All for one and some for all? Using EEG and the N400 response to track online scalar implicature calculation in adults

Research Director: Dr. Jesse Snedeker

While the presence of scalar implicature (SI) calculation is well-established in psycholinguistic research, their time-course information is not well understood. Some studies, such as Huang & Snedeker (2009), posit that the SI calculation- the application of pragmatic knowledge from a given situation to scalar terms like some- is delayed. Others, including Grodner et al. (2010), find that the pragmatic SI interpretation occurs immediately at the scalar term's utterance. These conflicting studies present evidence from visual world eye-tracking paradigms that do not provide the temporal resolution necessary to accurately distinguish between these two accounts. I aim to address this issue by using electroencephalography (EEG), a rapid automatic measure of neural activity, to detect a real-time neural response. I will be looking at the N400 event-related potential (ERP) response to semantic incongruencies. I adapt the Grodner et al. (2010) paradigm for ERP, instead presenting sentence prompts that elicit a SI violation, along with an analysis of the surrounding ERP. The additional violations allowed the N400 response to be a more reasonable and informative measure of the time-course of SI calculation. I predict an N400 response at the point of disambiguation between the violation and the pragmatically-licensed response, which would suggest an immediate calculation of the SI. Preliminary findings show a more negative N400 response at disambiguation, which is consistent with accounts of rapid SI calculation. This study provides evidence that ERP is a useful tool in understanding the time course of pragmatic processes that underlie SI.

Autonomic and brain activity concordance in patient/clinician dyads - a hyperscan fMRI study

Research Director: Dr. Vitaly Napadow

The patient-clinician interaction has been shown to modulate clinical outcomes both in acute and chronic disease, but its neural correlates—in particular the neural circuitry of empathy, concern, and social communication—are poorly understood. This thesis aims to use functional magnetic resonance imaging (fMRI) to explore how the patient-clinician interaction may modulate neural activation relating to empathy and analgesia in fibromyalgia patients, with the long-term goal of identifying a paradigm for clinically-effective interaction. We investigated this phenomenon within the framework of the social placebo effect, the concept that aspects of the therapeutic encounter outside of medicinal treatment can also play a role in healthcare efficacy, using qualitative assessment of behavioral intake videos via the Constitutional and Relational Empathy (CARE) Observer Scale and correlating video scoring metrics with brain imaging and autonomic concordance metrics. Specifically, we: 1) assessed clinical analgesic outcomes using a novel and naturalistic "Hyperscan" fMRI setup involving simultaneous scans of a patient and clinician, 2) examined activated neural and autonomic concordance and correlates of empathy and analgesia from fMRI scan data, and 3) ran correlations between variables rated in the patient-clinician interaction—including the use of appropriate humor, perceived warmth as measured from an adapted 9-factor warmth-competence scale, and clinician use of the patient's own words—and areas of neural activation in corresponding fMRI scans. The long-term goal is to use an optimal patient-clinician interaction paradigm to improve clinical analgesic outcomes, especially in cases where care is underprovided and clinical interaction may significantly affect pain management.

Characterizing the Morphology and Interactions of Glia in the Developing Cerebellum

Research Director: Dr. Jeff Lichtman

During developmental learning, neural circuitry is shaped by the elimination of immature neurons. The result of this cell death is a functional network that generates and influences behaviors. Of recent interest in the mechanisms driving apoptosis is a class of non-neuronal cells known as microglia. These cells are commonly referred to as the resident immune cells of the central nervous system because they play a phagocytic role in removing dying neurons and other cellular debris. Their highly ramified, motile branches allow them to constantly survey the brain for lesions and damage, as well as to make close associations with neuronal cell bodies. Here, we use serial-section electron microscopy techniques to reconstruct and characterize the morphology and interactions of a microglial cell, in order to investigate the role of glia in granule cell death in the developing cerebellum. We demonstrate that phagocytosis is facilitated by the high degree of glial branching which allows a microglial cell to engulf not only a neuronal cell body, but also the lengths of each neuronal process. This is contrary to the notion that “activated” microglia necessarily acquire an ameboid phenotype. Additionally, microglia are not self-repellent. Because microglia are implicated in cell death during early development and because deletion of microglia-specific genes is associated with the onset of neurodevelopmental disorders, an improved understanding of microglial function in the healthy brain may help to elucidate the mechanisms which are disrupted in disease.

