Analyzing Superior Collicular Neuronal Circuitry in Mouse Models of Rett Syndrome

Research Director: Dr. Michela Fagiolini

Rett Syndrome is a neurodevelopmental disorder resulting from a de novo mutation in the X-linked MECP2 gene. Onset occurs between 12-18 months, and patients appear to undergo normal development, followed by plateau and subsequent regression of sensory modality and cognition. In recent years, a selective visual impairment has been identified as measured by a loss of optomotor acuity in mouse models and loss of visual acuity in both mice and human patients. While the effect of MEPC2 deletion on the thalamus and visual cortex has been investigated, little research has been conducted to explore MECP2 deletion on neuronal circuitry in the superior colliculus, one of the subcortical structures governing the optomotor reflex. This thesis utilized wildtype and Mecp2 knockout mice from four ages that corresponded to the development and regression of optomotor acuity in mutant mice. Immunohistochemistry was conducted on coronal superior collicular sections to anatomically analyze feed forward input from the retina and the cortex with excitatory markers, Vglut1 and Vglut2, as well as inhibitory parvalbumin positive interneurons that have a demonstrated misregulation in the visual cortex. Further exploration of retinocollicular projections was conducted via fluorescent dye injections into the vitreous humor of the eye and examination of eye specific segregation patterns in the superior colliculus. Results indicated increased Vglut1, Vglut2, and parvalbumin levels beginning at P30, which approximately corresponded to the onset of optomotor regression. A retinocollicular eye specific segregation baseline was established in Mepc2 wildtype mice, and will be compared against that of Mecp2 knockout mice.

Characterizing CDKL5-mutant mouse models of Rett syndrome-like Disorders

Research Director: Dr. Michela Fagiolini

Rett syndrome is a neurodevelopmental disorder characterized by a rapid regression of social, behavioral, and intellectual development in early childhood. While most cases of Rett syndrome are caused by mutations in the methyl-CpG-binding protein 2 gene, mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene have been commonly identified in patients with the early-onset seizure variant of Rett syndrome. Given the clinical phenotype of patients with CDKL5 mutations, CDKL5 disorder was established as an independent clinical entity in 2013. However, the behavioral and biochemical abnormalities in recently developed CDKL5-mutant mouse models remain largely unknown and may provide insight into potential therapies for CDKL5 disorder. In this thesis, I developed a baseline behavioral phenotypic profile for two CDKL5-mutant mouse models, a Cdkl5-knockout mouse and a Cdkl5-knockin mouse, by characterizing their general locomotor activity, anxiety, memory, and visual acuity. Open field, elevated plus maze, novel object recognition, and optomotor testing revealed impaired locomotion and visual acuity in the Cdkl5-knockout mouse and decreased anxiety in the Cdkl5- knockin mouse. These findings of impaired visual acuity led to my subsequent quantification of the concentration of N-methyl-D-aspartate receptor subunits 1, 2A, and 2B in the visual cortex of juvenile and adult mice of both CDKL5-mutant mouse models. Western blot analysis revealed abnormal NMDAR subunit concentrations in the visual cortex suggestive of an impaired maturation of the visual system during the critical period.

Characterizing Trem2 expression for study of microglia-mediated synapse pruning

Research Director: Dr. Beth Stevens

During development, synapses form in excessive numbers and are pruned such that only the most functional synapses remain. Microglia, the brain's resident immune cells, are required for this pruning process; however, the mechanisms underlying microglia-mediated synapse elimination remain unclear. Recent research has shown that triggering receptor expressed on myeloid cells 2 (TREM2) regulates immune cell phagocytosis and may be expressed during peak pruning periods in mice. Therefore, we characterized TREM2's spatiotemporal expression pattern in the brain and across several organs to assess whether TREM2 expression correlates with periods of high synaptic pruning and to determine when and where to assess its role in microglia-mediated pruning. We optimized genotyping protocols for two TREM2 mouse models and employed fluorescence in situ hybridization (FISH) to assess TREM2 expression in microglia. We then validated primers for TREM2 and the microglia marker P2Y12 and employed quantitative PCR (qPCR) to characterize expression for both genes. We find that, within the brain, TREM2 is only expressed in cells positive for microglia markers; furthermore, expression of both TREM2 and P2Y12 mRNA begins as early as E12.5 in the brain and increases throughout postnatal development. These data suggest that TREM2 is expressed specifically within microglia in the developing brain and that TREM2 is expressed during periods of high synaptic pruning; however, further characterization is needed to determine TREM2's expression level per microglia and within distinct brain regions. Based on these data, we will assess the functional role of TREM2 in microglia-mediated pruning via a well-established microglia engulfment assay.

