Online Proceedings

SESSION 1C

SENSORY INTEGRATION, LEARNING, AND MOTOR CONTROL IN ANIMAL AND HUMAN MODELS

Session Moderator: Horacio de la Iglesia, Biology
MGH 231
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Shape Selectivity in V4 Using Novel Time Dynamic Stimuli
Alex Paul (Alex) Rockhill, Senior, Neurobiology, Applied & Computational Mathematical Sciences (Biological & Life Sciences)
UW Honors Program
Mentor: Wyeth Bair, Biological Structure
Mentor: Anitha Pasupathy

We hypothesize that neurons in the visual cortical area V4 have a distinct temporal integration time on the order 40 to 100 milliseconds that is required to perceive rapidly moving or changing shapes. If this is true, then we should be able to characterize these neurons using fast streams of dynamic stimuli. To test this, we created stimuli that change their boundary features rapidly by presenting different shapes in immediate temporal progression at various durations for visual fixations lasting just over one second. The duration for which each of the shapes was presented within the sequence was held constant for each fixation trial and varied randomly across trials for durations of 40, 80, 160 and 320 milliseconds. To access which shape durations were sufficient to cause differential, shape-tuned responses, we constructed tuning curves of the mean firing rate as a function of the duration of shape presentation. Our results suggest that shape tuning is consistent across durations from 80 to 320 milliseconds and exists at a lesser amount at 40 milliseconds. This raises the possibility that our future experimental design can involve showing stimuli for shorter durations, allowing us to characterize shape tuning for 3D stimuli, which requires the presentation of a greater number of stimulus images. It also suggests that V4 neurons may play an important role in encoding dynamic scenes as objects change shape or move or rotate with respect to the observer.

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SENSORY INTEGRATION, LEARNING, AND MOTOR CONTROL IN ANIMAL AND HUMAN MODELS

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Does Cerebellar Variability Compensate for Variable Motor Commands?
Dorothy Cabantan, Senior, Neurobiology
UW Honors Program
Mentor: Farrel Robinson, Biological Structure
Mentor: Amy Nowack, Otolaryngology

Normally, repeated voluntary movements to the same target are nearly identical to one another. If, however, the cerebellum is damaged, then repeated movements to the same targets have variable trajectories and end in different places. To investigate why cerebellar damage makes movements variable, we record in a rhesus monkey from the type of neurons in the cerebellum that we know most about, those that influence rapid eye movements (saccades). These neurons are in the medial nucleus of the cerebellum and send their axons to the saccade control center in the brainstem. The burst of action potentials fired by a single saccade-related cerebellar neuron can be long, short, fast, or slow. How do these variable signals from the cerebellum help create identical saccades? One commonly accepted idea is that the variability for each cerebellar neuron is not correlated with the variability of other cerebellar neurons. The uncorrelated variability of many cerebellar neurons produces a consistent summed signal that produces identical saccades. If this is true, then why are saccades variable when we damage the cerebellum? We propose an alternative hypothesis: the variability of saccade-related neurons is correlated. This correlation produces a variable summed output from the cerebellum for identical saccades. We think that the saccade-to-saccade variability of cerebellar output compensates for variability in the motor commands from the cerebral cortex. The signals from both the cerebellum and the cortex vary but their sum is consistent because they vary in opposite directions. Without cerebellar output,

only the variable motor command from the cortex drives saccades. Variable commands make variable saccades. To test our proposal, we record from several cerebellar neurons simultaneously to measure how correlated their variability is. If our proposal is correct, then the variability of cerebellar neurons is not random. It serves a previously unsuspected function to create consistent saccades.

POSTER SESSION 3

MGH 206, Easel 170

2:30 PM to 4:00 PM

Characterization of Hearing Loss Related to Impaired Mitochondrial Function in Mutant Zebrafish Models

Jonathan (Jonny) Ramos, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: David Raible, Biological Structure
Mentor: Sarah Pickett