Comparative synaptic characteristics between human and rodents

Research Director: Dr. Jeff Lichtman

In this paper, I established a baseline for human structural connectomic data and compared human synaptic data to previous rodent data. After using serial sectioning and scanning electron microscopy to virtually recreate a volume of human brain tissue, I used segmentation and annotation software to label, quantify, and measure dendrites, dendritic spines and synapses. The data showed that humans display a lesser synaptic density per unit volume in layer 5 of the neocortex compared to mice. This difference indicates a higher selectivity in human synaptic connectivity, possibly utilizing a greater number of neurons with greater specificity for more complex organization.

Data Visualization Software for DBS and Classification of Intraoperative Targets

Research Director: Dr. Todd Herrington

Neurosurgery and surgical treatments involving precise localization of specific targets, particularly deep targets, are challenging because they are often extremely small and never seen directly by the surgeon. During surgeries such as Deep Brain Stimulation (DBS) for conditions like Parkinson's disease, microelectrode recordings along an electrode trajectory (provided by stereotactic coordinates from preoperative planning with fMRI/CT imaging) are invaluable for verifying known physiological characteristics of desired areas and confirming the target. This thesis aims primarily to present a software solution developed to recapitulate the highly-structured nature of the data (multiple recordings along a depth axis, across multiple channels) to facilitate synchronized visualization and analysis of a given patient's data or surgery. The tool is built with a large amount of modularity in mind, and serves primarily to 1.) greatly enhance the ease of data exploration, multiple-resolution visualization, and annotation of data post-surgically and 2.) provide tailored methods of data representation helpful to the surgeon (such as audio playback of a recording) and potentially facilitate and ease the decision of electrode implantation intraoperatively. This thesis also aims to give a preliminary exploration of automatic classification of common brain targets by electrophysiology alone, using data drawn from a population of patients undergoing DBS and analyzed and annotated by the software presented.

Developing Proteomic Techniques: Uncovering Potential Biomarkers for Rett Syndrome using Proteomic Analysis of Cerebrospinal Fluid

Research Director: Dr. Hanno Steen

Rett Syndrome (RTT) is a neurodevelopmental disorder associated with an X-linked mutation in the MECP2 gene and affects 1 in every 10,000-15,000 girls in the world. Girls with RTT experience normal early development. However, they start to show regression in language, motor coordination, and intellectual abilities around two years of age. One of the major roadblocks in treating this disorder results from the delay in diagnosis – as it only occurs when symptoms have already manifested in the infant. The emerging field of proteomics, can serve as a tool for understanding the mechanisms of neurological disorders such as Rett Syndrome. Proteomics enables the study of differences in protein expression levels between diseased and control patients, which can identify potential biomarkers for disease. Previous studies have identified changes in enzyme, monoamine, and metabolite levels in blood and cerebral spinal fluid (CSF) of patients with neurodevelopmental disorders and controls. The goal of our study was to expand on this research by identifying specific neuropeptides that may be associated with Rett. We first optimized search parameters for proteomics data to account for the various post-translational modifications encountered in CSF. Next, we worked on optimization of CSF ultrafiltration/peptidomics protocols. We found that a decrease in search parameter specifications resulted in an increased yield of identified neuropeptides from the samples. We tested 4 ultrafiltration methods and found that the greater neuropeptide yield came from processing with acetonitrile and 10% formic acid. We used mass spectrometry to identify and quantify the peptide composition of the CSF samples.

