antagonist SR144528 blocked the anti-allodynic effects of JZL184. Additionally, analgesia was maintained over repeated JZL184 (8 mg/kg) dosing.

Conclusion – In the present study, dual MAGL and COX inhibition attenuated HPI-induced allodynia, indicating an additive drug interaction. The CB_2 antagonist, SR144528 blocked the anti-allodynic effects of JZL184, and the CB_2 agonist LY2828360 reduced HPI-induced allodynia suggesting a CB_2 receptor mechanism. These data support targeting the endocannabinoid and prostanoid enzymes for postoperative pain treatment.

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Poster Session 1 / No. 9

The Cellular Senescence Factor Extracellular HMGB1 Directly Inhibits Oligodendrocyte Progenitor Cell Differentiation and Impairs CNS Remyelination

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

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Poster Session 1 / No. 10

Regulation of BK channel surface abundance by an ARF6-associated clathrin-dependent endocytic pathway

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The BK channel (also known as Slo1) is a high-conductance K^+ channel gated by membrane voltage and cytosolic Ca^{2+}. In neurons, Slo1 is enriched at presynaptic sites where it serves as a potent inhibitory regulator of neurotransmitter release. Slo1 function potentially depends on the amount of its surface expression. However, the trafficking mechanism of Slo1 is essentially unknown. Here we demonstrate that Slo1 undergoes constitutive endocytosis in a clathrin- and dynamin-dependent manner in transfected HEK293 cells. We also found that the small G protein ADP-ribosylation factor 6 (ARF6) plays a crucial role in Slo1 endocytosis. Co-immunoprecipitation and immunostaining assays revealed that Slo1 interacts with ARF6, and endocytosis of Slo1 was inhibited by SecinH3, an inhibitor of small GTPases that activate ARF6. Expression of mutated ARF6 isoforms (T27N or Q67L), which prevents ARF6 activation by inhibiting the exchange of GDP to GTP, significantly increased the surface abundance of Slo1 without altering Slo1 total protein level. Internalized Slo1 colocalized with clathrin and this internalization was inhibited by the dynamin inhibitor dynasore, suggesting that Slo1 internalization is clathrin- and dynamin-dependent. Furthermore, similar results were obtained with mouse Slo1 and *C. elegans* SLO-1, suggesting that a conserved molecular pathway regulates Slo1 endocytosis. In conclusion, we have identified a molecular pathway that mediates Slo1 endocytosis through interactions with ARF6, dynamin, and clathrin. This molecular pathway potentially plays important roles in Slo1 physiological functions by regulating the amount of Slo1 surface expression.

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Poster Session 1 / No. 11

Molecular mechanisms of BK channel regulation by melatonin

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Melatonin, secreted by the pineal gland, produces its sleep-promoting effect via two G protein-coupled receptors: MT_1 and MT_2. These two receptors are expressed in a variety of brain structures, including the cerebral cortex, suprachiasmatic nucleus (SCN), and hippocampus. However, the downstream molecular target(s) mediating the sleep effect of melatonin has remained enigmatic. In our recent study (Niu et al., PNAS 2020), we found that melatonin promotes sleep by activating the BK channel SLO-1 through a specific melatonin receptor in *C. elegans*. In addition, we found that the human BK channel Slo1 may be activated by melatonin in the *Xenopus* oocyte heterologous expression system by binding to MT_1 but not MT_2, and that the activation results from the action of Gβγ subunits. These results suggest that melatonin might also activate Slo1 in mammalian neurons through a specific melatonin receptor. We have embarked on a project of testing this hypothesis by performing several types of experiments. First, we examined the effects of various chimeras of mouse MT_1 and MT_2 on mouse Slo1 single-channel open probability (P_O) using *Xenopus* oocytes as a heterologous expression system. We found that substitution of the N- but not C-terminus of MT_1 by that of MT_2 abolished the activation effect of melatonin on Slo1, suggesting that the N-terminus of MT_1 is required for Slo1 activation. Second, we isolated synaptosomes from wild-type mice, and determined whether they contained MT_1. We detected MT_1 in them by western blot. Because Slo1 is an important protein at presynaptic sites and its presence in synaptosomes has been confirmed in a previous study, our result suggests that MT_1 and Slo1 likely coexist in synaptosomes. Third, we performed coimmunoprecipitation (co-IP) assays with transfected HEK293T cells and mouse whole-brain lysates. We observed co-IP of Slo1 with MT_1, and found that the co-IP depended on an intracellular loop (S0-S1 loop) in Slo1, suggesting that the two proteins physically interact. Lastly, we have begun to determine whether melatonin may activate Slo1 through MT_1 but not MT_2 in SCN neurons by performing electrophysiological experiments with brain slices from CBA/CaJ mice, which, unlike most commonly used laboratory mouse strains, are proficient in melatonin secretion. In preliminary studies, we observed that administration of melatonin and paxilline (a specific Slo1 blocker) to the extracellular solution had opposite effects on the resting membrane potential (RMP), action potential (AP) firing rate, and paired-pulse ratio (PPR) of excitatory postsynaptic currents. Specifically, melatonin hyperpolarized the RMP, reduced AP frequency, and increased the PPR, whereas paxilline showed opposite effects, suggesting that activation of Slo1 by melatonin can inhibit neuronal excitability and inhibit neurotransmitter release. In addition, we observed increased excitability and AP firing rate in $MT_1^{-/-}$ mice compared with wild-type mice. In summary, our available results suggest that Slo1 may be activated by melatonin in mice as well as *C. elegans* .