Computational Study of Adaptive Behaviors in a Mouse Model of Motor Control

Research Director: Dr. Nao Uchida

A fundamental aspect of biological motor control is the ability to adapt one's actions dynamically to a changing environment. Recent work has shown that it is possible to begin studying the neural mechanisms behind adaptive motor control in a mouse model by combining behavioral tasks with optogenetic inhibition in primary somatosensory cortex (S1). This paradigm allows us to examine S1's specific contribution to the adaptive motor behavior that we observe, and articulate hypotheses regarding the structure of the requisite neural computations. ¶ In this work, we construct a computational model of motor adaptation, in which a biophysically plausible 2-joint model of the mouse arm is guided by a controller designed using ideas from the field of control theory. Most notably, we show that the adaptive control strategies employed by the mouse can be well modeled within the framework of Kalman filtering, and predict the modification of this behavior under optogenetic inhibition of S1. This leads us to consider the possibility that the mouse is learning about its environment by sampling the most salient points of the error within a trajectory. Finally, we consider the fit of this model to the observed data using information-theoretic measures suited to the analysis of high dimensional data such as our own. From this analysis, we conclude that control-theoretic concepts such as Kalman Filtering provide appropriate and illuminating frameworks that can inform further inquiries into the mechanisms behind biological motor control.

Developing silencing strategies for unmyelinated mechanoreceptors and assessing the behavioral implications

Research Director: Dr. David Ginty

Unmyelinated mechanoreceptors, or C-fibers, are one of several classes of tactile afferents used to sense touch. While high-threshold C-fibers are established as playing an important role in nociception, their low-threshold counterparts, called C-low threshold mechanoreceptors (C-LTMRs) are not as well understood. Building off of recent research identifying key genetic features of C-LTMRs, the principal objectives of this thesis were to evaluate the efficiency of genetic strategies for silencing these afferents in mice, and to examine possible behavioral changes that may result from such silencing. We used two dual recombinase methods to silence the unmyelinated afferent population—RC::PFTox expression and diphtheria toxin receptor (DTR) expression—to attempt silencing through vesicle-release inhibition as well as ablation, respectively. Silencing strategies were evaluated using immunohistochemistry (IHC) to examine mouse dorsal root ganglia (DRGs). To test for possible behavioral effects of C-LTMR silencing we ran a wide range of established behavioral assays as well as developed novel assays to examine self-grooming, and responses to acute pain and itch. Currently our data has been gathered and analysis is nearly complete, with results and conclusions forthcoming.

Early Biomarkers for Anxiety: An event-related potential study on emotional face processing and internalizing traits in three-year olds

Research Director: Dr. Chuck Nelson

Anxiety disorders are among the most prevalent mental health disorders in children. One of the ongoing goals of current research is to investigate biomarkers for anxiety that can be measured at a young age, so that children at higher risk for these disorders can be identified and supported as early as possible. This study explored the use of brain activation recordings and genetic information to predict internalizing behaviors, which are often precursors to anxiety, in typically-developing three-year-olds (n=63). Event-related potentials (ERPs) were recorded during a passive viewing task of emotional face stimuli, and four face-sensitive components were identified for analysis: the P1, N170, P400, and Nc. Subjects were also genotyped for single nucleotide polymorphisms (SNPs) associated with anxiety, including COMT rs4680, NPSR1 rs324981, FAAH rs324420, and RGS2 rs4606. Using the Infant Toddler Social and Emotional Assessment (ITSEA) as a measurement for internalizing behaviors, I investigated the predictive power of these potential biomarkers for internalizing behaviors in three-year-olds. At this point, analysis and results are forthcoming.

Effects of inflammation on brain development in Bangladeshi children

Research Director: Dr. Chuck Nelson

Early postnatal brain development is influenced by environment. Exposure to prolonged and severe adversity in many parts of the world make these early developmental years a time of great vulnerability. One such adversity is lack of sanitation and basic healthcare which increases exposure to and duration of infections. In my thesis, I sought to examine the associations between elevated levels of inflammatory cytokines and brain development as measured using behavioral assessments of cognitive development and neural imaging in Bangladeshi children. Using near-infrared spectroscopy (NIRS) and culturally adapted versions of the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) and the Mullen Scales of Early Learning (MSEL), I conducted statistical tests to quantify differences between children of varying levels of pro-inflammatory biomarkers at 18 weeks and their respective developmental outcomes. I found the following: 1) analysis of cytokines and behavioral outcomes upheld Jiang et al.'s finding of increased inflammation correlating negatively with developmental outcomes as measured using behavioral assessments, 2) analysis of NIRS responses to the silent stimulus revealed significant hemodynamic responses which correlated positively with levels of IL-1β over the anterior area of the arrays, corresponding to the prefrontal cortex, and 3) analysis of NIRS responses to the control stimulus revealed significant hemodynamic responses which correlated positively with levels of IL-6 over the posterior area of the arrays, corresponding to the temporal parietal junction or the occipital lobe. Taken together, these findings suggest that inflammation is indeed detrimental to development, though the underlying neural activity is not understood.

