Characterization of the Differences in Food Odor Naive Preference and Learning in C. elegans Males and Hermaphrodites

Research Director: Dr. Yun Zhang

Olfaction is an important means for animals to communicate with the environment, and experience can profoundly shape the representation of olfactory cues to an animal. Previous results suggest that males and females process odors differently. However, it is not clear how sex differences influence olfactory learning and the neural mechanisms underlying these differences.

Because learning to avoid harmful food is essential for survival, conditioned avoidance of odors associated with toxicity or infection is a robust form of olfactory learning in C. elegans. Previously, we have shown that naive hermaphrodites prefer the smell of pathogenic bacteria Pseudomonas aeruginosa (PA14) in comparison with the smell of the common lab food Escherichia coli (OP50), and brief training with PA14 induces a learned olfactory aversion to PA14 (Ha et al., 2010; Zhang et al., 2005). Here, we ask whether sex differences regulate olfactory learning.

We used a high throughput micro-droplet assay to, first, measure the ability for males to naively prefer food odors in the same manner as hermaphrodites and, second, to assess the ability of males to learn to avoid the smell of PA14. We demonstrate males to both exhibit both reduced naive food odor preference and olfactory learning to food odor PA14, suggesting sex differences in olfactory behavior and olfactory plasticity. Two olfactory neurons in C. elegans, AWB and AWC, are each required for olfactory preference to PA14 in hermaphrodites and AWB/AWC sensorimotor circuits are required for driving aversive olfactory learning (Ha et al., 2010). Thus, we hypothesize that sex differences in this olfactory network underlies the behavioral difference.

Characterizing the Roles of Cholinergic and GABAergic Neurons in C. elegans Head Movement

Research Director: Dr. Yun Zhang

The balance between excitatory and inhibitory neurotransmission is important for proper nervous system function. In Caenorhabditis elegans, this balance is necessary for the animal to modulate its head deflections so that it can sample the environment and move forward. Previous studies have postulated that cholinergic head motor neurons promote head deflections, while GABAergic head motor neurons limit head deflections. However, the exact interaction between these neurons is unclear.

This study aims to examine how cholinergic and GABAergic head motor neurons interact with each other to regulate C. elegans head bending. Specifically, we hypothesized that GABAergic neurons communicate with cholinergic neurons to restrain head bending. By performing genetic rescue experiments and intracellular calcium imaging, we found that GABAergic neurons down-regulate cholinergic neurons via GABA(B) receptors to restrict head bending.

Next, we hypothesized that cholinergic neurons activate GABAergic neurons to increase head bending. By expressing tetanus toxin in cholinergic head motor neurons and examining the calcium activity in GABAergic neurons, we found that cholinergic neurons provide input into GABAergic neurons to promote head bending.

We also examined the functional implications of head bending by performing thermotaxis navigational assays. Preliminary results suggest that worms with increased head deflections are defective in thermophilic behavior, but not in cryophilic or isothermal tracking abilities. This suggests that head bending is important for certain advanced navigational abilities.

This research hopes to elucidate the complex mechanisms and implications of inhibitory and excitatory control, and to provide a framework for how this control can underlie mammalian locomotion.

Circadian Activity Rhythm Disturbances and Suprachiasmatic Nucleus Cell Population Size in Elderly Humans

Research Director: Dr. Clifford Saper

Aging is associated with changes in circadian function, which are aggravated in Alzheimer's disease (AD). In model organisms, the suprachiasmatic nucleus (SCN) plays a key role in driving circadian rhythmicity. However, whether interindividual differences in SCN integrity are an important determinant of differences in circadian rhythmicity in older humans is unknown. In this study, we investigated whether the parameters of circadian rest-activity rhythms in older humans are associated with cell counts in specific neuronal populations in the SCN.

METHODS:
We collected up to 10 days of actigraphy data from 17 older adults with and without Alzheimer Disease (mean [SD] age 89.6 [5.5]) participating in the Rush Memory and Aging Project, a longitudinal cohort study of the common conditions of aging. We performed empirical mode decomposition analysis to estimate circadian activity rhythms. Upon death (which occurred a mean [SD] of 9.0 [4.5] months after actigraphy), post-mortem hypothalamic blocks from these subjects were sectioned and stained immunohistochemically for vasopressin (AVP) and vasoactive intestinal peptide (VIP). Numbers of VIP neurons in the ventrolateral and AVP neurons in the dorsomedial portions of the SCN were counted stereologically.

RESULTS:
There was a significant ($p<0.008$) positive correlation between the number of VIP neurons in the ventrolateral SCN and circadian amplitude. By contrast, there were no correlations with any circadian measure and the number of AVP neurons in the dorsomedial compartment. AD subjects had significantly delayed activity acrophases and nadirs in comparison to healthy controls, but diagnosis of AD was not a significant predictor of circadian amplitude or SCN cell numbers.

CONCLUSION:
The retinorecipient VIP neurons are important for maintaining the amplitude of rest-activity rhythms in older humans. Loss of these neurons with the fragmentation of sleep previously found in AD patients may underlie their inability to maintain a normal angle of phase entrainment.

Consolidation of Motor and Spatial Memory during Sleep in Relation to EEG Features, The

Research Director: Dr. Erin Wamsley

Although no function has been definitively proven, using PSG and other methods researchers recently revealed that sleeping potentially serves purposes such as ontogenesis, restoration, and memory processing. Overnight recordings PSG demonstrated that the brain passes through complex cycles of activity known as sleep stages during the night. Moreover, particular features of the EEG may be associated with memory enhancement during sleep: two such features are sleep spindles, 12-15 Hz synchronous oscillations during stage 2 NREM sleep, and theta power, 4-7 Hz activity during REM sleep. Previous studies observed a positive correlation between next day performance on a motor memory task and the prevalence of sleep spindles. This research supports the aforementioned and, for the first time, demonstrates that quantity of sleep spindles and theta power correlates with performance on a spatial navigation task. Participants performed a 3-D maze video game and a typing sequence task; the sleep group initially did both tasks in the evening then spent the night in the lab monitored by low-density polysomnography, while the wake group was first exposed in the morning. Nine hours later, both groups retested on the tasks. Significant improvement occurred in the subjects that slept, while subjects that remained awake performed near baseline levels. Statistical data analyses showed that both spatial and motor memory processing are associated with spindles and that theta power was proportional spatial learning improvements, possibly due to the emotionally-charged maze elements. These results suggest memory consolidation is a primary evolutionary benefit of human sleep.