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Poster Session 1 / No. 12

Modeling of Aryl Hydrocarbon Receptor Pathway Intrinsic Immunometabolic Role using Glioblastoma Stem Cells and Patient-Derived Organoids

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We investigated the ability of patient derived glioma stem cells organoids to model the role of AHR in glioblastoma stem cells (GSCs) function and immune molecular programs in the dish as a patient's avatar. GSCs are highly self-renewing, resistant to therapy, and form lethal tumors. Organoids are defined as 3D *in vitro* tissue-like constructs derived from stem cells which mimic their corresponding *in vivo* organ. Aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, and key regulator of infiltrating immune cells in gliomas and associated with poor prognosis, but its role in GSC and GBMO biology is unknown. Generation of glioblastoma organoids (GBMOs) from patient's resected GBM surgical tissue. A total of 555 organoids were generated for the study. We used limited dilution analysis for GSCs, in vivo transplantation of GSCs inactivated for AHR, small animal MRI. We used RNA microarray of GBMO treated with AHR agonist and antagonist, and CRISPR-Cas9 genetic ablation for AHR promote region in GSCs and GBMOs for validation of AHR immune gene targets. We show that AHR is a patient-specific regulator of the glioma intrinsic gene program in GSCs and GBMO that are enriched for AHR. We find that AHR is required for GSC self-renewal, GBMO expansion, radial glia-like cell proliferation, and expression of immune mediators seen in the mesenchymal subtype. CRISPR-Cas9 genetic ablation and pharmacological inhibition revealed that AHR regulates genes linked to intrinsic immunity, proliferation, and migration in GBMO. Genomic analysis of GBMO treated with AHR inhibitors identified expression signatures and candidate markers associated with survival of gliomas. Our work defines the glioma intrinsic function of AHR in a model of early GBM formation, offering a rationale for clinical exploration of a potential 'two-hit' target of both GBM cells and infiltrating immune cells in patients with GBM expressing high levels of AHR.

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Poster Session 2 / No. 13

Loss of LRP10 causes behavioral deficits and neurodegeneration

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In recent decades, loss-of-function variants in the low-density lipoprotein receptor-related protein 10 (LRP10) gene have been associated with autosomal-dominant Parkinson's disease (PD), PD dementia, and dementia with Lewy bodies (DLB). Moreover, LRP10 variants have been found in patients with progressive supranuclear palsy and amyotrophic lateral sclerosis. Moreover, LRP10 is identified as the driver of a specific molecular subtype of Alzheimer's disease (AD). These shreds of evidence suggest potential roles for LRP10 across a broader spectrum of neurodegenerative diseases. In spite of these initial genetic studies, little is known about LRP10 function and no LRP10 animal models have been developed and characterized. We have generated LRP10 knockout (KO) mice. LRP10 KO mice exhibited a battery of behavioral deficits including locomotor function defects and cognitive changes. Besides, we also observed significant axon and neuron degeneration, accompanying with tau pathologies and synucleinopathies in KO mice. To further investigate the cellular regulatory role in which LRP10 is involved, proteomic analysis was performed to identify the LRP10-targeting proteins and pathways. Thus, these mice provide the first LRP10 mouse model to investigate the loss of function of LRP10 in neurodegenerative diseases and an important platform to study the molecular mechanisms of how LRP10 induces neurodegeneration.

