1I

DEVELOPMENTAL NEUROPLASTICITY

Session Moderator: Sheri Mizumori, Psychology
251 MGH
1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Evoked Electrical Events Behave Like Spontaneous Propagating Events in Developing Hindbrains

Bethanny Patricia (Bethanny) Danskin, Senior, Neurobiology
Mary Gates Scholar
Mentor: Martha Bosma, Biology
Mentor: Hiro Watari, Neurobiology & Behavior
Mentor: Amanda Tose, Biology

During embryonic neural development mouse hindbrains exhibit spontaneous depolarizations that propagate from cell to cell across the tissue in waves. There is a discrete, anatomically identifiable population of cells responsible for generating these waves: the cells of the initiation zone (InZ). Spontaneous waves of activity have been shown to affect neuronal migration, pathfinding, and differentiation, among other developmental sequences. Spontaneous depolarizing events, in general, allow passage of ions into and out of the cell; of developmental importance are calcium ions, which are involved with regulating gene expression. During critical windows of development these calcium fluxes help to determine the adult fate of a neuron. The spontaneous and variable nature of the propagating neuronal events makes them difficult to manipulate experimentally. Were these propagating events able to be elicited experimentally and discretely that would provide both a vehicle for further study and an indication of what properties of the initiator population allows the cells to generate propagating events. I have used a combination of several techniques, including calcium-dye imaging, whole-cell patch clamp, and extracellular electrical stimulation, to probe the details of the cells' activity. These experiments have identified separate populations of cells that are capable of initiating propagating waves, both spontaneously and elicited by external electrical stimulation. One population includes the previously identified cells of the InZ, and another population exists at the isthmus between the midbrain and hindbrain. Cells in these populations respond differentially to a variety of electrical stimuli. Within each of these initiator populations may exist single, highly-networked neurons capable of initiating propagating electrical activity evoked by intracellular stimulation, which is the subject of further investigation.

Quantifying Microglial and Macrophage Cells in Cortex Ischemic Preconditioning Pulse

Ryan Edward (Ryan) Dodge, Senior, Neurobiology
Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) is a robust neuroprotective phenomenon in which a brief ischemic exposure increases resistance to the injurious effects of subsequent prolonged ischemia. Characterizing the cellular and molecular mechanisms of IPC is an active area of investigation in stroke research. Microglia are the resident immune cells of the central nervous system and are the primary cell type responsible for post-stroke neuroinflammation in the brain. Several lines of evidence support a role for microglia in IPC. Previous research from the Weinstein laboratory has demonstrated using ex vivo flow cytometry that IPC induces a robust increase in the number of microglia/macrophages that can be identified and sorted from the preconditioned (ipsilateral) cortex. However, it is uncertain if this increase reflects a true change in cell number (due to proliferation or migration) or if it reflects a novel characteristic of the preconditioned microglia to better survive the enzymatic and mechanical tissue digestion that precedes the flow cytometry processing. In order to address this question, we are carrying out quantitative immunofluorescent microscopy on tissue sections from preconditioned or naïve mouse brains that have been stained with a fluorochrome-conjugated antibody (anti-Iba1) specific for identifying microglia/macrophages. My contribution to this work will include tissue preparation and sectioning, antibody staining, microscopy image capture and cell quantification using custom calibrated image analysis software. Answering this initial yet fundamental question will be an important starting point for additional investigations into the function and phenotype of microglia/macrophages in the preconditioned brain and may shed light new light on the mechanism of IPC.

Expression of Tbr1 and Tbr2 in Embryonic Mouse Hypothalamus

Michelle Anna Ninh (Michelle) La, Senior, Neurobiology, Biochemistry
Mentor: Robert Hevner, Neurological Surgery

Brain development is highly regulated by transcription factors that control often-variable levels of gene expression. Two transcription factors, Tbr1 and Tbr2, are expressed by neurons in the embryonic vertebrate brain during different stages. Abnormalities in Tbr1 and Tbr2 expression have been linked to autism, a developmental disorder characterized by impaired communication abilities with an incidence rate of 1.1% in the United States. Therefore, these transcription factors are of significant research interest. Tbr1 indicates fully differentiated postmitotic projection neurons that form a matrix through which newer neurons migrate. Tbr2 is a marker of intermediate progenitors, or unipotent neural cells, committed to a glutamatergic fate. In cortical development, neurons migrate from the brain interior toward the cortical plate and express Tbr1 and Tbr2 in well-documented time-dependent layers. However, less is known about Tbr1 and Tbr2 in the hypothalamus, also part of the forebrain. The adult hypothalamus performs diverse roles such as endocrine hormone secretion, hunger regulation, and circadian cycles. Because peak hypothalamic neurogenesis occurs during embryonic days (E) 12-14, I have examined mouse tissues from that period with techniques including immunohistochemical (IHC) fluorescent staining and Nissl staining in order to better identify the types of cells that express these transcription factors. IHC staining uses fluorescent antibodies to tag Tbr1 and Tbr2 as antigens, thus rendering cells that express the transcription factors highly visible under certain wavelengths of light. I also hope to gain information about the adult cells these progenitors eventually develop into. My results may enable the pursuit of research avenues such as migratory behavior of hypothalamic neurons, and a greater understanding of Tbr1 and Tbr2 may contribute to our current knowledge of autism associations.