Effects of Microbubble-Enhanced MR-Guided Focused Ultrasound on the Expression of P-glycoprotein in the Blood-Brain Barrier

Research Director: Dr. Nathan McDannold

The blood-brain barrier prevents the passage of many blood-borne substances into the brain, including a variety of therapeutic agents for diseases of the central nervous system. Efflux transporters embedded in this barrier, such as Permeability glycoprotein (Pgp), contribute to its selective permeability. Knocking out Pgp in mice has been shown to increase levels of Pgp substrates in their brains. Similarly, focused ultrasound in combination with circulating microbubbles has been shown to disrupt the blood-brain barrier, increasing its permeability to otherwise impenetrable molecules. This project served to investigate whether modulation of Pgp expression is an effect of using focused ultrasound and microbubbles to disrupt the blood-brain barrier. Pgp expression was assessed using a monoclonal mouse anti-Pgp antibody (C219, Dako). We found that microbubble-enhanced focused ultrasound suppresses Pgp expression. When disruption of the blood-brain barrier was produced at 0.55 MPa, expression levels of Pgp were suppressed for up to 48 hours and restored by 72 hours. At 0.81MPa, Pgp expression was suppressed for 72 hours or longer. These results suggest that the suppression of Pgp, and consequent minimized drug efflux, is a mechanism by which microbubble-enhanced focused ultrasound treatment disrupts the blood-brain barrier.

Evaluating Phase-Amplitude Coupling As a Marker of Cognitive Development in Rural Pakistani Children

Research Director: Dr. Chuck Nelson

Children in low and low-middle income countries are often exposed to adverse conditions that can impair early brain development, though the neurological underpinnings of differences in cognition observed in these children are not well understood. We investigated the longitudinal effects of the Pakistan Early Child Development Scale Up (PEDS), an early nutrition and responsive parenting intervention program, on development in rural communities in southeast Pakistan. Previous analyses have shown an association between EEG gamma power and verbal IQ in girls and gamma power and executive function in all children in the sample, but no combined interaction for intervention group, neurological measures, and behavioral outcomes in this sample. However, previous findings in children suggest phase amplitude coupling in general, and theta-phase gamma-amplitude coupling in particular, may provide information about the development of attention regulation and memory not shown by analysis of individual frequency bands in isolation. We will compare spatial and temporal distributions of theta-phase gamma-amplitude coupling (TGC) between intervention groups during rest and examine the relationship between TGC patterns and behavioral outcomes related to attention control, executive function, and memory.

Face ERPs at 9 Months Predict Later Social Behaviors

Research Director: Dr. Chuck Nelson

It is invaluable that we find biomarkers indicative of Autism Spectrum Disorder to allocate resources to those lost likely to develop the disorder. However, ASD is diagnosed only after the observance of behavior symptoms, usually around 50 months of age. The earlier we identify individuals at risk for ASD and begin intervention, the better their outcomes in life. Therefore, electrophysiological methods, such as Event Related Potentials, may help us detect abnormalities in early development that are predictive of later behavioral phenotypes. We analyzed ERPs in response to face familiarity in a 9-month population at high risk of developing Autism Spectrum Disorder. We also examined the correlation of these neural responses to the social behavior at 18 months to determine if these electrophysiological results may be predictive of later observable behavior. The siblings of individuals with ASD are at greater risk of developing the disorder themselves. Even when they do not develop the disorder, they may present sub-threshold behavioral symptoms. Therefore, this group of individuals may allow us to learn more about the development of the disorder. We compare between-group differences in infant ERPs to mother and stranger faces in a low-risk control group, an infant sibling non-ASD group, and an infant sibling ASD group. Specifically, we compared responses to the face-sensitive components NC, P1, N290 and P400. If differences are present, it will support the use of electrophysiological methods as markers for the later development of ASD and ASD-related symptoms. While there are similar ERP studies on infant sibling populations, none have compared those that develop ASD themselves and those that do not meet criteria for any psychopathology. A correlation between the severity of ERP differences and behavioral scores on social components of behavioral evaluations would support the theory that detectable differences in neural activation early in life are responsible for the behavioral difference at a later age.

Impact of Increased Dorsolateral Prefrontal Cortex Activity on Impulsivity

Research Director: Dr. Josh Buckholtz

The dorsolateral prefrontal cortex (DLPFC) has been shown to play a significant role in cognitive control of reward-mediated impulsivity. In the present study, we explored the region's impact on perception of risk in gambling scenarios. Participants were presented with a series of lottery games that differed in levels of risk and ambiguity. Participants were then asked to rank willingness to pay, likelihood of winning, and certainty of likelihood estimate before and after stimulation of the DLPFC using transcranial direct current stimulation.