Development of a Novel Oncolytic Virus Targeting the PD-1/PD-L1 Pathway for Glioblastoma Treatment

Research Director: Dr. Sean Lawler

Oncolytic virotherapy—a type of immunotherapy which uses viral infection as a means of increasing immune response to cancer cells—presents a promising approach to the treatment of the brain tumor glioblastoma and other tumor types. Oncolytic herpes simplex virus (oHSV) is a well-established candidate for the treatment of neurological cancers, as multiple studies have confirmed its ability to selectively infect neurological tumor cells while avoiding healthy tissue. Also, its large genome allows for substantial modifications. This thesis analyzes the in vitro and in vivo activity of the NG34scFvPD-1 oHSV, which contains two novel modifications. First, the viral genome contains the GADD34 gene—the non-neurotoxic human orthologue to the ICP34.5 localization sequence present in wildtype HSV. Second, the scFvPD-1 modification codes for the single chain variable fragment (scFv) of the anti-PD-1 antibody. This antibody is designed to competitively inhibit the interactions between the PD-L1 ligand expressed on tumor cells and PD-1, a T cell membrane bound receptor. The PD-1/PD-L1 interaction inhibits immune response normally triggered by interactions between the major histocompatibility complex II (MHCII) and T cell receptor (TCR). The scFvPD-1 antibody disrupts this inhibition thus reactivating T cell immune responses. In vitro experiments revealed comparable infectivity, replicability, and toxicity of the scFvPD-1 and NG34 viruses in multiple murine and human glioma cell lines. In vivo experiments with C57BL/6 mice showed successful scFvPD-1 viral infectivity and increased survival rates in GL261Nectin1 engrafted mice. Similar experiments with athymic nude mice showed successful infectivity and replication in human cell lines, but without increased survival, implying that the functionality of the virotherapy depends on T cell response.

Effects of Family History and Home Literacy Environment on Cortical Brain Structures in Pre-Reading Age Children

Research Director: Dr. Nadine Gaab

Despite being the most prevalent of all reading disabilities, much is still unknown about the factors that influence developmental dyslexia onset. Although studies have shown significant structural brain differences between individuals with and without dyslexia, minimal research has been done investigating the cause of these differences. To address this, recent studies have focused on pre-reading age children in an effort to distinguish whether these structural differences arise as compensatory mechanisms developed during the reading learning period, or if they are established earlier due to genetic or environmental influences. The present study aimed to further elucidate this distinction by analyzing the effect of home literacy environment (HLE) on pre-reading age children with and without a family history of dyslexia. Collected fMRI scans of children aged 5-6 years (n=58) were analyzed using the FreeSurfer Image Analysis Suite to determine cortical thickness, volume, and area measurements. In addition, parental questionnaires were obtained for each subject for HLE and socioeconomic status information. Correlational analysis run between the structural brain measurements and HLE information revealed that certain factors of HLE were significantly correlated to structural brain differences. Specifically, the ages at which a child was first read to, first asked to be read to, and first looked at books alone were all significantly negatively correlated with cortical thickness measurements in multiple brain regions. These results illustrate that cortical structure differences in individuals with and without dyslexia do not arise purely due to compensatory mechanisms and suggest possible factors that could influence this distinction earlier on.

Effects of Growth Stunting on Neural Functional Connectivity: An fNIRS Study of 3-year-olds in Bangladesh