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Poster Session 2 / No. 14

Modeling and Predicting Human Perceptual Sensitivity of Speech Recognition in Natural Environmental Noise

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Being able to recognize sounds in competing noise is a critical task of the auditory system. While spectral content of competing noise has been shown to energetically mask foreground stimuli, high-order structure in the modulation content of sounds may also contribute towards masking. We enrolled participants in a psychoacoustic study to assess how the spectrum and modulation content of natural background sounds mask the recognition of vocalized digits (0-9). Native English speakers with normal hearing (0-20 dB threshold, 0.25-8 kHz) listened to digits in various original and perturbed natural maskers (e.g., water, bird babble, construction noise, speaker babble, etc.) at 72 dB SPL and -9 dB SNR. Phase randomized (PR, retains the spectrum but distort the modulations) and spectrum equalized (SE, preserves the modulations and distort the spectrum) perturbations were used to dissociate spectrum vs. modulation masking effects. Behavioral responses show differences across distinct backgrounds and perturbations, indicating some of the masking can be attributed the modulation content and its high-order structure. For instance, the PR 8 speaker babble exhibits an increase in recognition accuracy, indicating that the modulation content is a major masking component. For construction noise, by comparison, the comodulating background tend to improve the accuracy. Results across each ecologically relevant background suggest that modulation content in the background either improves or impairs speech recognition.

We next developed an auditory midbrain model to determine whether masker interference in a physiologically inspired modulation space could predict the perceptual trends. Sounds were decomposed through a cochlear filterbank and a subsequent set of spectro-temporal receptive fields that model modulation sensitivity and map the waveform into temporal and spectral modulation. These outputs were then sent to a logistic regression model estimate perceptual transfer functions and to predict response accuracy. Cross validated and optimized predictions demonstrate that the model accounts for ~90% of the perceptual response variance using 18 biologically inspired features. The model also outperformed predictions obtained using a cochlear model, which accounted only for ~ 67 % of the variance. Perceptually derived transfer functions subsequently allow us to identify salient cues that impact recognition in noise. For instance, comodulated backgrounds tend to improve recognition, while slow background modulations (<8 Hz) tended to reduce accuracy.

The finding demonstrate that the modulation content of environmental sounds can have adversarial masking outcomes on speech recognition and that an auditory midbrain inspired representation can predict and identify high-order cues that contribute to listening in noise.

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Poster Session 2 / No. 15

Presenilin promotes neurotransmitter release through the ryanodine receptor in *C. elegans*

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Mutations in presenilin (PS) are the primary cause of early-onset familial Alzheimer's disease (FAD), but the underlying molecular mechanisms remain controversial. Previous studies have shown that conditional double knockout (cDKO) of *PS1* and *PS2* in CA3 but not CA1 neurons in mouse hippocampus inhibits neurotransmitter release by reducing ryanodine receptor (RyR)-mediated Ca^{2+} release from the endoplasmic reticulum, and that this effect of the cDKO on RyR function resulted from decreased RyR protein levels. However, it is unknown how PS may regulate RyR expression. While humans and mice have three different *RyR* genes (*RyR1*, *RyR2*, and *RyR3*), *C. elegans* has only one such gene, *unc-68*, which makes *C. elegans* an attractive system for studying the function and regulation of RyRs. In preliminary studies, we found that mutations of either *unc-68* or *sel-12*, which encodes a presenilin homolog, caused decreased neurotransmitter release at the *C. elegans* neuromuscular junction (NMJ), as revealed by analyses of miniature and evoked postsynaptic currents (minis and ePSCs). The effects of the *sel-12* and *unc-68* mutations on synaptic transmission were non-additive in *sel-12;unc-68* double mutant, and the synaptic phenotypes of the mutants could be rescued by expressing a corresponding wild-type gene in neurons (presynaptic), but not body-wall muscle cells (postsynaptic), suggesting that SEL-12 and UNC-68 act in a common molecular pathway to regulate neurotransmitter release. In addition, we observed greatly decreased expression of GFP-tagged endogenous UNC-68 in a *sel-12* mutant, which resembled the effect of PS cDKO on RyR expression in the mouse brain. We hypothesize that SEL-12 upregulates UNC-68 expression by acting through an intermediate molecule(s), and that such a molecule(s) might be identified by studying those that are either up- or downregulated in *sel-12* mutants. We have started to identify candidates for the putative intermediate molecule(s) by comparing RNA seq data between wild type and *sel-12* mutants. NAS-21 and CLA-1 are among the candidates thus identified. We found that loss-of-function mutations of either *nas-21* or *cla-1* inhibited minis and ePSCs at the *C. elegans* NMJ, which are reminiscent of the effects of *sel-12* and *unc-68* mutations on synaptic transmission. NAS-21 is related to a metalloendopeptidase, while CLA-1 has been implicated in maintaining presynaptic active zone structure. We are trying to determine whether these two proteins are involved in the functions of SEL-12 and UNC-68. Our ultimate goal is to understand how mutations of presenilins may cause FAD.