MicroRNA Processing by *Dicer-1* Regulates Sensory Neuronal Morphology

Marvin Eduarte Nayan, Senior, Neurobiology, Biochemistry
EIP Scholar, Howard Hughes Scholar, Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee, Washington Research Foundation Fellow
Mentor: Jay Parrish, Biology

Since neuronal morphology is linked to neuronal function, the nervous system goes to great lengths to ensure that neurons achieve their proper shape, and failure to do so is associated with many cognitive disorders. However, the precise regulatory mechanisms underlying the coordinated expression of certain genes in a given cell type, at the right amount, and at the exact developmental time is one of the longstanding questions in developmental biology. MicroRNA biogenesis is a common mechanism by which cells regulate gene expression, often by "tuning" levels of target gene products, allowing for fine regulation of protein levels over a broad dynamic range. From a genetic screen for mutations in fruit flies that affect dendrite morphology in sensory neurons, we identified a novel *Dicer-1* allele that causes stereotyped dendrite defects, including deregulated growth and defects in dendrite self-avoidance. *Dicer-1* encodes a type III RNA endonuclease that is required for processing many types of small regulatory RNAs, including microRNAs. Time-lapse imaging and quantitative analysis of the dendrite patterning in *Dicer-1* mutant sensory neurons reveals that there is a significant increase in the number and overall length of dendrite branches as well as an increase in dendrite-dendrite crossing-over, especially at late stages of larval development. Interestingly, mutation in a gene (*pasha*) that functions upstream of *Dicer-1* to process a subset of miRNAs recapitulates the exuberant branching, but not the dendrite crossing phenotype of *Dicer-1* mutants. We hypothesize that different classes of miRNAs regulate dendrite branching and dendrite self-avoidance and we plan to use microarray analysis of *Dicer-1* and *pasha* mutants to identify the pathways regulated by these different classes of miRNAs.

Seasonal Plasticity in an Avian Song Control System: An Examination of Neuronal Recruitment and Apoptosis During Transition from Breeding to Nonbreeding Seasons

Nivretta Murlidharan (Nivi) Thatra, Senior, Neurobiology
Mentor: Eliot Brenowitz, Psychology
Mentor: Tracy Larson, Biology, University of Virginia

Ongoing neurogenesis in the adult brain is a fundamental process of neural plasticity. The songbird is an established model for studying neuroprotection, neurogenesis, and neuronal turnover due to dramatic plastic changes in morphology and function in the nuclei that control avian song production. HVC, a region of the brain that times the production of song, doubles in size at the beginning of the breeding season, largely as a result of an increase in new neuron incorporation. In the nonbreeding season the song control circuit rapidly regresses in size. Within 7 days HVC regresses to non-breeding condition volume, and neuron number decreases by around 25% ($>$ 68,000 neurons) via neuronal apoptosis. We tested the hypothesis that regression of HVC via neuronal apoptosis upon transition into nonbreeding conditions is tightly linked to proliferation of neural stem cells (NSC) in the nearby ventricular zone and changes in singing behavior. We also asked whether new neurons recently incorporated into the song control system were retained or lost through transition into non-breeding conditions. We rapidly transitioned birds from breeding to nonbreeding conditions and measured neuronal death and survival, NSC proliferation, and song behav-

ior over a time-course of 28 days. We found that some but not all neurons incorporated into HVC during the previous breeding season persist at least 28 days into the nonbreeding season, suggesting that both new and mature neurons must undergo apoptosis during transition into nonbreeding conditions. Interestingly, we found that proliferation in the VZ was tightly linked to the amount of cell death occurring within HVC. We also quantified song degradation following rapid transition into nonbreeding conditions and correlated the observed changes in behavior to the cellular changes occurring within HVC. These findings demonstrate a relationship between cell death and neural stem cell proliferation.

Properties of Spontaneous Waves of Activity in Developing Cerebral Cortex Studied with a Microscopy-Compatible Microfluidic Electrode Array

Keiko Weir, Senior, Economics, Neurobiology
Howard Hughes Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: William Moody, Biology

During early development, spontaneous waves of electrical activity propagate across many structures in the central nervous system. These waves are believed to regulate neuronal migration, physiological maturation, and synaptic connectivity. In the developing mouse cerebral cortex such waves manifest as increases in intracellular calcium and bursts of actions potentials that occur simultaneously in the majority of neurons in the cortex. We have developed a microfluidic multi-electrode array for use in the study of these waves of activity. This array records extracellular signals from twelve 50 micron microfluidic apertures that can also be used for focal electrical or chemical stimulation. The array is transparent and is compatible with simultaneous fluorescent imaging of intracellular calcium signals at single-cell resolution. We have used this array to study propagation patterns and mechanisms of cortical waves in postnatal (P) day 1 to day 5 brain slices. Waves initiate in the ventral (piriform) cortex and are detected by the array sequentially at each of the apertures placed along the ventral-dorsal axis of the slice, and by simultaneous calcium imaging as the spread of a fluorescence signal along the same propagation axis. The calcium signals outlast the electrical events by several seconds. We used a power spectral density analysis to identify the unique frequency components of different types of cortical waves. The signals from the dorsal regions show a prominent low-frequency oscillation late in the signal that is not present in ventral initiator regions. We have also used this device to study the contributions of the neurotransmitter GABA to network development by investigating a transgenic mouse missing the primary enzyme that creates GABA. Use of this device will lead to a greater understanding of the development of neural networks in the mouse cortex.