Contributions of estrogen and oral contraceptive use to sex differences in functional responding during fear extinction recall

Research Director: Dr. Mohammed Milad

Data from rodent and human studies have identified a network of brain regions involved in conditioned fear extinction. Recently, studies have emerged examining sex differences in the acquisition and expression of fear memories and their subsequent extinction, and in the functional activation of the fear extinction network. There is, however, inconsistency in the literature on whether or not sex differences exist in the functional activation of this network. During the past few years, the Milad laboratory has collected data showing that estrogen enhances the consolidation of fear extinction memories and increases the activation of the fear extinction network in women. My thesis work aims at extending these efforts by examining whether or not estrogen, and hormonal contraceptives (HC) use, contributes to the presence or absence of sex differences in this network. To achieve this, I have analyzed a sample of XX men and XX women that underwent a 2-day fear conditioning and extinction paradigm while in a functional magnetic resonance imaging (fMRI) scanner. The sample included women that underwent extinction learning in a high estrogen state, low estrogen state, or using HCs. Skin conductance response was collected as the index of fear extinction. Results show that sex differences are masked when disregarding variation in estrogen levels and HCs use of the women, but do emerge when differentiating the women by hormonal state. These results highlight the importance of considering women's hormonal state when responding to emotionally salient stimuli, raising questions regarding the possible interactions between sex hormones and anxiety disorders.

Deconstructing the Moral Mind: An fMRI Investigation Mapping the Foundations of Moral Judgment

Research Director: Dr. Joshua Greene

Several attempts have been made to organize moral judgments into conceptual categories. Haidt (2007) proposes five foundations on which moral judgments are made: harm, fairness, in-group loyalty, deference to authority, and purity. In the present study, we use functional magnetic resonance imaging (fMRI) to assess the neural basis of these proposed categorizations. 19 participants rated how immoral 100 behaviors were while their blood oxygenation level-dependent (BOLD) responses were recorded. We conducted a representational similarity searchlight analysis to determine whether distinct moral categories are uniquely represented in the brain. We found significant correlations between a behavioral model of moral organization and neural representational profiles in regions associated with social cognitive processing, including the inferior parietal lobe (bilaterally), the precuneus, the posterior cingulate, and the right superior frontal cortex. This partially replicates a preliminary study that demonstrated unique BOLD responses in these regions to different moral behaviors. In the same study, contrasts across Haidt's moral domains demonstrated no significant difference between responses to harm and fairness violations, nor between any pairing of in-group, authority, or purity violations; however, there were significant differences in responses to harm or fairness and in-group, authority, or purity violations in the medial prefrontal cortex and insula. In ongoing work we attempt to replicate results from this contrast analysis. We are also investigating whether individual differences in personality, which have been shown to correspond to differences in values across Haidt's moral categories, are reflected in activation patterns. These findings may elucidate the neural basis of distinct moral beliefs.

Development and neural bases of happy face processing in infants: A study in fNIRS and temperament

Research Director: Dr. Charles A. Nelson

Accurate decoding of facial expressions is critical for human communication, particularly during infancy before formal language has developed. Differentiation of brain responses to different emotional faces develops within the first months of life. However, there are broad individual differences in such responses. In the current project we seek to examine such differences by studying the relation between neural response and temperament. We do so using functional near-infrared spectroscopy (fNIRS) to measure oxyHb responses to happy face stimuli. Seven-month-old infants (n=11, study in progress) were shown images of happy faces, and neural activity was recorded using fNIRS, which measures hemoglobin concentrations in response to stimulus events. Greater oxyHb response is associated with greater local brain activation. Temperament data were collected using the Revised Infant Behavior Questionnaire (Gartstein & Rothbart, 2003), which assesses three temperament factors: Surgency/Extraversion, Negative Emotionality, and Orienting/Regulation.

Across several channels over the prefrontal cortex, we found that oxyHb response was correlated with temperament. For three channels over the left prefrontal cortex, there was a positive correlation between Surgency/Extraversion and oxyHb response ($r=.739$, $p=.009$; $r=.705$, $p=.015$; $r=.736$, $p=.015$). Over the right prefrontal cortex, three channels showed negative correlations between Negative Emotionality and oxyHb activity ($r=-.741$, $p=.009$; $r=-.715$, $p=.013$; $r=-.709$, $p=.015$). This second effect was driven by negative deflections in oxyHb concentrations.

These results suggest that individual temperament differences are associated with differential oxyHb responses to happy faces, putatively subserved by the orbitofrontal cortex. Infants with higher Surgency/Extraversion and lower Negative Emotionality characteristics show greater brain responses to happy faces.

Development of Spatial Memory Abilities in Infants and Children: A Behavioral and Electrophysiological Study, The

Research Director: Dr. Charles A. Nelson

Declarative memory, the memory for facts and events, is fundamental for defining who we are. It has been shown that memory abilities develop gradually throughout the first years of life. Interestingly, the neural structures underlying these functions also have shown a prolonged developmental profile. No studies to date, however, have established the link between the behavioral and structural maturation of declarative memory functions. Therefore, I aimed to demonstrate this link using electrophysiological and behavioral techniques in 9-month old infants, 2-3 year old children, and adults.