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Poster Session 2 / No. 16

Regeneration and neuroprotection of optic nerve axons in a blast injury model of traumatic optic neuropathy

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Closed head blast injuries modeled in mice can disrupt vision, but rarely visualize the extent of axon damage using axonal tracers. Blast injuries cause retrograde axon degeneration which leads to retinal ganglion cell (RGC) death. Retrograde degeneration has been studied extensively in optic nerve crush (ONC) using axonal tracers, but this model lacks the extent of the chronic secondary damage that occurs from closed head injuries. We have developed a closed head blast model of traumatic optic neuropathy (TAI), which includes acute and chronic damage, to test experimental therapeutics which we identified that elicit RGC protection and axon regeneration after ONC. This model will allow us to characterize the secondary chronic components of blast and evaluate the extent of protection and regeneration our therapeutics may have on this injury model. A blast pressure wave is generated using a shock tube cannons system, which was designed and built by the US Navy engineers. Mice are anesthetized and placed into a modified PVC apparatus (which we designed and built) with their torso shielded using a 1-inch acrylic barrier to protect the lungs from blast injury. Animals are attached perpendicular to the shock tube exit with the left side of the cranium placed directly in front of the cannon exit. Animals are subjected to a single 75 psi blast to the left cranium to induce a closed head injury, which affects the white matter including the optic nerve. Animals are placed immediately onto a heating pad until awake. One day prior to sacrifice, mice were intravitreally injected with Cholera toxin subunit B (CTB) to visualize the retinal ganglion cell axons, in order to determine if/where along the central visual pathway the axons are disrupted. Mice are sacrificed and transcardially perfused using 0.9% saline followed by 4% paraformaldehyde (PFA). Optic nerves, chiasm, optic tracts, and eyes are dissected out and postfixed in PFA and sucrose at 4 °C. Post fixed tissue then embedded in OCT tissue Tek Medium and cryosectioned onto a coated glass slide for immunohistochemistry. 5 days after blast injury, we found an increase in GFAP and Iba1 immunofluorescent signal in the left optic nerve, which is on the side on of the head that was subjected to the blast. These markers indicate activation of astrocytes and microglia, which is characteristic of chronic secondary damage. 14 days after blast injury, we found axonal varicosities and bulb formations (visualized by axonal CTB signal) in the left optic nerve, which are indicative of traumatic axonal injury (TAI). GFAP and Iba1 immunofluorescent signal remained increased in the injured optic nerve at 14 days after blast injury. 16 weeks after blast, we found evidence of axonal disruption (visualized by axonal CTB signal). Severed axons were found close to the left optic nerve head, throughout the optic nerve, chiasm, and optic tracts. Our non-penetrating closed head blast model is well-suited for testing IP-protected experimental therapeutics, which we found elicit RGC neuroprotection and axon regeneration after acute focal ONC.