Hair cells are mechanosensory receptors of the auditory and vestibular systems in vertebrates. Loss of hair cells or hair cell dysfunction, leads to defects in hearing and balance. There are several mitochondrial gene mutations that lead to hearing loss, however, whether these mutations affect hair cells directly remains unknown. Two mitochondrial proteins known to be involved in sensorineural hearing loss, CLPP protease, and Timm8a translocate, were chosen as the focus of this study. To investigate the role of these proteins in hair cell function and susceptibility, I used the zebrafish lateral line as a model. Lateral line hair cells are structurally and functionally similar to those in the mammalian inner ear, but instead detect changes in water flow. CRIPSPR/Cas9 was used to introduce dsDNA breaks in CLPP and Timm8a in zebrafish embryos. Non-homologous end repair following dsDNA breaks is error prone, and can introduce small insertions or deletions (indels) in the protein coding sequence which often leads to a loss of function in the protein. In order to quantify hair cell susceptibility, I treated embryos 5 days postfertilization with varying concentrations of neomycin, a well characterized ototoxin, and counted the hair cells remaining after treatment. Future characterization of these mutants will involve examining mitochondrial activity and oxidation levels. These studies will provide insight into how mutations in CLPP and Timm8a affect hair cell function and susceptibility to damage. Additionally, they will more broadly inform our understanding of how changes in mitochondrial function can cause deafness.

POSTER SESSION 3

Balcony, Easel 108

2:30 PM to 4:00 PM

Expression of DCX in Inner Ear of Embryonic and Postnatal Mice

Isabel Y. Lee, Senior, Neurobiology
Mentor: Olivia Bermingham-McDonogh, Biological Structure
Mentor: Heather Zebroski, Biological Structure

Inner ear hair cells are the sensory receptors in the auditory and vestibular system of vertebrates. Damage to these hair cells permanently decrease one's hearing and balance due to inability to regenerate these cells. Transdifferentiation of inner ear support cells into hair cells is a potential method to regenerate hair cells. The Doublecortin(DCX) gene is exclusively expressed in support cells of the cristae in adult mice. We are interested in examining whether there is a correlation between DCX expression and the plasticity of inner ear progenitor. However, we must first examine the temporal and spatial expression of this gene. We hypothesized that DCX expression begins in all inner ear progenitor cells and later gets restricted to support cells. An alternative hypothesis is that only some of the progenitor cells express DCX and these go on to differentiate into support cells. To study this question, we obtained inner ear tissue from mice of varying embryonic and postnatal ages that are DCX-Cre ER heterozygous and Tdtomato homozygous positive. We fed pregnant female mice with tamoxifen one day prior to euthanization, which will activate the tomato reporter causing the DCX expressing cells to look red. We then used immunostaining to identify hair cells and support cells and look for double labeled cells. By better understanding DCX expression, we can develop a protocol using DCX to FACS (Fluorescence activated cell sorting) support cells and/or progenitor cells from the vestibular organs. In addition, we can continue our research to determine the importance of DCX in transdifferentiation of support cells.

POSTER SESSION 4

MGH 206, Easel 166

4:00 PM to 6:00 PM

Fusion Proteins: An Approach to Distinguishing "Itch" and "Pain" Neurons

Becka Marie Warfield, Senior, Chemistry: Biochemistry (Bothell)
Innovations in Pain Research Scholar
Mentor: Ajay Dhaka, Biological Structure
Mentor: Kali Esancy, Biological Structure

Contrary to the previous belief that itch (pruritis) is simply a less intense form of pain, recent findings suggest that itch and pain are distinct sensations mediated via independent neuronal circuits. Nevertheless, itch and pain share similar molecular machinery. In mammals, itch sensations are the product of the coupling of pruritic receptors, usually GPCRs,

and TRP ion channels such as TRPA1, nociceptors that normally encode noxious, painful stimuli. We identified an itch selective compound, imiquimod (IMQ), which was found to mediate pruritic responses via direct activation of TRPA1. However, other TRPA1 agonists such as allyl isothiocyanate (AITC) specifically evoke nociceptive responses. We found that IMQ is a weak agonist of TRPA1 and specifically activates itch selective neurons. These itch selective neurons are primed to respond to TRPA1 agonists while having no effect on TRPA1-expressing nociceptors. We hypothesized that the differences in sensitivity of itch-selective versus nociceptive sensory neurons to TRPA1 agonists could be caused by differences in the amount of TRPA1 channels they contain, the activation mechanism of TRPA1, or the trafficking of TRPA1 following activation. Thus, we developed a strategy to visualize TRPA1 by creating fusion proteins that tether various indicators to TRPA1. We have created a fusion construct of TRPA1 and green fluorescent protein (GFP) by using overlap polymerase chain reactions (PCR) to amplify segments of DNA and tethering the two pieces together via direct, rigid, and flexible linkers. This experiment will lead to the fusion of TRPA1 to other molecules, such as genetically-encoded calcium indicators, GCaMP and CaMPARI. These fusions will give us the ability to visualize the localization, expression, activation, and trafficking of TRPA1. These studies will help elucidate the molecular mechanisms underpinning itch versus pain sensation and show how a single ion channel can mediate distinct sensations via differential activation.