Research Director: Dr. Chuck Nelson

Growth stunting is one of the most globally prevalent and detrimental early childhood health conditions, with lifelong cognitive, educational, and economic effects. Bangladesh has a high rate of growth stunting; over 1 in 3 children were growth stunted in 2014. However, not yet known are the neural mechanisms by which growth stunting has such detrimental effects. This study explored the neural effects of growth stunting, using functional connectivity as a measure of neurodevelopment. 131 3-year-olds from the NIH Provide cohort in Dhaka, Bangladesh participated in Functional Near-Infrared Spectroscopy (fNIRS), a brain-imaging method ideal for low-resource contexts due to its high portability and spatial resolution. Using fNIRS data, functional connectivity was quantified as the correlations between cortical Regions of Interest (ROIs) during resting-state spontaneous fluctuations. Examining the inferior frontal, superior temporal, and middle temporal lobes, it was found that growth stunted children exhibit a significant decrease in the strength of functional connectivity between ROIs. Thus, we conclude that growth stunting indeed has significant impacts on the connectivity of the brain, and measuring the growth of a child is not only important as a measure of nutrition, but as a proxy for cortical connectivity. Moreover, this validates the use of fNIRS to measure the neurodevelopmental impacts associated with adverse environmental conditions. These findings have the potential to inform and improve the efficacy of future interventions which target growth stunting.

Elucidating the Therapeutic Benefits of Docosahexaenoic Acid for Major Depressive Disorder in Human iPSC-Derived Neuronal Cell Models

Research Director: Dr. Steve Haggarty

Major depressive disorder (MDD), a common mental disorder that affects 10-15% of the population worldwide, has become an increasing public health concern. The leading medical treatments for MDD—selective serotonin reuptake inhibitors (SSRIs)—show inadequate benefit with undesirable side effects for many patients. Given these limitations, some MDD patients manage their symptoms with complementary and alternative medicines (CAMs). However, the molecular and cellular mechanisms of most CAMs have not been established. We began to address this knowledge gap by gaining insight into the efficacy of the widely-used CAM Docosahexaenoic acid (DHA), an omega-3 fatty acid with reported antidepressant activity, in potentially disease-relevant cell types—human iPSC-derived neural progenitor cells (NPCs) and post-mitotic neurons.

Utilizing these ex vivo cellular models, we observed that DHA treatment increased neuronal survival, promoted neurite outgrowth, and enhanced dendritic arborization with prolonged exposure. We further identified that DHA enhanced both WNT and CREB signaling pathways that play important roles in neuronal development, adult neurogenesis, and memory. To generate a molecular signature of DHA, we applied multiplexed gene expression profiling technology (L1000) to identify and validate additional genes regulated by DHA, including the key neuroplasticity and memory gene EGR1 and multiple stress-related genes. Finally, we showed that DHA could counteract the detrimental effect of oxidative stress in patient-derived MDD cell lines. In conclusion, our study indicates that DHA may elicit multiple mechanisms for its antidepressant effect. Future investigation holds promise for providing insight into the mechanisms of other CAM natural products, with implications for treatment efficacy.

Functional genetic screen in Drosophila on proteasome-modulated tau toxicity, A

Research Director: Dr. Mel Feany

At a molecular level, the deleterious neurodegenerative pathology associated with Alzheimer's disease is correlated with elevated levels of neurofibrillary tangles composed of tau protein and senile plaques constituted by beta-amyloid peptide. Putatively, the ubiquitin-proteasome system (UPS) plays a crucial role in protein dynamics, and examining the role of the proteasome in the context of the cellular antioxidant response pathway may elucidate molecular pathways underlying Alzheimer's onset and progression. Drosophila melanogaster models of tau-induced Alzheimer's disease are highly susceptible to cytotoxic stress, displaying adult-onset progressive neurodegeneration, early death, and accumulation of abnormal tau, consistent with human Alzheimer's pathology. Using the UAS/GAL4 system, human tau (τWT28) was expressed ectopically in Drosophila comcomitant with proteasomal activity suppressors to deduce molecular interactions between proteasome activity and subsequent AD toxicity. Due to overwhelming levels of toxicity, it remains unclear whether proteasomal activity contributes additively or multiplicatively to cytotoxicity, but future experiments may yet examine the role of proteasomal activators as therapeutic targets for Alzheimer's-like tauopathies.