I specifically investigated the development of spatial memory, a fundamental component of declarative memory. Using a Visual-Paired Comparison task and the recording of event related potentials, I studied the development of two spatial memory abilities: egocentric spatial memory (location encoded in relation to oneself) and allocentric spatial memory (location encoded in relation to global cues). Egocentric spatial memory is subserved by the parahippocampal cortex, believed to be mature by birth, and allocentric spatial memory is subserved by the hippocampal formation, which continues to develop beyond the first years of life. Taken together, I hypothesized that egocentric spatial memory abilities would be apparent in all age groups but allocentric spatial memory abilities would only be apparent in 2-3 year olds and adults. This study will provide a better understanding of the typical development of declarative memory abilities and the corresponding brain regions. This data may help to inform future studies regarding neurodevelopmental disorders involving these brain regions, such as developmental amnesia, epilepsy, and learning disabilities.

Developmental Regulation of TRP Channel Expression in Oligodendrocytes, The

Research Director: Dr. Paul A. Rosenberg

Brain injury in premature infants is a major public health concern because of its long-term, often irreversible impact on the neurodevelopment of children who survive prematurity. Cerebral Palsy (CP) is one particular condition that results from periventricular white matter injury (PVL) during the perinatal period, when developing premyelinating oligodendrocytes (pre-OLs) are the predominant white matter cell type in the brain and particularly susceptible to injury. Transient receptor potential (TRP) channels may play an important role in this cell injury by allowing cations to flow into cells and activate death pathways. Specifically, two members of the TRPM family, TRPM2 and TRPM7, have been implicated in cell death during ischemia and oxidative stress in the central nervous system, but little is known about the expression and function of TRP channels in the developing nervous system. Here we tested the hypothesis that TRPM2 and TRPM7 are expressed in developing oligodendrocytes, and that their expression changes across development. We utilized cell cultures of immature oligodendrocytes, immunocytochemistry, and Western Blot analysis to determine the relative expression levels of these channels in developing and mature oligodendrocytes in vitro. As a result, we identified TRPM2 and TRPM7 expression in developing oligodendrocytes, and observed an upregulation in TRP channel expression during the pre-OL stage of oligodendrocyte development. These data support that TRP channel expression may contribute to the particular vulnerability of developing pre-OLs to injury. Further studies investigating the in vivo expression and function of TRP channels in developing oligodendrocytes are warranted.

Diffusion tensor imaging of the stria terminalis in first episode schizophrenia

Research Director: Dr. Martha E. Shenton

In this study the aim is to use DTI to quantify the stria terminalis in 21 patients diagnosed with a first episode of schizophrenia and 20 healthy controls matched on age, gender, and parental socioeconomic status. The method to be used is streamline tractography to identify the stria terminalis and to quantify the coherence of these fibers using a diffusion index known as fractional anisotropy, which provides information about the directionality and coherence of fibers. We predict that patients will show less coherence and directionality as measured by fractional anisotropy compared to healthy controls and we further predict that these abnormalities will be correlated with both negative and positive symptoms in schizophrenia.

Effects of CFEOM3 mutations in beta tubulin isotype III on cranial nerve development

Research Director: Dr. Elizabeth Engle

Congenital fibrosis of the extraocular muscles type 3 (CFEOM3) is a disorder characterized by restricted eye motility, strabismus, and ptosis. CFEOM3 is believed to be due to dysinnervation of extraocular muscles by the oculomotor nerve. CFEOM3 has been linked to mutations in TUBB3, which encodes for the neuronal specific beta-tubulin, isotype III. Previous studies in TUBB3R262C/R262C mice have revealed a misguided oculomotor nerve but it is not known if this misguidance appears in other TUBB3 amino acid substitutions, how this misguidance arises, and if other cranial nerves are affected. This thesis aims to help answer those questions by using mouse models harboring the R262C, R380C, and E410K amino acid substitutions. To examine and compare cranial nerve abnormalities whole-mount neurofilament staining was performed on islGFP+ E11.5 wild-type, TUBB3R262C/R262C, TUBB3R380C/R380C, and TUBB3E410K/+ mice. To look at how the mutation affects growth cone behavior, an oculomotor explant culture was performed on wild-type and TUBB3E410K/+ mice. Live cell imaging was performed on the culture after 17 hours. Whole-mount staining revealed various cranial nerve abnormalities such as a misguided, abnormal turn of the oculomotor nerve, thinning of the facial nerve in TUBB3R262C/R262C and TUBB3R380C/R380C mutants, and thinning of the trigeminal nerve in TUBB3R380C/R380C mutants. Oculomotor explant culture revealed no significant differences in growth cone behavior between mutants and wildtype. This suggests a common mechanism across the amino acid substitutions that leads to the misguidance of the oculomotor nerve and that this misguidance is not due to inherent behavior of the developing oculomotor nerve.

Emergence of Orientation Invariant Representations Within the Visual Cortex, The

Research Director: Dr. George Alvarez

It's surprisingly easy to recognize objects at different sizes, orientations, and positions in the visual field. Robust object recognition across such transformations suggests that the visual system achieves an (almost) invariant representation at some level. While understanding the computations that underlie view-invariant representation is an ongoing topic of research, it remains unclear exactly where and how invariance is achieved. Here, we aim to provide some insight into this question by exploring the emergence of orientation invariant representations within the visual system using fMRI. We used a rapid-event related paradigm in order to attain highly reliable brain patterns for 40 items (8 distinct objects at 5 orientations each; average reliability across items and subjects, r=.79). We then divided the cortex into several regions of interest, including V1-V3, lateral occipital complex (LOC), and the broader occipitotemporal cortex (OTC). We divided the data set into all possible halves of the 12 runs, and computed the correlation between item patterns for each half for all combinations of the 40 items (1600 correlations). This analysis revealed strong orientation dependence in V1-V3: for a given object, pattern similarity decreased as the difference in orientation between items increased. In contrast, LOC showed evidence for complete invariance: the average pattern for a particular object on one half of runs (e.g., upright face) was just as similar to the same object at the same orientation (upright face) as it was to the same object at any other orientation (45, 90, 135, 180 deg) on the other half of runs. The same degree of orientation invariance was observed in OTC. These results show that object responses in occipitotemporal cortex are completely invariant, not just tolerant, to changes in orientation, and suggest that these invariant representations emerge abruptly in the transition between early visual cortex and LOC.