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Poster Session 2 / No. 17

Transcriptomic Analysis Reveals Common Age Independent Immunomodulatory Proteins as a Mode of Neuroprotection in P2X4R KO Mice after Ischemic Stroke

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Identification of a new potential drug target protein and their plausible mechanism for stroke treatment is critically needed. We earlier showed that genetic deletion as well as short term pharmacological inhibition of P2X4R, a purinergic receptor for adenosine triphosphate (ATP), provides acute neuroprotection and thus can be potential drug targets to treat ischemic stroke. However, potential mechanisms remain unknown. Therefore, in this study, we employed RNA-seq technology to identify the gene expression profiles, pathways analysis and qPCR validation of differentially expressed genes (DEGs). This analysis will identify role of those DEGs in certain biological processes responsible for P2X4R dependent neuroprotection after stroke. We subjected young (8-12 weeks-old n= 4/group), and aged (12-18-month-old; n=8/group) male and female Global P2X4 KO and littermate WT mice to right middle cerebral artery occlusion (MCAo) for 60 min followed by 3 day of reperfusion. After 3 days, mice were sacrificed and prefrontal cortex tissue was isolated to extract total RNA using Trizol and used for RNA-seq sample preparations as well as for validation by Nanostring mediated qPCR technique. DESeq2 and Gene Ontology (GO) and /or Ingenuity Pathway Analysis (IPA) were used to identify mRNA transcript expression profiles and biological pathway. qPCR was analyzed with nSolver Data Analysis Support system. We found 2246 DEGs in P2X4R KO vs WT tissue after stroke. Out of these DEGs 1920 gene were downregulated and 325 genes were upregulated in KO. GO/IPA analysis of top 300 DEGs suggests an enrichment of ion channel transport system, inflammation, and extracellular matrix component genes. QPCR validation of top 30 DEGs revealed down regulation of two common age independent genes: Lnterleukin-6 (*IL-6)*, an inflammatory cytokine and Cytotoxic T Lymphocyte-Associated Protein 2 alpha (*Ctla2a*), an immunosuppressive factor KO group. This data suggests P2X4R mediated neuroprotection after stroke is brought by attenuation of immune modulatory pathways in both young and aged mice of both sexes. Future studies will delineate the detailed role of *IL-6* and *Ctla2a* in P2X4R mediated neuroprotection mechanisms after stroke.

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Poster Session 2 / No. 18

Enhancing expression of CX3CL1-C-terimnus for reducing neurodegeneration in an HD mouse model

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Huntington's disease (HD) is a fatal genetic neurodegenerative disorder, which is caused by an increased number of CAG repeats in the Huntingtin gene (Htt). Mouse models carrying mutant Htt genes share many pathological features, including behavioral disturbances, cognitive decline, and motor disorders, similar to that seen in HD patients. There are currently no disease modifying treatments for HD, and it is essential to explore treatment options that will decrease HD pathologies and improve HD patients' quality life. We recently discovered that the intracellular domain of CX3CL1 (CX3CL1-ICD), released from membrane bound CX3CL1 by γ-secretase, is able to enhance neurogenesis in the adult as well as promote neuronal survival, due largely to altered gene transcription pathways initiated from the TGFβ2/3, Smad2/3 and the insulin receptor. Considering the fact that HD exhibit age-dependent neurodegeneration and defects in neurogenesis and neurodevelopment, we attempt to explore whether increased expression of CX3CL1-C-terminal fragment will mitigate neuronal loss in HD mouse models and whether behavioral phenotypes will be ameliorated. In order to generate our HD model, we first crossed YAC128 mice with inducible transgenic mice overexpressing the CX3CL1-C-terminus under the CAMKIIA promotor. Starting at 3-months, mice of both sexes underwent behavioral tests including the open-field, Y-maze and Rota-rod every three months. Although no significant differences were observed in the open-field or Y-maze at both 3 and 6-months, we noted a significant improvement in Rota-rod performance in mice overexpressing the CX3CL1-C-terminus compared to controls at 6-months of age ($p=0.0031$ and 0.0022, 2-way ANOVA) as well as improved working memory on the Y-maze at 9-months. This study provides first evidence that overexpression of the CX3CL1-C-terminus in YAC128 HD mice ameliorates motor deficits. Future studies are planned to evaluate the potential role in cognitive and histopathological outcomes as well as attempting to elucidate the mechanism via cell culture experiments. Additional studies including the use of knock-in HD mice, Q175, are also underway. Together, enhancing expression of CX3CL1-ICD is likely a novel approach for HD treatment.