POSTER SESSION 4

Balcony, Easel 120

4:00 PM to 6:00 PM

The Impact of Retinal Progenitor microRNAs on Müller Glia in Postnatal Mouse Retinal Explants

Ellen Bercaw, Senior, Biochemistry
Mentor: Stefanie Wohl, Biological Structure
Mentor: Thomas Reh, Biological Structure

Müller glia (MG) are the predominant glia of the neural retina. In fish and birds, retinal injury causes these glial cells to de-differentiate into retinal progenitor cells (RPCs), and these RPCs repair the damaged tissue by generating new neurons. This process does not happen in mammals, and so retinal damage is permanent. microRNAs (miRNAs, miRs) are small RNA molecules that regulate gene expression. Several miRNAs are expressed in the RPCs, but not in the Müller glia, and we call these RPC-miRs. This study focused on if artificially expressing the RPC-miRs would stimulate mammalian MG to regenerate new neurons in postnatal mouse retinal explants. Two leading questions were explored: Will the Müller glia treated with RPC miRNAs regenerate new neurons? What is the best time window to convert Müller glia into retinal progenitors with high efficiency? Retinas from postnatal (P) 4 up to P12 wild type or transgenic reporter mice were dissected from surrounding eye tissue and electroporated to introduce RPC artificial miRNAs (such as miR-15a, miR-17, miR-19, and miR-20a mimics) or control miRNA mimics, along with a reporter mRNA. Explants were cultured for 3/4 or 6/7 days ex vitro (DEV), fixed, cross sectioned, stained (immunofluorescent labeling) and analyzed using confocal microscopy. The proliferation marker EdU was added to track cell proliferation. Our data showed that RPC-miRs led to an increased proliferative response in the Müller glia, indicating that the cells were adopting an RPC-like state. Moreover, the age of the explants was an important factor; the areas that were successfully electroporated became smaller in older explants and therefore fewer Müller glia can be targeted in older tissue. Our data shows that miRNAs from RPCs can promote Müller glia to adopt some RPC-like characteristics and therefore could be used to stimulate regeneration in the retina.

POSTER SESSION 4

MGH 206, Easel 165

4:00 PM to 6:00 PM

CRISPR Based Behavioral Screening for Genes Affecting Nociception in Zebrafish

Nicolas Germanos, Senior, Neurobiology
Mary Gates Scholar, UW Honors Program
Mentor: Ajay Dhaka, Biological Structure
Mentor: Andrew Curtright, Biological Structure

Chronic pain affects millions of people worldwide, and current treatments are often ineffective and come with unwanted side effects. Modern research focuses on studying the mechanisms of pain sensation in the nervous system, with the goal of using our improved knowledge to develop more efficient analgesics. Our research focuses on neurons located in the Trigeminal and Dorsal Root Ganglia (TRG and DRG), as they are known to play an important role in the sensation of touch and pain, and on genes regulating the development and function of these neurons. Recent advances in gene editing methods, particularly the CRISPR/Cas9 system, allow us to mutate specific genes of interest a living animal and monitor the effect of such mutations within a matter of days. To this end, we have developed a behavioral assay which determines the effect of CRISPR induced mutations on pain sensation in zebrafish larvae. CRISPR injected larvae are exposed to noxious stimuli such as AITC (mustard oil) and harmful temperatures. The CRISPR treated larvae's response to the painful stimuli determines the impact of the specific gene we targeted on nociception. Through this assay we have identified two genes, zmat4b and zbtb7b, as potentially important to the sensation of noxious stimuli. Further experiments are now required to determine the precise mechanisms through which these genes play a role in the sensation of pain.