Expert Symbol Learning, Invariant Visual Object Recognition and Value-Based Generalization

Research Director: Dr. Margaret Livingstone

Expert visual learning constitutes a critical component of primate social behavior. Through behavioral research in rhesus macaque monkeys, this thesis investigates visual expertise, the inversion effect associated with visual object learning, and generalization across category-specific knowledge manifest in the object recognition performance of non-human primates. Previous studies in our lab have shown that intensive early experience can influence the development of specialized modular organization in macaque inferior temporal lobe (IT) that responds selectively to trained sets of unnatural symbol stimuli. To investigate the degree to which expert symbol recognition is viewpoint-dependent or invariant, four male macaques were trained to recognize and discriminate between exemplar images using a delayed match-to-sample task in which choice symbol stimuli were identically rotated following an upright sample symbol. Further, a value-based choose-between task was designed to test whether the macaques could generalize knowledge across distinct sets of previously trained symbol stimuli. Statistical analysis of reaction times and trial responses revealed an absence of the inversion effect, as stimulus rotation did not impede performance relative to the learned upright orientation. Monkeys immediately performed the choose-between task to high levels of accuracy. These results suggest that the neural pathways in IT specialized for processing unnatural stimuli are fundamentally more diffuse and invariant than the functional architecture underlying specialized recognition pathways for natural stimuli such as faces.

How does Socioeconomic Status Affect Children's Executive Function? Examining Motivation and Neural Structure as Possible Mediating Factors

Research Director: Dr. Margaret Sheridan

Research has made it clear that children raised in poor socioeconomic environments have worse executive function skills, however it is still unclear what factors mediate this relationship. In the first part of this analysis, we examined the possibility that socioeconomic disparities in performance are rooted in differences in motivation by assessing the effect of external incentives, internal motivation, and socioeconomic status (SES) on two executive function tasks requiring inhibitory control and working memory. In our population, children who received external motivation performed worse, while those with higher internal motivation performed better. Interestingly, socioeconomic status was not associated with either intrinsic motivation or task performance, however there was some evidence to suggest that that higher SES children outperformed their lower SES counterparts when given external incentives, whereas lower SES children with more intrinsic motivation outperformed their higher SES counterparts. In the second half of our analysis, we examined the hypothesis that the SES gradient in executive function might be explained by neural correlates using structural MRI analysis. We found that in several networks of the brain, including the prefrontal region, SES was positively associated with both baseline cortical thickness and cortical thinning across development, an indicator of improved executive function. Together, these results suggest that disparities in executive function across the SES gradient cannot be explained by motivation or eliminated by extrinsic motivators, but are likely to be the result of biological embedding of early social circumstances. If this is the case, then it is clear that policies and programs should focus on reducing early inequalities in order to prevent the establishment of lifelong disparities.

Hyper-aggression, Long-term Consequences of Social Defeat, and Resilience in Drosophila melanogaster

Research Director: Dr. Edward A. Kravitz

How are innate behavioral phenotypes like aggression, differentially and temporally influenced by genetics and experience? Recent work with the Drosophila melanogaster model system has shown that past losing experiences can play a significant role in generating a "loser mentality." Male flies that lose one fight (former losers) show reduced aggression in fights fought shortly after and have a much lower probability of winning a fight, when compared to winners or socially naive flies (Yurkovic et al., 2006). A similar effect was also found in a highly aggressive strain of Drosophila referred to as "bully flies" (Penn et al., 2010). This strain of hyper-aggressive flies is generated by selecting winners of fights for over 35 generations. Normally, bully flies, have a competitive advantage and win at a 90% frequency when fought against wild-type Canton S flies. After experiencing a single social defeat, bully flies completely lose this competitive advantage when fought 30 minutes post defeat. However, long-term consequences of single social defeat in bully flies are yet to be determined. In the following study, we examine the persistence of "loser mentality" in bullies by subjecting both winners and losers to subsequence second fights at different time intervals against Canton S flies. We demonstrate that the loser mentality is an experience- dependent phenotype by showing that bully flies do in fact "forget" their loser status and return to their hyper-aggressive state 24 hours after experiencing defeat. We also tracked the behaviors exhibited in the first and second fights to study what experiences could build a loser mentality, and how those experiences change a loser's second fight fighting strategies. Loser flies that experienced more mid to high intensity interactions, such as lunging and boxing, won significantly more of their second fights, perhaps revealing a population of resilient or non-learning flies that despite their previous defeat experiences, continue to behave in subsequent fights like naive flies. Surprisingly, in comparison to past winners, past losers establish dominance in their second fights after significantly less encounters. We thus have found that a single social defeat can overcome the genetically inbred hyper-aggressive phenotype in bully flies; however after a day, they regain their bully phenotype. We also show that similar to olfactory learning social learning shows inter-individual differences, and can vary in inducing and maintaining a learned state.

Impact of Stress on Reward Responsiveness in Individuals with Remitted Major Depressive Disorder: An EEG Study, The

Research Director: Dr. Diego A. Pizzagalli

Patients with major depressive disorder (MDD) exhibit reduced reward responsiveness marked by decreased activation in dopaminergic brain regions encoding reward such as the caudate and nucleus accumbens. Research also indicates that uncontrollable stressors can cause decreased activity in the dopamine reward circuitry, and that MDD patients are more sensitive to the effects of stress. However it is unclear whether these patterns persist in patients with remitted MDD (rMDD). As a result, the goal of this study was to determine whether rMDD patients exhibit (1) differences in their reward responsiveness relative to never-depressed controls and (2) differences in the interaction of stress and reward responsiveness relative to never-depressed controls. To do this, a behavioral task measuring reward responsiveness (Probabilistic Reward Task - PRT) was given to 11 rMDD patients and 19 healthy controls before and after a psychosocial stressor (Maastricht Acute Stress Test - MAST). During this task neural electroencephalography data (EEG) was recorded and saliva samples for cortisol measurement were obtained periodically. A repeated measures ANOVA show no significant effect of group or interaction. However, there is a modest trend suggesting an effect of group on reward responsiveness ($p = .20$). HPA-axis activation during the session, and EEG analysis of phasic dopamine response across groups is forthcoming as well. This data suggest that there may be a differential impact of stress on reward resposiveness in rMDD patients and never-depressed controls. However, continued experimentation is needed to fully validate this hypothesis.