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Poster Session 2 / No. 19

Ampyra (drug used to alleviate motor symptoms of multiple sclerosis) Potently Modulates Ethanol-Induced Effects on the Neuronal Spontaneous Electrical Activity

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Significant research effort is invested in finding additional chronic pharmacotherapy that would promote reductions in alcohol (Ethanol) intake. However, a pharmacotherapy for treating acutely intoxicated patients (for example, young people after party) and saving their lives in the emergency room is currently missing. Keeping such patients awake, agile and respiratory-compensated with a help of adjuvant drugs may be useful. We found an alcohol-induced depression of synaptically evoked population voltage (network) responses in all layers of the mouse frontal and parietal cerebral cortex; this depression can be reversed by application of 4-aminopyridine aka dalfampridine (Ampyra™). Ampyra is a "*neuron excitability booster*" used to improve walking in patients with multiple sclerosis. In our hands, in brain slices pretreated with debilitating concentrations of Ethanol (20 mM), bath application of Ampyra restores the amplitude and propagation of evoked synaptic depolarizations. The aforementioned experiments have addressed the synaptic transmission specifically, because our population voltage signals are dominated by EPSPs occurring in massive dendritic trees of cortical pyramidal neurons, while the neuronal action potentials (spikes) are filtered out (do not contribute to the optical signals significantly). To address neuronal spiking specifically, we investigated the effects of Ethanol and Ampyra on cultured mouse neurons. We found that at a concentration of 20 mM, Ethanol caused a significant increase in the amount of spontaneous neuronal activity (spiking), while at a higher concentration of 40 mM, it actually decreased the number of spikes in the neuronal culture. Interestingly, in the presence of either 20 mM or 40 mM of Ethanol, bath application of Ampyra was able to increase the spiking frequency of the cultured cortical neurons. Our findings suggest that Ampyra is a potent modulator of Ethanol-induced changes in the cerebral cortex. Even in the presence of Ethanol-induced increase in spontaneous spiking, Ampyra was able to further increase the spiking frequencies of the neurons. Notably, at 20 mM concentration, Ethanol decreases the amplitude of EPSPs in brain slice preparations but increases the spiking of neurons in cell culture. Overall, our results indicate that Ampyra could be a promising treatment for acute alcohol intoxication, as it may help alleviate the suppressive effect of alcohol on neuronal functions (EPSPs and spiking).

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Poster Session 2 / No. 20

Unraveling the Hypercitrullination-PAD4 Axis in Injury and Genetic Models of Degenerative Retinal Disease

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Many common retinal diseases, such as diabetic retinopathy and age-related macular degeneration (AMD), as well as rare genetic disorders, such as retinitis pigmentosa and Leber congenital amaurosis share common pathological features attributed to reactive gliosis. A hallmark of reactive gliosis is the overexpression of type III intermediate filament (IF) proteins in Muller glia (MG), the principal reactive cell type of the retina. During chronic reactive gliosis MG become hypertrophic and engage in fibrosis that leads to retinal scarring. It remains unknown how MG contribute to retinal scarring, a pathology for which there are no FDA approved drugs for ocular application. Scarring is an irreversible process that both disrupts retinal cellular organization and the transparency of this neural tissue, hence the contribution of MG to this enigma is now an active area of investigation. Our lab has employed the murine laser-induced model of retinal gliosis as an experimental model for AMD and shown that the IF protein, glial fibrillary acidic protein (GFAP) becomes overexpressed and post-translationally modified by a process known as citrullination. Citrullination is an irreversible modification of arginine residues to citrulline. This enzymatic reaction is catalyzed by peptidyl arginine deiminases (PADs), and we demonstrated that PAD4 expression increases in reactive MG in coordination with reactive gliosis. Employing glial-specific conditional PAD4 knockout (PAD4cKO) mice subjected to laser injury we have also shown that both hypercitrullination and GFAP overexpression in MG are significantly reduced illuminating this enzyme as a potential target for treatment (Palko, et al. https://doi.org/10.1073/pnas.2121875119). The causal role of PAD4 in subretinal fibrosis was further elaborated in a subsequent study showing that PAD4cKO mice are also significantly protected from developing subretinal fibrosis in the laser injury model (Palko, et al. https://doi.org/10.1002/jnr.25158). In attempts to model therapeutic approaches to antagonize PAD4, we have delayed the onset of PAD4 genetic deficiency extended 15 days post-injury and assessed levels of retinal hypercitrullination and gliosis in the laser injury model. In the paradigm of delayed PAD4 knockout, retinal hypercitrullination and gliosis were also significantly reduced, unraveling a clinical opportunity for the first time in this pre-clinical expedition. To further help translate these findings to a non-injury genetic disease paradigm, we investigated citrullination and fibrosis in a genetic model of spontaneous retinal degeneration using the *Crb1*^{rd8}*/Jak3*^{m1J} double mutant mouse (JR5558 line). The JR5558 mice model human Leber congenital amaurosis, and as such, develop retinal lesions spontaneously, leading to adult-onset retinal degeneration in a manner similar to the progressive nature of retinal pathology seen in humans. JR5558 mice as early as 1 month of age show lesion-specific hypercitrullination and GFAP expression in MG compared to age-matched wild-type controls (Palko, et al. https://doi.org/10.1073/pnas.2121875119). Moreover, JR5558 mice aged 2 and 4 months also develop subretinal fibrosis from retinal hypercitrullination similar to that observed in the laser injury model. *Taken together, the laser injury and JR5558 paradigms demonstrate that MG hypercitrullination is a broadly conserved molecular process underlying retinal pathology and that PAD4 serves as a potential druggable target that warrants further investigation using the JR5558 mouse line*.