Impact of Maternal Stress and Neighborhood Socioeconomic Status on Infant Neurodevelopment at Two and Six Months

Research Director: Dr. Chuck Nelson

Early experiences, such as prolonged toxic stress exposure, have the potential to influence long term physical and mental health outcomes. To determine whether electroencephalogram (EEG) or oxidative stress markers are biomarkers of early toxic stress exposure in the first year of life, I recorded baseline EEG and collected urine samples from infants when they were two (n=26) and six (n=14) months of age, on average. Using simple linear regression, I examined the relationship between stress exposure—quantified by maternal scores on the perceived stress scale (PSS) and levels of neighborhood socioeconomic status (NSES)—and both frontal and whole brain EEG power and urine F2a-IsoProstanes (F2a-IsoP). Stress exposure did not predict differences in two-month EEG power. However, lower two-month NSES predicted lower frontal theta power (4-6Hz) in the six-month EEG, while higher scores on the six-month PSS predicted lower levels of high alpha power (9-13Hz). Theta power in infancy is associated with limbic system plasticity and attention, and alpha power has been found to be modulated by adversity. F2a-IsoP urine data are currently being processed by collaborators and, time permitting, these data and analyses will be included in the final thesis. Data indicate that by six-months differences in exposure to stress can predict specific differences in frontal theta and whole-brain alpha power. These findings suggest that specific EEG patterns observed could be markers of stress at six months. Results will also be discussed in terms of their limitations and applicability for intervention assessment.

Investigating the Relationship Between L2 Acquisition & White Matter Integrity: A Diffusion Tensor Imaging Study

Research Director: Dr. Gigi Luk

Previous research in older adults and young children has shown that bilingualism is associated with higher white matter (WM) integrity, a possible neural correlate of higher levels of white matter maintenance, or "cognitive reserve," in the former and of higher levels of cognitive control in the latter. The literature evaluating WM integrity in young adults, however, is mixed, and thus this metric as a life-long neural correlate has been called into question. The present study investigates this in two ways: via a group comparison and behavior-structural correlations. Given the existing developmental literature, we tested the hypothesis that the age of second language (L2) acquisition would be correlated with WM in young adults. Using diffusion tensor imaging, we found a significant inverse correlation between WM integrity in the corpus callosum extending to the superior longitudinal fasciculi and age of L2 acquisition as measured by fractional anisotropy (FA). However, analyzing the same data as a group comparison suggested no statistical difference in FA values in the same WM tracts. These results highlight the importance of considering the heterogeneity within bilingualism, and suggest that a correlational approach is more meaningful than a group comparison with respect to this neural correlate.

Investigating the Role of Microglia in Synapse Loss in Alzheimer's Disease Mouse Models

Research Director: Dr. Beth Stevens

Microglia, the resident macrophages of the CNS, have been implicated in Alzheimer's disease (AD) pathology, which is hallmarked by amyloid beta plaques, tau tangles, and synapse loss. Interestingly, the mechanisms surrounding synapse loss are not well understood, despite the established relationship between this deficit and cognitive decline. Recently published data from the Stevens lab suggest that microglia and complement contribute to pre-plaque synapse loss in AD mouse models. Specifically, genetic and antibody-mediated inhibition of complement protected synapses in AD mouse models. Additionally, challenging wildtype (WT) mice with synaptotoxic AB oligomers (oAB) led adult microglia in vivo to eliminate synaptic material through complement receptor, CR3.

- These findings raise the need to understand mechanisms of how microglia eliminate synapses in early AD pathology. To assess whether microglia are necessary for synapse loss, we utilized a genetic means to ablate microglia from adult mice. However, we found that the ablation was incomplete; furthermore, this means of microglial ablation led to changes in astrocytic and neuronal numbers. Next, considering the Stevens Lab findings that genetic inhibition of CR3 protected synapses from oAB-induced microglial engulfment, I assessed whether CR3 would be upregulated in a region-specific manner in AD mouse models. Using immunohistochemistry, we found an increase of CR3 in the hippocampi of pre-plaque 1-month-old J20 and oAB-injected WT mice.
- Lastly, I asked what other immune markers on microglia may accompany oAB-induced synapse loss. Preliminary data using fluorescence-activated cell sorting indicated changes in CD45 on isolated microglia with oAB. Using immunohistochemistry, I observed a region-specific increase in immunoreactivity of IgG, CD45, and CD68 in 3 mo mice challenged with oAB. Interestingly, these increases were exacerbated with aging, in 19-month-old mice. My data to date suggest that certain immune markers on microglia are upregulated when challenged with AB, and aging increases this association. I am currently investigating whether aging exacerbates oAB-induced synapse loss. These results also put forth a potential role for the adaptive immune system in AD-related microglial and synaptic pathology. To this end, I am exploring the possibility of central-peripheral crosstalk through other immune cell types that may be infiltrating the brain and producing the present observations.