Interfacing three-dimensional macroporous nanowire nanoelectronic scaffolds with neural tissue in vivo

Research Director: Dr. Charles M. Lieber

Biocompatible integration of electronic devices that can record extracellular and or intracellular potentials from individual neurons and groups of neurons with tissue at the 3-D level is crucial for neuroscience research and in clinical applications for neuro-prosthetic devices. However, such electronic devices have yet to be delivered and integrated with brain tissue in non-invasively, as most of such delivery methods require implantation or use hard probes as substrates for delivery, which disrupts functional tissue. This disruption prevents the device from conducting chronic recordings from the same group of neurons for weeks or months without degradation, reduced functionality and reliability. For clinical applications, chronic compatibility is critical for behavioral and physiological feedback. Here, we report the development of a 3-D macroporous nanowire nanoelectronic scaffold, flexible enough to be delivered through a 100uM inner diameter needle, to be injected into brain tissue. We show that this free-standing and self-organizing electronic scaffold, when injected into brain tissue of mice, unfolded within the lateral ventricle and within the tissue-dense cortex and hippocampus, and incited little gliosis and chronic immunoreactivity. Furthermore, we show this injectable electronics device did not degrade from the host immune system over a 4-5 week period and neural cells (subventricular zone astrocytes and neural stem cells) began to grow into and through the scaffold from one edge of the lateral ventricle to the other. This technology presents a novel delivery method for neural stem cell therapy and makes possible the realization of chronic and functionally selective brain-machine interfaces.

Investigating Cell Surface Receptors of the Vagus Nerve

Research Director: Dr. Stephen Liberles

The vagus nerve is critical for monitoring a variety of physiological states in the body to coordinate autonomic and behavioral responses. The vagus nerve is the tenth cranial nerve and named for its "wandering" trajectory, travels from the brainstem through the thoracic and abdominal cavities to innervate many visceral tissues. Sensory fibers of the vagus nerve include mechanosensors and chemosensors to sense a variety of stimuli in the internal organs. In preliminary data, the Liberles lab performed deep sequencing analysis on individual vagus nerve sensory neurons, revealing an abundance of novel cell surface receptors, signaling molecules, developmental factors, and transcription factors. For my thesis, I used in situ hybridization analysis to further characterize expression of identified cell surface receptors. I identified several genes expressed in subsets of vagal afferents, suggesting a role in specific aspects of vagus nerve physiology. Among these genes was p75, a receptor for the neurotrophin family of neurotropic factors. I next used two-color fluorescent in situ hybridization to look for co-expression of p75 with auxiliary signaling molecules and other markers of vagal afferent subsets. I found that discrete neuron populations express particular combinations of receptors for neurotropic factors, suggesting that these factors control the survival and diversity of vagus nerve sensory fibers. Moreover, these genes could provide valuable markers for future anatomical and functional analysis.

Investigating effects of the Behavioral Inhibition temperament construct on stimulus-driven affective attention and attention-shifting in 7-month old infants: An ERP study

Research Director: Dr. Charles A. Nelson

The Behavioral Inhibition (BI) temperamental construct refers to a set of behaviors characterized by withdrawal and uneasiness in response to unfamiliar situations. The presence of BI in infancy has been associated, before, with the occurrence of anxiety and internalizing disorders in adolescence and adulthood. The present study aimed to investigate whether differences in attentional behavior exist between Behaviorally Inhibited infants and uninhibited infants, similar to those that have been found between anxious and non-anxious adults and adolescents. We created a novel temperament scale, using items from a maternal report temperament questionnaire, to assign 7-month olds indexical scores of BI-like tendencies. We then used recordings of the right hemispheric Nc, obtained through the administration of an ERP task, as a physiological measure of stimulus-driven attentional behavior, and recordings of eye movement, obtained through the administration of a disengagement task, as a measure of attention-switching. Infants displaying stronger BI-like tendencies exhibited slightly heightened stimulus-driven attention towards emotional faces, as compared to infants displaying a weaker tendency towards BI. As well, a trend was observed whereby failure to disengage from fearful stimuli increased with increasing index of inhibition.

The results of the present study suggest, but do not conclusively prove, that differences in stimulus-driven attentional behavior, and differences in attention-switching, may exist between inhibited and non-inhibited 7-month olds. Further, this suggests that these attentional differences may play a role in connecting the Behavioral Inhibition of infancy across time, to the anxiety and internalizing disorders of adolescence and adulthood.

Investigating molecular mechanisms over subtype-specific neocortical projection neuron dendritic development

Research Director: Dr. Jeffrey Macklis

The mammalian neocortex is a complex six-layered structure responsible for cognition and higher-level learning, processing sensory input, and controlling motor output. The diversity of neuronal subtypes that compose circuits is generated through complex molecular programs in a precise spatiotemporal manner. As molecular controls over development and migration of neuronal subtypes become elucidated, the great diversity of neuronal subtypes that coexist intermingled with each other in the neocortex has limited progress in understanding development of cortical projection neurons. Furthermore, while much work has been done to elucidate regulators of subtype specific axonal projection patterns, molecular controls over dendritic morphology are still largely uncharted territory. In this thesis, I investigate molecular mechanisms controlling subtype-specific neocortical projection neuron dendritic development, taking advantage of previously uncharacterized subtype-specific transgenic Cre lines.