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Poster Session 2 / No. 21

Ex Vivo Gamma Frequency (40 Hz) Entrainment in the Mouse Model of Alzheimer's Disease

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In Alzheimer's disease (AD), accumulation of pathological proteins (beta-amyloid and tau) is known to disrupt normal neuronal functions in the brain, leading to unbalanced neuronal excitatory-inhibitory tone, distorted neuronal synchrony, and altered network oscillations. Interestingly, the interaction is bidirectional. On one hand, amyloid and tau accumulation alters the brain physiology (an increase in electrical activity), while on the other hand, the increased electrical activity facilitates accumulation of amyloid and tau. In other words, there is a bidirectional link between brain pathology and brain physiology. Most interestingly, an induction of synchronized 40 Hz gamma oscillation of neuronal networks by sensory stimulation reverses AD-related pathological markers in transgenic mice carrying AD-related human pathological genes. Only the 40 Hz inputs are effective, all other frequencies do NOT mitigate AD pathology. What is so special about 40 Hz? We hypothesized that 40 Hz stimuli propagate more efficiently between cortical layers and along cortical laminas than 20 Hz or 83 Hz inputs do. In brain slices of AD mouse model animals (5xFAD) we delivered extracellular (synaptic) stimuli in the cortical layer 4 (L4), where the majority of thalamocortical projections make afferent connections with the cortical circuit. Genetically encoded voltage indicator (VSFP-Butterfly), expressed in cortical pyramidal neurons, allowed us to monitor evoked cortical depolarizations at many sites simultaneously. Experimental animals (age 90 – 130 days) were divided into 4 experimental groups: AD-Female, CTRL-Female, AD-Male, and CTRL-Male. We found no difference between sex-matched AD versus CTRL data at 3 frequencies tested (20, 40 and 83 Hz). Except, at 40 Hz (and not any other frequency) the propagation of signals through cortical neuropil was significantly less in the AD Group compared to the healthy littermates (CTRL). Our data supports the previous reports about the special relation between AD pathology and cortical oscillations at only one segment of the gamma band, 40 Hz.

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Poster Session 2 / No. 22

Investigating CXCL14's role in Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia worldwide and currently lacks disease-modifying therapies. Abnormal accumulation of extracellular amyloid beta (Aβ) leads to a cascade of neuropathological changes resulting in gliosis, impaired synaptic function, neuroinflammation, and neuronal death. Previous work has shown that astrocyte-specific knockout (KO) of beta-site APP cleaving enzyme 1 (BACE1), the enzyme responsible for generation of Aβ, significantly reduces Aβ pathology in a mouse model of AD. Analysis of differentially expressed genes from single-cell transcriptomic studies on BACE1 KO astrocytes reveals a significant upregulation of chemokine (C-X-C) ligand 14 (CXCL14). CXCL14 is a secreted chemoattractant protein for peripheral immune cells such as macrophages and neutrophils. Interestingly, genome-wide association studies have identified several single nucleotide polymorphisms near the CXCL14 gene locus associated with increased risk of AD pathology. However, the role of CXCL14 in AD and glial cell function is unknown. Here, we investigated the effect of CXCL14 on Aβ-treated microglia *in vitro*. We demonstrated that CXCL14 significantly increased microglial migration, uptake of Aβ, and phagocytic machinery involved in the degradation of Aβ. Additionally, we outline the development of a transgenic mouse model overexpressing CXCL14 under the tetracycline- transactivator controlled promoter. We will cross this overexpression mouse line with two widely used AD mouse models (5xFAD and PS19) to test the effect of CXCL14 on alleviating AD-related memory deficits and AD pathology *in vivo*. These studies could help identify CXCL14 as a novel therapeutic target for AD.