Genomic Imprinting: Analysis of dynamic genomic imprinting at the resolution of single cells

Research Director: Dr. Catherine Dulac

Imprinting is a form of epigenetic regulation in which one allele is expressed preferentially based on parent of origin. While imprinting has been generally understood as a complete silencing of one parental allele, the expression of many imprinted genes shows a strong parental bias. We do not know the significance of allelic imbalance at the single cell level. Most imprinted genes are consistently expressed but in low levels, suggesting that a sensitive single-cell technique for further analyzing imprinted gene expression is necessary. Additionally, imprinted genes play crucial functions in neuronal development, such as apoptosis. One such gene is BCL-X, an anti-apoptotic gene. In collaboration with the Hensch lab, we have found that BCL-X affects plasticity in a specific layer of excitatory neurons in the visual cortex. We therefore sought to understand the spatiotemporal dynamics of imprinting of BCL-X. We have established an in situ hybridization technique on cortical tissue from uniparental mouse deletions of BCL-X, that allows us to directly quantify mRNA at the single cell level. We compared expression of BCL-X in different cell types of the cortex to determine whether BCL-X is monoallelically or biallelically expressed in distinct populations. In the second aim of my project, we evaluated patterns of transcriptional activity for genes clustered with Bcl-X and other known imprinted genes that are implicated in social behaviors, in order to understand when imprinting is established in neurons, and in which cell types. Our results indicate that BCL-X is imprinted differently in distinct cell types in the cortex, and that developmental time points may impact the imprinting patterns of both the genes in the BCLX cluster, and those involved in social behaviors. Our results shed light on how imprinting occurs at the single cell level, and suggests that changes in gene imprinting patterns across time and cell types may affect brain development and function.

Impact of Maternal Depression on the Relationship Between EEG and Behavioral Inhibition Through the First Three Years of Life

Research Director: Dr. Chuck Nelson

There are considerable individual differences among infants and toddlers in their socioemotional development. These patterns of regulation and response can be attributed to distinct temperamental profiles, which have been defined as genetic and biological dispositions that underlie an individual's socioemotional development and behaviour. Research investigating these patterns have used a wide variety of methods, including behavioural coding, parental report, and frontal EEG asymmetry. Many previous studies with diverse samples have confirmed effects of early temperament on behavioural problems in later childhood and adulthood. Likewise, similar investigations have also shown markedly changed patterns of behaviour over time. More specifically, attributes of the psychosocial environment and the caregiving atmosphere have been shown to influence these temperamental profiles and development. Thus, a better understanding of stability and change of temperament has become an important focus for understanding emotional development and behaviour in infants and toddlers. Identifying whether additional factors influence these profiles and trajectories over time can have important implications in preventing and predicting later psychopathology or poor socioemotional outcomes. This research proposes to examine continuity and discontinuity in both behavioural (temperament coding) and neural correlates (frontal EEG asymmetry) of temperament over the first three years of life, critically investigating the extent to which psychosocial factors in the early infant caregiving environment, such as maternal depression, influence stability and change in temperamental profiles across development.

Influence of Early-Life Stress on Sleep and Attentional Functions in Mice

Research Director: Dr. Takao Hensch

Children who have experienced early life stress (ELS) such as emotional and behavioral disturbances have increased rates of behavioral features that are consistent with Attention Deficit/Hyperactivity Disorder (ADHD). Research on ELS in animal models has shown altered brain development and associated changes in psychological/behavioral functioning. The objectives of this study were to explore the impact of ELS on attentional performance and sleep in mice.

Mice were raised with either control care or the fragmented care paradigm (limited nesting and bedding material) and then administered the 2-choice visual attention task with and without sleep deprivation (SD), to assess attentional performance in control and ELS mice and to evaluate their sensitivities to SD. To investigate the effect of ELS on sleep as well as the associated cortical activity, mice underwent EEG/EMG recordings after surgical implantation of electrodes over the right prefrontal cortex. ELS mice showed decreased attention levels, but showed no reduction in attentional performance post-SD, which was different from the trend seen in control mice. This effect was evidence of resistance to attentional decline in ELS mice when they had increased sleep need. In addition, while circadian modulation of wake/sleep rhythms were largely unchanged in ELS mice, these mice exhibited heightened power of gamma oscillation during wake periods, potentially being the neural basis of altered attention.