Longitudinal Deep Phenotyping of the Individual: Dynamics Associated with Real-World Stress

Research Director: Dr. Randy Buckner

An individual's moods and behaviors dynamically fluctuate over time based on a variety of experiences specific to that individual. Mood, sleep, and cognitive task performance have all been shown to vary by condition of high or low stress in individuals. While these factors have been studied in isolation, little has been done to study the impact of stress in individuals longitudinally to dynamically capture the experience and effects of real-world stress. Stress is ubiquitous on college campuses, and 63% of college students report that daily stress significantly interferes with their work. Therefore, it is important to understand how stress affects students' moods, behaviors, and cognitive abilities in a real-world, contextualized manner. In the first phase of this study, sixteen healthy college students were deeply phenotyped over the course of one academic semester. Participants completed a daily survey of 40 questions related to their physical health, emotional state, social behaviors, and stress level and interference in their day. In addition, objective measures of sleep, physical activity, and sociability were measured using an actigraphy wrist monitor and custom smartphone application research platform. Each individual attended 12 sessions scheduled in periods of high and low stress leading up to and following examinations. During each session, participants completed a cognitive battery of tasks selected to probe emotional face perception, reward response, and executive function. In the second phase, ten healthy college students enrolled in intensive 7-week summer courses were recruited and deeply phenotyped. Participants received similar daily surveys and objective measurements were collected from actigraphy monitors and the smartphone application. Finally, each participant attended four functional MRI sessions, two on days prior to an examination and two days directly following an examination, to examine stressrelated shifts in the organization of functional brain networks and their relationship to real-world behaviors. Results revealed meaningful relationships between measures including increased selfreported stress and time spent studying in periods leading up to examinations. Additionally, in some subjects, increases in stress and cramming were related to shifts in subjective and objective measures of sleep as well as other behaviors. We explored imaging data related to patterns of activity in cognitive domains shown in the literature to be impacted by stress including emotional salience and reward processing, working memory, and executive function. This study further illustrates the vast impacts of changes in stress state on behaviors, moods, and emotions in individuals over time. Moreover, these results validate the importance of dense, longitudinal deep phenotyping of individuals to best capture idiosyncratic profiles of individuals as a framework for better understanding changes across numerous facets of their lives based on real-world stress events.

Modeling and Preventing Inclusions That Result from Alpha-Synuclein Dyshomeostasis

Research Director: Dr. Dennis Selkoe

Parkinson's disease (PD) is a neurodegenerative movement disorder that affects close to one million people in the United States alone (Parkinson's Disease Foundation, 2016). As the second most prevalent neurodegenerative disease just behind Alzheimer's disease, the development of therapeutics for the prevention and treatment of PD is a central ambition for PD research. A growing body of research has found that the primary aggregating protein in PD-linked Lewy Body inclusions, alpha-synuclein (αS), is particularly involved in the pathogenesis of both familial and sporadic PD. Preventing the formation or reducing the presence of αS inclusions presents a promising avenue for PD treatment. To this end, we completed a candidate-based drug screen of over fifty compounds involved in such cellular functions as autophagy, vesicle trafficking, and membrane stability. Using YFP- tagged human neuroblastoma cells transduced with virus containing full-length αS mutated to overexpress upon induction, we were able to monitor the drugs' capacity to prevent or reduce αS inclusion formation via imaging with the IncuCyte ZOOM system. We found that Trifluoperazine, E64d, FK506, NAB2, and Drug X most significantly attenuated αS inclusion growth and persistence. Further exploration of their mechanism revealed [LEFT BLANK AT TIME OF SUBMISSION]. This evidence suggests that these five drugs may re-solubilize αS and increase the quantity of tetrameric αS, thereby demonstrating potential as therapeutics for PD and other synucleinopathies.

Modeling Trail Tracking Behaviors and Antennae Dynamics in Camponotus pennsylvanicus

Research Director: Dr. Venki Murthy

The manner by which animals detect sensory information in their environment and then make decisions is an unresolved problem. Here, we chose the robust trail following behavior of black carpenter ants in order to address this question. Specifically, we characterized olfactory- based behaviors and antennae dynamics in these ants during trail tracking by first recording high spatial and temporal resolution videos of ants introduced to artificial odorant trails. We then segmented and analyzed these videos. We first found that these ants employ various distinct behaviors while trail tracking, here named 'probing', 'sinusoidal movement', and 'trail hugging', which can be characterized by the velocity of the ant and its movement relative to the trail. Within these behaviors, the ants utilized their antenna differently, both in the sample space occupied by the antenna and the directional motions of the antenna. Furthermore, during an overall tracking event, ants used their antenna in an anti-correlated fashion with one antenna often kept closer to trail and thus receiving more odor. Thus, these ants exhibit specific behavior to both follow the trail and sample the odor environment. Altogether, we have developed a phenomenological model that helps us to understands the behaviors and antenna-based strategies used by ants while solving the problem of tracking an odorant trail.