I first investigate subtype specificity of two previously uncharacterized Rbp4-Cre and Ntsr1-Cre lines for use towards investigating molecular controls over dendritic development. Using immunocytochemistry, genetic manipulation, and retrograde labeling, I find Rbp4-Cre and Ntsr1-Cre display subtype specificity and distinct expression patterns for subcerebral projection neurons and corticothalamic projection neurons, respectively. Temporal analysis further shows that these lines are ideal for studying dendritic development, as Cre expression begins later in development. These two subtypes of Corticofugal Projection Neurons (CFuPN), subcerebral projection neurons (SCPN) and corticothalamic projection neurons (CThPN), are important populations. SCPN, of which corticospinal motor neurons (CSMNs) are a subtype, extend axons subcerebrally. CSMN control voluntary motor movement, and their degeneration is central to amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia (HSP), and primary lateral sclerosis (PLS), and CSMN damage is central to loss of motor function in spinal cord injury. Corticothalamic projection neurons extend axons to the thalamus, and are critical for feedforward and feedback cortical processing. These Cre lines enable direct genetic access to SCPN and CThPN, which is highly useful for studying molecular mechanisms controlling subtype-specific development.

I have identified Ptprk (protein-tyrosine phosphatase receptor-type kappa) as a candidate molecular control over callosal projection neuron (CPN) dendritic development. CPN extend axons to the contralateral cortical hemisphere, and aberrations in cortical dendritic arborization have been described in patients with mental retardation-associated neurological diseases, such as Down, Rett, and fragile-X syndromes. Expression analysis of Ptprk shows high CPN specificity, and temporally mirrors transcription factor Cux1, a known regulator over dendritic morphology, which suggests it may be a Cux1 downstream target. Using overexpression and knockdown experiments, I am analyzing effects of PTPRK on dendritic development. Experiments and analysis are still in progress. As PTPRK has been associated with neurite outgrowth, I hypothesize the knockdown may result in decreased axonal branching and arborization.

Investigating Neural Activation to Repetition and Non-Repetition Grammar Patterns in 7-Month Olds at High Risk for Autism

Research Director: Dr. Charles A. Nelson

Individuals with autism spectrum disorders (ASD), a disorder typically diagnosed around 24 months of age, commonly experience language impairments. In previous research, early intervention therapies have been shown to ameliorate the social, communicative, and behavioral impairments that characterize ASD. Therefore, in this study we examined the language perception capabilities present in 6 month old infants who were at high-and-low-risk of developing autism. Utilizing functional Near-Infrared Spectroscopy (fNIRS) as a measure of neural activation, participants listened to randomly played blocks of words containing either an ABB (e.g. balolo) or ABC (e.g. baloti) pattern. We found that while infants in the low-risk group showed significantly different patterns of activation between the two grammars in the right posterior, left anterior and left posterior channels overlying the temporal lobe, the high-risk group exhibited no such differentiation. Moreover, comparing both grammars to their respective baseline, the high-risk group activated fewer channels, over a smaller area, and only channels overlying the left hemisphere, unlike the low-risk group which exhibited bilateral activation. These results suggest that abstract rule learning manifests itself neurally as early as 6 months, and differences between children at-risk for ASD are evident at this young age. These results add to the growing canon of literature examining early neural markers of ASD in at-risk populations. Further research should aim to understand the developmental trajectory of the neural differences between at-risk infants who age into a diagnosis and those who do not, in order to inform interventions developed for children with ASD.

Investigating the carboxypeptidase function of Alzheimer's gamma secretase through substrate deletion mutants

Research Director: Dr. Michael S. Wolfe

Alzheimer's disease is the sixth leading cause of death in the United States, affecting more than five million individuals nationwide. One characteristic of the disease is amyloid plaques, which are deposits of 42-residue Aß protein (Aß42). Gamma secretase first cleaves within the transmembrane domain of APP CTFß at the ε site to produce long Aß peptides (primarily of Aß49), which it then trims ~3 residues at a time to the secreted, shorter Aß38-42 peptides. Understanding this process is important, as the Aß42 peptide is more prone to forming neurotoxic aggregates than Aß40.

Here, to elucidate the trimming mechanism, I test the hypothesis that deletion of one, two, or three residues from the C-terminus of the CTFß transmembrane domain will result in shifts in the ε cleavage site and subsequent final products. Specifically, deletion of one residue would shift ε cleavage to Aß48 with a final product of Aß42; deletion of 2 residues would result in cleavage at Aß47 with a final product of Aß41; and deletion of 3 residues would 'reset the register' resulting in cleavage at Aß46 and a final product of Aß40.

To test this hypothesis, I created three CTFß deletion mutants and performed gamma secretase activity assays. Production of Aß peptides was analyzed using ELISA and long urea-polyacrylamide gels. Results show that these selective deletions in the substrate do not affect the reading frame of gamma secretase. These preliminary findings suggest that regions upstream of the deleted residues are primary determinants of cleavage site specificity. Further research is necessary in order to understand the production of neurotoxic Aß.

Mapping a Signal Transduction Pathway for Olfactory Preference in C. elegans

Research Director: Dr. Yun Zhang

Olfaction is an indispensible sensory modality for a wide range of organisms and plays an essential role in many ecologically important behaviors, such as locating a food source. Despite knowledge of the key features of sensory neurons used in olfactory transduction and behavioral responses to pure chemical odors in vertebrate and invertebrate systems, the mechanisms involved in linking sensory molecular machinery to changes in sensory physiology during odor exposure is not completely understood. In Caenorhabditis elegans, olfactory cues allow the nematode to navigate towards attractive stimuli and away from repulsive ones in the environment. Mutant nematodes expressing altered secondary messenger molecules in olfactory neurons show behavioral deficits in preference of complex food odors. In this study, we attempted to link sensory molecules to changes in the intracellular calcium dynamics in key olfactory neurons required for food odor preference. Using a genetically encoded calcium indicator, we examined neural activity changes in AWB, an olfactory sensory neuron required for food preference, and investigated the link between AWB sensory transduction molecules and food odor evoked calcium dynamics in vivo. Our study has implicated a cGMP dependent pathway in shaping calcium dynamics during food odor sensation. Specifically, we identify a receptor guanylyl cyclase and a CNG-gated channel each responsible for distinct physiological changes during odor responses. This indicates that sensory transduction molecules regulate specific properties of sensory neuron physiology. These findings are congruent with and provide a mechanism underlying the results from behavioral chemotaxis assays. This study provides a link between behavior and property of sensory neurons for future research.