In conclusion, this study demonstrated a link between ELS and lack of attentional decline post-SD, which may suggest abnormal neurodevelopment during critical periods of early development and consequent impairment of attention and sleep regulation.

Influence of Social Dominance on Competitive Foraging Behavior in Wild-Type and Shank3-Deficient Mice

Research Director: Dr. Ziv Williams

Social interactions play a key role in both human and animal behavior, and are commonly involved in neurocognitive disorders such as schizophrenia and autism. Despite the importance of interactive social behavior and its dysfunction, its single-neuronal basis and causal underpinnings are not well understood. One significant instance of social interaction dynamics between individuals is competitive foraging within groups of animals. In this study, we sought to elucidate the dynamics of competitive foraging behavior within groups of mice and investigate its neural underpinnings. Specifically, we observed the influences of social dominance hierarchies in groups of familiar mice performing a competitive food foraging task. Preliminary data indicates that social dominance influences the order in which mice access food, and this influence changes depending on the abundance of food. Next, we will use single-unit electrode recording to monitor neural activity in the anterior cingulate cortex, a center for processing information involved in competition, while the task is underway in order to link differences in behavior and socialization to different patterns of neural activity. Finally, we will observe differences in behavior and ACC activity between wild-type mice and Shank3 genetic knockouts during the task; Shank3 knockouts serve as a model of Autism Spectrum Disorder in mice, and allow us to investigate the effect of ASD symptoms on competitive foraging.

This research will give insight into the social and neurobiological mechanics of competition and success, allowing us to better understand the basics of group competitive behavior and how it differs from competition between two animals. Our hope is to open the door for further studies into competition in large and complex groups, the patterns of brain activity associated with such behaviors, and the effect of disease on competitive outcomes.

Insights into amyloid-β42 aggregate structure from large-scale mutational data

Research Director: Dr. Sean Eddy

Alzheimer's Disease (AD) has long been associated with amyloid-β (aβ) aggregation in the brain into plaques–clusters of long insoluble fibrils, also identified as amyloids for their repeated cross-β structure. It has been shown that structural variation in these fibrils, as well as soluble precursors, namely oligomers and photofibrils, correlates with both varying levels of neurotoxicity, and variation in AD phenotype. Thus, a thorough understanding of the various aggregate forms of aβ, and the structural details that contribute to their varied toxicities may assist in revealing the exact molecular interactions leading to neurodegeneration. Here, we performed a deep mutation scan (DMS) to yield general insights into aβ-42 aggregate structure, and provide a basis of knowledge for future, more detailed investigation into specific aggregate forms. In this high-throughput assay, we exploited yeast's dependence on dihydrofolate reductase (DHFR) for growth, using the metabolic enzyme as a reporter of the aggregation propensity of ~15,000 upstream fused aβ variants. We found four regions (Y10-Q15, L17-E22, I31-V36 and V39-A42) important for aggregation on account of their high sensitivity to mutation. We hypothesize that these regions form tightly bound β-sheets while in aggregate form. We also found that mutation is most disruptive at hydrophobic residues. Finally, by pairing DMS scores of single- and double-mutants, we propose likely epistatic interactions within and between aggregate monomers. In a second line of analysis, we present a framework with which to benchmark proposed structures of protein aggregates derived from in-vitro experimentation against cell-based DMS data. With an expanding list of proteins capable of self-assembling in disease-causing fashion, this may prove useful in corroborating future structural models ultimately critical for therapeutic development.