Neural Basis of Social Hiearchy in Mus Musculus

Research Director: Dr. Catherine Dulac

From mice to primates and even humans, social hierarchy is displayed as higher-ranking members exhibiting enhanced aggressive behavior. Identifying the specific behaviors and neural mechanisms underlying hierarchy in mice can provide novel insights into certain neuropsychiatric disorders, which often manifest as deficits in social interactions. Preliminary lab research implicates the Medial Dorsal Thalamus, a structure that receives inputs from olfactory centers and sends efferent projections to the Pre-Frontal Cortex, in the acquisition of social hierarchy. Using MDT lesions and a two-step behavioral paradigm, I determined a role of the MDT on social hierarchy, with lesioned mice exhibiting decreased hierarchy stability as compared to WT mice. I identified specific behavioral correlates of hierarchy rank. Then, using a social memory test and lesion studies, I interrogated the role of the MDT in social memory, one facet of social hierarchy, since deficits in social memory could affect social hierarchy stability. In this assay, lesioned mice did not display deficits in social memory as compared to wild type controls. To continue interrogating the role of the MDT in social memory, I plan to repeat the social memory setup but will employ reversible neuronal inhibition using a conditional DREADD virus. Altogether, these results will provide insight on the role of the MDT neural circuit and specific defensive behaviors in the establishment, stabilization, and maintenance of social hierarchy.

Regulating inhibitory interneuron circuits through the choroid plexus: A potential therapeutic target for Rett syndrome

Research Director: Dr. Takao Hensch

Rett syndrome (RTT), a neurodevelopmental disorder caused by de novo mutations in the X-linked gene MECP2, is characterized by rapid developmental regression early in the lives of affected female patients, resulting in profound cognitive, motor, and social disability. Mecp2-knockout mouse models of RTT exhibit various behavioral and neurobiological abnormalities, including disruption of the excitatory/inhibitory balance in the cortex. Although it is known that widespread re-expression of Mecp2 reverses RTT-like symptoms in Mecp2-knockout mice, controlling dosage of the X-linked Mecp2 gene has proved elusive, and toxicity of Mecp2 overexpression poses a significant challenge to the translatability of Mecp2 re-expression therapies to human patients. Because it is known that parvalbumin-positive (PV) interneurons, which mediate cortical excitatory/inhibitory balance, mature precociously in Mecp2-knockout mice, this project first compared brains of RTT mouse models and postmortem human RTT patients through neuroanatomical analysis of DAB staining for parvalbumin, confirming that the neurobiological abnormalities of Mecp2-knockout mice accurately recapitulate the human phenotype. Because of the complications of Mecp2 re-expression therapies, we studied OTX2, a non-cell-autonomous factor produced in the choroid plexus and known to regulate PV neuron maturation, as a potential therapeutic target for RTT that bypasses MECP2 control. Specifically, we sought to reduce OTX2 levels by modulating its production in the choroid plexus, evaluating the effectiveness of choroid-specific reduction of OTX2 in Mecp2-knockout mice on RTT-like behavioral and neurobiological abnormalities through phenotypic Rett scoring and immunohistochemical analysis of inhibitory puncta. We then compared OTX2 levels in Mecp2-knockout mice and postmortem human RTT patients through ELISA, in order to assess whether modulation of OTX2 in mouse models may be translatable to treatment of human RTT. Taken together, we establish that OTX2 modulation through the choroid plexus may reverse many RTT-like behavioral symptoms in Mecp2-knockout mouse models and rescue the hypermaturation phenotype of PV circuits. Since we also establish that the PV neuron hypermaturation noted in RTT mouse models can also be observed in human RTT patients, this project supports OTX2 modulation through the choroid plexus for further study as a potentially viable therapeutic target for RTT that, compared to MECP2 re-expression therapies, allows for improved dosage and spatial control.

Role of Microglia in the Regulation of Synapse Development and Survival of Adult-born Neurons in the Olfactory Bulb

Research Director: Dr. Venki Murthy

The adult brain's plasticity allows for the ability to adapt to our diverse environment, and it varies from changes in neurons at the subcellular level to alterations in whole brain systems. Among the many mechanisms of plasticity within the adult brain, adult neurogenesis – the birth of new neurons – only occurs in two brain regions: the olfactory bulb and the dentate gyrus. These adult-born neurons face the challenge of integrating into circuits that already exist within the brain without disrupting the circuit's ability to process information. The process, therefore, is highly regulated, although little is known about the mechanisms that affect both the survival and synapse formation of these neurons. In this study we aim to determine the role of microglia in the survival and synaptic development of adult born neurons in the olfactory bulb. Microglia ablation was performed using two methods: diphtheria toxin and CSF1R receptor inhibition. An average of 82% ablation was achieved in the olfactory bulb through diphtheria toxin administration, while CSF1R inhibition resulted in the ablation of an average of 91.5%. Adult-born neurons were labeled using lentiviral injections and spine density and morphology was quantified through the neuron tracing software Imaris. Mice were injected with BrdU and survival levels were determined after 28 days through immunostaining. Microglia ablation resulted in alterations in spine length and spine head volume in adult-born neurons. These results suggest that microglia play a role in the synaptic regulation and integration of adult-born neurons.