Multimodal Neuroimaging and Behavioral Studies on Auditory-Motor Deficiencies and Compensatory Mechanisms

Research Director: Dr. Gottfried Schlaug

In spite of the complex and precise abilities required for music, musical competence is ubiquitous across cultures and spontaneously develops during childhood. However, there are individuals in our society who experience music-specific deficiencies, a condition commonly referred to as tone-deafness (TD). The affected brain network includes the superior temporal gyrus (STG), middle temporal gyrus (MTG), and inferior frontal (IFG) regions, connected by white matter in the arcuate fasciculus. In the present study, I asked what intrinsic functional neural substrates might be underlying the auditory-motor system and its possible disruptions in tone-deafness. Twenty subjects (10 TD) underwent resting-state functional MRI (rs-fMRI). Rs-fMRI data showed decreased functional connectivity between the right IFG and STG in TD subjects, as well as decreased functional connectivity between the right and left STG. Moreover, DTI analysis showed decreased mean Fractional Anisotrophy (FA) values in the right IFG and STG tract, and decreased volume in the right and left STG tract in TD subjects. In addition, graph theory analyses on pairwise functional correlations obtained from atlas-based parcellations of the resting-state fMRI data showed that TD subjects had decreased degrees, clustering, strength, and local efficiency of functional correlations across the whole brain. Interestingly, TD subjects showed increased functional connectivity between the frontal and the occipital lobe, suggesting visual compensatory mechanisms for this auditory deficit. Taken together, results suggest that tone-deafness is a product of intrinsic global disruption of network connectivity in the brain, with effects centering around frontotemporal regions, accompanied by possible compensatory mechanisms in the visual modality.

Neuron type specific tracing from Dopaminergic neurons in the Ventral Tegmental Area in Transgenic Mice

Research Director: Dr. Naoshige Uchida

The midbrain dopaminergic system processes reward information and plays a critical role in reward, expectation, decision making, motivation, addiction, and reward based learning. The biggest dopaminergic nuclei are the Ventral Tegmental Area (VTA) and the SNc, which are located in the midbrain, an area of the brain located near the anterior end of the brain stem. The VTA is part of a vast network that extends throughout the entire brain. Though advances in electrophysiology and optical imaging have allowed us to better measure VTA output, progress in finding VTA input has been much slower. Previous research in the Uchida lab has identified inputs from autonomic, motor, and somatosensory areas, including the lateral hypothalamus, which is involved in pleasure seeking. However, we do not know the cell types of these input neurons due to limitations in previous methods of tracing. This project attempts to trace direct input to dopaminergic neurons in the VTA using a retrograde rabies virus in three lines of transgenic mice: vGlut2-GFP, D1-GFP, and GAD67-GFP. I identified inputs from areas consistent with previous research. In addition, I found that some areas like the dSt, the BNST, and the RMTG had mostly GABAergic inputs while other areas like the EP and the VP had mostly Glutamatergic inputs. I concluded that using the rabies virus in conjunction with transgenic mice is a viable way of identifying the type of input neurons. Knowing cell types of input to the VTA gives us a better understanding of the midbrain dopamine circuitry.

Novel study on miR-505 in glioblastoma metabolism and cell survival, A

Research Director: Dr. Mark Johnson

A growing number of studies have revealed an important role for metabolism in glioblastoma. MicroRNAs are short non-coding RNAs that regulate biological function by binding to complementary sequences in the 3'-untranslated region (UTR) of mRNAs to repress protein translation. An important glycolytic enzyme, 6-phosphofructo-2-kinase/fructose-2,6- biphosphatase 4 (PFKFB4), was found highly associated with glioblastoma cell survival. Based on bioinformatics analyses, I predicted that miR-505 targets the 3'-UTR of PFKFB4. Here, the principal objective was to determine whether miR-505 targets PFKFB4 to significantly inhibit the survival of both established human glioblastoma cell lines and primary human glioblastoma- initiating stem-like cells (GSCs). Using Western blots and luciferase assays, I validated the predicted miR-505/PFKFB4 mRNA interaction. Specifically, exposure to miR-505 significantly decreased PFKFB4 protein expression in established glioblastoma cell lines and in primary human GSCs. Growth assays further revealed that miR-505 represses glioblastoma cell growth by downregulating PFKFB4. Seeing as PFKFB4 mediates shifts in glycolytic flux, I proposed that miR-505 treatment would increase the production of reactive oxygen species, thereby triggering apoptosis and halting the aggressive proliferation that is characteristic of GBM. As predicted, mir-505 significantly increased glioblastoma cell death in a live/dead cell apoptosis assay. Taken together, these data suggested that miR-505 directly targets and downregulates PFKFB4, thereby decreasing glioblastoma cell survival. Notably, I found that patient survival was positively correlated with miR-505 expression and negatively correlated with PFKFB4 mRNA expression. Thus, the targeting of PFKFB4-dependent metabolism via miR-505 may represent a promising new therapeutic approach in glioblastoma.

Optogenetic interrogation of the role of the hippocampal CA3 region in fear learning

Research Director: Dr. Uwe Rudolph

Fear conditioning is a popular animal model for anxiety disorders, and therefore much research has focused on elucidating the circuitry that underlies fear learning. While traditional techniques such as lesion studies, pharmacological inactivation studies, and gene-knockout studies have identified brain regions that play a role in fear learning, these techniques lack temporal specificity. One region that has been shown to be involved in contextual fear conditioning is hippocampal CA3. In order to determine the time specific role of the region, we used optogenetic technology to inhibit glutamatergic neurons in CA3 prior to shock administration. We used a mouse strain, in which an Archaerhodopsin-GFP fusion protein was specifically expressed in CA3 glutamatergic cells; consequently, illumination of these cells via a fiberoptic cannula reversibly inhibited their action potentials. These mice demonstrated a reduction in freezing compared to control mice both in the period immediately after the shock and when returned to the same context 24 hours later. This indicates that normal CA3 activity is necessary for acquisition of the environmental context in fear learning. Precisely understanding the hippocampal sub-circuits that are activated during the acquisition, consolidation, retrieval, and extinction of learned fear, can allow us to find new, specific targets for therapeutic anti-anxiety drugs.