Investigating GDF11's Permeability through the Blood-Brain Barrier and its Effect on Cerebral Vasculature

Research Director: Dr. Lee Rubin

Recent studies have shown that factors circulating in the blood can positively and negatively affect the health of multiple tissues during aging. Research thus far has demonstrated that restoring “youthful” levels of circulating protein GDF11 leads to the reversal of age-related hypertrophy in the heart, promotes neurogenesis and increases blood flow in the adult brain, and has even been associated with rejuvenation in bone, muscle, liver, and pancreatic tissue. Focusing on GDF11’s angiogenic and neurogenic properties, the molecule could help combat dementia, neurodegenerative diseases, and stroke. We hypothesize that GDF11 either crosses or interacts with the blood-brain-barrier (BBB) to carry out these downstream effects. This study explores the effect of GDF11 on the BBB and the cells of the brain vasculature in three separate experiments. First, an in vitro model of the barrier is constructed on hanging cell-culture inserts using a co-culture of brain vascular endothelial cells and astrocytes, two of the cell types that comprise the BBB. The permeability of the barrier is evaluated upon addition of GDF11, testing whether GDF11 may alter BBB integrity by affecting the tight junctions, cell density, or other aspects of BBB composition. Preliminary experiments have shown that co-culturing astrocytes with endothelial cells as well as culturing endothelial cells in astrocyte culture media increases the density of endothelial cells at confluence, improving the integrity of the BBB model. Further, we utilize a tube formation assay on Matrigel to evaluate GDF11’s effect on angiogenesis, potentially forming new capillaries in the brain to increase blood flow to the tissues. Furthermore, TGF-B and GDF11 showed an increase in tube formation while TGF-B inhibitor SB-431542 disrupted formation of tubes. Finally, the study uses an in vivo mouse model to perform time-course injections of GDF11 in order to test the activation of its target cells and its penetration into the brain in vivo. Homogenization and differential centrifugation of brain tissue are used to separate the parenchyma from the vasculature to look at GDF11 levels in these specific tissues via western blot analysis, revealing whether GDF11 crosses the BBB from the vasculature into the parenchyma. Understanding the effects of GDF11 on the cells of the brain vasculature and BBB will help reveal its, and other associated factors’, potential therapeutic effects on alleviating the burden of various neurodegenerative diseases.

Investigating the Relationship between Self-Punishment, Self-Criticism, the Experience of Pain and Non-Suicidal Self-Injury

Research Director: Dr. Jill Hooley

Non-suicidal self injury (NSSI) is a prevalent behavior with significant emotional and physical consequences. NSSI has been shown to alleviate feelings of distress, but it does so with a negative consequence of pain. As such, it is believed that the experience of pain may be functional in this coping mechanism. This research seeks to explore how the experience of pain may differ across a sample of adults with and without a history of NSSI, and how that may contribute to some individuals employing NSSI as a coping mechanism while others do not. Previous research suggests that the pain of NSSI may function as a form of self-punishment, in which mood improvement results from a feeling of atonement, but this association appeared to be limited to individuals who rated high on self-criticism. The present study hypothesized that this association would also be found in individuals who rated high on neuroticism, orderliness, and internalized shame, and those with a heightened belief in a just world. The study utilized self-report measures of self-criticism, neuroticism, orderliness, shame, and belief in a just world, as well as a self-report questionnaire about self-injurious thoughts and behaviors. In the lab, participants completed a negative mood induction followed by a task involving the self-administration of pain, in which the participant was in full control of the duration of pain. If neuroticism, orderliness, shame, and belief in a just world are found to be mediators of the valence of the experience of pain, this would suggest clinical focuses in treating this behavior.