Role of the SVA Retrotransposon Insertion in the Manifestation of X-Linked Dystonia Parkinsonism

Research Director: Dr. Cris Bragg

X-Linked Dystonia Parkinsonism (XDP) is an extremely rare genetic neurodegenerative disease endemic to the island of Panay in the Philippines. The XDP haplotype consists of seven variations in a 297kb stretch of DNA: five disease-specific sequence changes, a 48-base pair deletion, and an SVA retrotransposon insertion. These mutations are located either within the TAF1 gene or within the flanking multitranscript system. A previous study show significantly decreased TAF1 in patients. The high prevalence of XDP in a single geographical locus combined with its nonfatal nature suggest that perhaps only one of the seven mutations led to the manifestation of the disease in the XDP proband. Because of intrinsic repetitive qualities of the SVA retrotransposon, which have capabilities of modifying transcriptional activity in its vicinity, we believe that the SVA retrotransposon insertion of the XDP haplotype may cause abnormalities of TAF1 levels, and therefore disease manifestation, though its mechanisms remain unknown. In this project, we observe the SVA retrotransposon insertion in a firefly Luciferase vector and compared it to the negative control as well as the positive control to identify intrinsic promoter activity. Our findings support the knowledge that SVA retrotransposon insertion reduces transcriptional activity when oriented in the same direction as its naturally-occurring transcription. During the experiment, it was also discovered that the length of CT hexamer repeat, a variable component of the SVA retrotransposon, differentially affects the severity of dystonic symptoms. Additional experiments, which include RT-PCR of Luciferase and RT-PCR of CT repeats reveal that the decrease in Luciferase data previously acquired was a result of a frameshift misread in translation, rather than the effect of repressor activity. Our findings seem to invalidate the theory of G4 structure formations but encourage the theory that the SVA retrotransposon may be the culprit TAF1 abnormalities and the manifestation of XDP.

- Promoter/enhancer/rpressor activity in xdp forward and reverse.
- Antisense had less luciferace activity than sense sva
- Then we found CT repeats correlate to severity of disease.
- Then we chose to look at promoter/enhancer/repressor activity in sva f and r for medium length and short length.
- Results were incomparable and insignificant.
- Perhaps luciferace being made but out of frame

Screening for clonal biases in the generation of retinal neural subtypes

Research Director: Dr. Ryan Draft

We sought to determine whether clonal groups demonstrated a bias in the generation of retinal neural subtypes in chickens. Prior studies of retinal development have addressed the generation of the six classes of neurons in the retina, but have failed to examine biases at the subtype level—an important distinction given the functional diversity of these subtypes. To screen for such biases, we electroporated chick embryos with an updated brainbow transgene called multi-addressable genome-integrated color markers (MAGIC markers), and performed immunohistochemical labeling of subtypes and subtype groups after sacrificing the embryos at embryonic stage E14. We screened for these biases at both the proliferative and neurogenic stages, to more precisely place the time scale of the bias, by using either Self-Excising Cre at E1.5 (proliferative) or Er-Cre-Er with tamoxifen injection at E4.5 (neurogenic). We selected a number of retinal neural subtypes and subtype groups based on access to antibodies, information, and functional interest, including: two amacrine cell subtypes (parvalbumin-associated and tyrosine-hydroxylase-associated); calbindin-associated amacrine and RGC subtypes (related subtype group); and a parvalbumin + rhodopsin subtype group (rod photoreceptors + rod-feedback amacrines). Clonal groups were identified by unique color values and spatial arrangement. [Statistical analysis yet to be performed]. Evidence for either a bias or lack of bias across clonal groups in the generation of these subtypes will improve our understanding of neurogenesis, give insight into how best to proceed with future neural developmental research, and could prove useful in stem cell treatments of retinopathies in the future.

Sensory Gating: From elevators to schizophrenia

Research Director: Dr. Bob Stickgold

Introduction:The role of the thalamic reticular nucleus (TRn) in wake and sleep is a complex one, and its dysfunction now appears to be associated with schizophrenia. During sleep, the TRn plays a critical role in the generation of EEG sleep spindles, while in wake it mediates the transfer of sensory information through the thalamus, impacting performance on tasks involving sensory gating and divided attention. Deficits in sleep spindles, sensory gating, and divided attention are seen in Schizophrenic patients, potentially contributing to both their positive symptoms and cognitive deficits. Thus, this study aimed to investigate whether these neurobiological markers are reflected in our novel Elevator Dilemma survey assessing normal sensory gating in healthy control patients.