Phase-Amplitude Coupling in Infant Resting-State EEG as a Potential Marker for Compensatory Activity in Autism Spectrum Disorder

Research Director: Dr. Charles A. Nelson

The application of signal processing measures to electroencephalographic (EEG) data offers new methods of examining brain connectivity. One such measure is phase-amplitude coupling (PAC), the modulation of the amplitude of high frequency activity by the phase of low frequency activity. The current study explored the properties of PAC in infants, in particular its developmental trajectory and its relationship to risk for autism spectrum disorder (ASD) and the development of ASD. As part of the ongoing Infant Sibling Project, high-density EEG was collected during an eyes-open resting state from infants, who were sorted into risk groups based on whether they had an older sibling with an ASD diagnosis (high-risk) or a typically developing older sibling (low-risk). PAC levels were measured between theta and gamma activity for each electrode. When infants reached 36 months, the presence of an ASD diagnosis was determined using behavioral measures with clinical correlation. Increased PAC was found in right frontal and posterior temporal regions, and there appeared to be an upward trend in PAC at 36 months in the high-risk infants who developed ASD. However, a high degree of variability within groups and within subjects questioned the robustness of this difference. This was the first study to explore PAC in infants, and further refinements of the measure may allow it to shed light on connectivity in the infant brain. Establishing differences in PAC between infants who develop ASD and those who do not may also allow it be used as a biomarker of the disorder.

Role of the Retrograde Response Gene Bcl-w in the Auditory System, The

Research Director: Dr. Rosalind A. Segal

The human sense of hearing relies on the precise wiring of spiral ganglion neurons within the cochlea during development and their maintenance during aging. Formation of these precisely organized circuits depends on proper expression of Trk receptors and stimulation by neurotrophins, suggesting that the neurotrophin-induced survival pathways seen in other areas of the nervous system, such as the dorsal root ganglia, may also be salient within the inner ear. Since spiral ganglia are the sole input of auditory information to the brain, understanding the pathways that initiate their assembly and ensure their maintenance over time is of particular importance to auditory research. Here we report by whole mount in situ hybridization and immunofluorescence that Bcl-w, a neurotrophin-induced pro-survival member of the Bcl-2 family, is expressed in spiral ganglion neurons along an apical-to-basal gradient. We also show by behavioral analysis of the preyer reflex that BLK6 Bclw-/- mice develop an earlier onset of hearing loss compared to controls. This evidence suggests that Bcl-w may provide a mechanism by which peripheral processes within the cochlea are maintained over time and protected against the progressive retrograde degeneration observed during age-related hearing loss. Together, these findings contribute to our understanding of the pathways involved in maintenance of stable circuits within the inner ear, which may accelerate efforts to enhance the benefits of cochlear implants and advance ongoing research to exploit stem cells as a viable solution for spiral ganglion replacement therapy.

Visual Feedback Latency Reduces the Retention of Visuomotor Learning and Alters Its Internal Representation

Research Director: Dr. Maurice Smith

Visual feedback is essential for the acquisition of goal-driven motor adaptation. Studies suggest that endpoint-only visual feedback requires close temporal pairing with the motor movement in order to drive motor adaptation. Visual delays impaired motor learning when visual feedback was given 50ms after movement completion. However, endpoint-only trials do not accurately represent the continuous visual feedback in daily motor learning. We explored the effects of a continuous visual feedback delay (VFD) on the learning and application of a motor task.

Reverse hierarchy theory suggests that high-level motor learning generalizes broadly to untrained conditions and low-level learning generalizes locally to the trained condition. We hypothesize that adaptation at small VFDs will show local generalization, indicating low-level motor learning, while larger VFDs will result in broader generalization, indicating high-level motor learning.

We trained 38 subjects in a visuomotor rotation (VMR) task, in which they adapted a point-to-point reaching movement to a 30° rotation with a VFD of 0ms, 25ms, 62ms or 275ms. The rate of VMR adaptation did not vary significantly across VFD groups, but the 62ms and 275ms groups did show significantly less VMR retention than the 0ms group after a 60s delay in training. All groups showed similar global generalization to untrained directions, however the 62ms and 275ms groups displayed significantly less generalization locally than the 0ms group ($p=0.007$ and $p= 0.0001$ respectively). Because VFDs as small as 62ms result in an increased global to local generalization ratio, our results indicate that the timing of visual feedback may alter the internal representation of motor learning.

Your Brain on SET: An Exploration of the Neural Basis of Expertise in Rule-Based Pattern Recognition

Research Director: Dr. George Alvarez

Perceptual expertise is an integral part of the visual cognitive system in most animals, but consciously trained expertise in a complex, rule-based paradigm is a skill usually only naturally obtained by humans. Specialized brain areas for recognition of faces, words, bodies, scenes, and objects have been localized using fMRI, but the neural underpinnings of this more abstract process – rule-based pattern recognition – have not been explained nor extensively investigated. Therefore, this study examined the neural activity associated with complex perceptual expertise in the card game SET, which requires rapid visual processing of multiple features belonging to three groups of colored and textured shapes. SET experts were significantly faster than SET novices at recognizing "sets" (three cards whose features satisfied the rule of the game) and also showed evidence of non-serial visual processing of the features. fMRI data from SET experts viewing sets, nonsets, and individual shape elements did not localize an area trained to differentiate between sets and nonsets; however, an area near the right superior intraparietal cortex showed a significant difference between sets and nonsets in the prediction accuracy of activation patterns of the "whole" (three cards together) from those of its constituent parts. This finding highlights new information about the sIPS – which has already been implicated in visual feature integration – and sheds light on how expertise in rule-based pattern recognition manifests at the neural level.