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Poster Session 2 / No. 23

Transcriptomic profiling of retinal cells reveals a subpopulation of microglia/macrophages expressing Rbpms marker of retinal ganglion cells (RGCs) that confounds identification of RGCs

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Analysis of retinal ganglion cells (RGCs) by single cell RNA sequencing (scRNA-seq) is emerging as a state-of-the-art method for studying RGC biology and subtypes, as well as for studying the mechanisms of neuroprotection and axon regeneration in the central nervous system (CNS). RNA-binding protein with multiple splicing (Rbpms) has been established as a pan-RGC marker, in the retina and Osteopontin (Spp1) has been established as an αRGC type marker as well as a macrophage marker. Here, we analyzed by scRNA-seq retinal microglia and macrophages, and found an Rbpms+ subpopulation of retinal microglia/macrophages, which poses a potential pitfall in scRNA-seq studies involving RGCs. We performed comparative analysis of cellular identity of the presumed RGC cells isolated in recent scRNA-seq studies, and found that Rbpms+ microglia/macrophages confounded the identification of RGCs. We also showed using immunohistological analysis that, Rbpms protein localizes to stress granules in a subpopulation of retinal microglia after optic nerve injury, which was further supported by bioinformatics analysis identifying stress granule-associated genes enriched in the Rbpms+ microglia/macrophages. Our findings suggest that the identification of Rbpms+ RGCs by immunostaining after optic nerve injury should exclude cells in which Rbpms signal is restricted to a subcellular granule, and include only those cells in which the Rbpms signal is labeling cell soma diffusely. Finally, we provide solutions for circumventing this potential pitfall of Rbpm-expressing microglia/macrophages in scRNA-seq studies, by including in RGC and αRGC selection criteria other pan-RGC and αRGC markers.

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Poster Session 2 / No. 24

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

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Axonal injury in the mammalian adult central nervous system (CNS) results in permanent disabilities due to the failure of axons to regenerate and rebuild functional connections with their original targets. Like other CNS projection neurons, retinal ganglion cells (RGCs) do not spontaneously regenerate axons after optic nerve crush (ONC) injury. Several intracellular and extracellular factors have been discovered to affect axon regeneration, but the manipulation of even the most potent factors results in less 1% of axons regenerating the full-length to their post-synaptic targets.

Here, we investigated why the regeneration of axons that respond to regenerative treatments stalls before reaching postsynaptic targets in the brain. During CNS development, axon myelination is delayed until the axons reach their postsynaptic targets in the brain. However, optic nerve axons experimentally-induced to regenerate after injury interact with oligodendrocytes and are myelinated even while they are still growing, and eventually the growth stalls. We hypothesized that interaction of the axons (that are experimentally-stimulated to regenerate) with newly born oligodendrocytes (which were absent during developmental axon growth) stalls axonal regeneration even after the axons bypassed the glial scar and grew over the pre-injury myelin.

To test this hypothesis, we used single cell RNA-seq and immunohistological analysis to investigate whether post-injury born oligodendrocytes integrate into the glial scar after optic nerve injury. Then, we used a multiple sclerosis model of demyelination concurrently with the stimulation of axon regeneration by Pten knockdown (KD) in projection neurons after optic nerve injury. We found that post-injury born oligodendrocytes integrate into the glial scar, where they are susceptible to the demyelination treatment, which reduced their integration into the glial scar, and thereby enhanced Pten KD-stimulated axon regeneration.

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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!

WE LOOK FORWARD TO SEEING YOU NEXT YEAR!

Spring 2024