- Method: One-thousand and thirty subjects completed our novel Elevator Dilemma questionnaire and a magical ideation questionnaire to assess schizotypal traits. Another 14 subjects underwent a 90-minute electroencephalographic nap recording (56 electrodes) in addition to the previous questionnaires. Sleep spindles were analyzed during the nap and sensory gating task.
- Expected Results: 1) Sensory Gating was reduced after exposure to the elevator intervention. 2) The first sensory gating task was correlated with sleep spindle density.
- Conclusions: The interrelationships amongst these multiple measures of schizophrenia and TRn function in healthy subjects may reflect the roles of TRn function and the gene CACNA1I in the development and expression of Schizophrenia.

Tradeoff between cost and accuracy in model-free and model-based reinforcement learning systems

Research Director: Dr. Sam Gershman

The dual-process theory of decision making proposes that humans act in order to maximize reward and minimize punishment through two distinct brain mechanisms: the habitual and the goal-directed systems. The habitual system corresponds to a model-free reinforcement learning strategy where an agent relies on cached estimates of state-action values from past trial-and-error interactions with the environment. The goal-directed system corresponds to a model-based reinforcement learning strategy where an agent generates choices through an internal cognitive map of the environment that describes the relationship between different states. Unlike the model-free strategy, the model-based strategy is able to deliberatively plan and flexibly adapt to a changing environment. Since the model-based strategy estimates the consequences of taking particular actions in advance, it is more cognitively demanding but also more accurate than the model-free strategy. One significant question remains largely unexplored—how do we arbitrate between these two systems, or in other words, how do we decide how to decide? A wide body of literature suggests various factors that influence an individual's tendency to use one system over another for a given task. One factor, dopamine has a well establish role in learning. In particular, phasic dopamine in the striatum codes reward prediction errors in model-free learning with bursts indicating a greater reward than expected. The role of tonic dopamine in model-based and model-free learning has not been well studied. This current study seeks to better understand the relationship between tonic dopamine levels, measured through resting eye blink rate, and model-based and model-free control of decision making, measured through the novel two-step task.

What to do when your baby monkey falls asleep in the scanner: A study on resting-state and stimulus-state functional connectivity in infant macaques

Research Director: Dr. Marge Livingstone

Functional connectivity of the brain has been extensively assessed in adult humans, adult monkeys, and to a lesser extent, in infant humans. There is evidence that functional networks present in infant humans are different from those identified in adult humans, but it remains unclear whether this difference is due to experience-dependent learning or a fundamental developmental time course (Fair 2009, Fransson 2007). This study aims to identify functional connectivity networks in infant macaques and compare with those previously reported in adult macaques. Data-driven spatial independent component analyses (ICA), cross-hemisphere seed-based correlations guided by a probabilistic anatomical atlas, and clustering were applied to the fMRI data that was acquired during rest periods and passive visual stimulus presentation (Fig. 1). The spatial ICA approach yielded approximately eight robust bilateral cortical networks, a somatomotor cortical-subcortical network, and a frontal eye field lateral intraparietal sulcus (FEF-LIP) network, all of which were identified previously in adult macaques. The most robust cortical networks were foveal visual, peripheral visual, occipetoparietal, dorsal temporal, auditory, dorsal parietal, somatomotor, ventral premotor, and FEF-LIP (Fig. 2). The seed-based analysis yielded clusters of correlated activity within and across hemispheres, which closely matched the regions identified by ICA. A k-means analysis for each subject revealed a variable number of clusters for each data set, ranging from 2 to 8 (Fig. 3). Notably, the FEF-LIP network was similar to the attentional salience network previously identified in adult macaques (Seeley et al 2007), and a default-mode network was not consistently identified (Buckner 2008) (Fig. 4). These results support the hypothesis that a substantial portion of the primate brain?s functional network connectivity is already established within one week of life.

What's normal anyway? Day to day variation in brain structure and behavior of healthy individuals

Research Director: Dr. Randy Buckner

The human brain is remarkable for its ability to alter its structure in response to environmental changes and skill-learning, and the brain maintains this capacity for structural change throughout the course of life. Much work has been done on cross-sectional differences in brain structure across populations, as well as on longitudinal differences in brain structure within individuals over the long-term (months to years) as a result of behavioral interventions or normal aging. Evidence from this research suggests that longitudinal changes in brain structure are not uniform across brain regions, and there is considerable variation across individuals. Additionally, day-to-day variations (that is, "normal" variations that occur independently of any research intervention) in brain structure have not been well-studied. To account for these inter-individual and day-to-day intra-individual variations, our study utilizes a "deep phenotyping" approach, which seeks to gather as much information as possible about a small number of subjects in order to understand and classify brain-behavior phenotypes in greater detail. To this end, we collected actigraphy, daily survey, GPS, and phone use data for 4 subjects over the course of 4 months, in addition to fMRI scans of all subjects every 3-4 days. In this investigation, we hope to find meaningful, intra-individual variations in brain structure that may be attributable to behavioral variation. Specifically, we investigate regional and global changes in volume and cortical thickness that may be due to variations in sleep and activity patterns