Investigation of Altered Neural Response Variability as a Key Marker of Disordered Neural Development in Children with 16p11.2 Copy Number

Research Director: Dr. Chuck Nelson

Background: Copy number variants (CNVs) at chromosome16p11.2 increase the risk for a variety of neurodevelopmental disorders. 16p CNVs affect circuit-level excitability via altered glutamate binding and result in neural response variability. Leblanc and Nelson (2016) reported that, compared to typically developing children the amplitude of the visually-evoked potential (P100) was larger in deletion carriers and reduced in duplication carriers. Amplitudes are averaged across numerous trials, so both excessive trial-by-trial response variability and low trial-by-trial amplitudes impact P100 amplitude. We sought to investigate the magnitude of response variability and its impact on P100 amplitude across groups and neural frequencies.

Methods: Inter-trial phase coherence (ITPC) measures trial-by-trial response variability. Participants included children with 16p11.2 deletions (n=19) or duplications (n=9) participating in the Simons Variation in Individuals Project, and typically developing children (n=13). Using a visual evoked potential paradigm, we quantified P100 amplitude and ITPC for each participant across multiple frequency bands.

Results:. Spearman rho correlations revealed significant positive correlations between ITPC magnitude and P100 amplitude across groups in the alpha frequency band ($r^2 = 0.175$, $p = 0.000128$), but ITPC magnitude across frequency bands did not differ significantly based on 16p copy number. Percent impact of ITPC on P100 amplitude differed by genotype.

Conclusions: Neural response variability varies by frequency and 16p copy number. The between group P100 amplitude differences reported by Leblanc and Nelson (2016) are largely explained by differences in frequency band activity rather than response variability. Response variability plays a previously unrecognized role in between group neural response differences in children with 16p CNVs. These findings are important as neural response variability is implicated in sensory perception and learning.

Lynx1 Enables Attentional Suppression under Sleep Need

Research Director: Dr. Takao Hensch

Attention, an essential precognitive ability, forms an important foundation for cognitive abilities such as learning, memory, and decision making. Certain behaviors and drugs, such as sleep deprivation and nicotine, are known to alter attention levels, but the exact mechanisms behind these phenomena are unknown. We sought to understand the role of the cholinergic system in regulating attentional levels particularly following changes in arousal state. One factor known to modulate cholinergic activity is lynx1, an endogenous protein which binds and depresses activity of nicotinic acetylcholine receptors (nAChRs), making it an interesting target for attentional studies. We measured attentional level in lynx1 knockout mice using two choice visual attention (2-CVA) and reversal learning tasks. Following this task, we performed circuit dissection in the anterior cingulate cortex (ACC) by immunohistochemistry. We also measured attentional levels of sleep deprived lynx1 knockout mice using the 2-CVA task. Finally, we quantified expressional changes of nAChRs following sleep deprivation by qPCR and in situ hybridization. Lynx1 mice, in addition to having higher attentional ability than wildtype mice, also are resistant to decreases in attentional ability following sleep deprivation. Following the 2-CVA task, knockout mice showed higher activity in PV and SST inhibitory interneurons than knockout mice. Finally, one subtype of nAChR, α7, was specifically decreased in excitatory neurons in the prefrontal cortex following sleep deprivation. We therefore suggest a model in which nicotinic activity in the prefrontal cortex is essential for attention and modulation of that nicotinic activity by lynx1 and sleep need is what drives the change in attentional state.

Neural and Behavioral Effects of an Olfactory Masking Agent

Research Director: Dr. Venki Murthy

Off-flavors provide key olfactory cues regarding potential dangers associated with our environment, ranging from toxic algae in water supplies to rancid foods. These olfactory compounds have particularly important global health implications, as off-flavors can negatively influence food and drink consumption habits and, therefore, contribute to the growing, worldwide food waste problem. Many studies point to the apparent aversive and masking nature of off-flavors; however, little is known about the neural and subsequent behavioral responses to these compounds. I examined the cellular response profiles of neurons that provide input to olfactory bulb (OB) glomeruli in the presence of 2,4,6-trichloroanisole (TCA), one of the most potent off-flavors, using 2-photon microscopy. Further, I assessed the behavioral responses of mice to TCA using a free-running arena. I found that TCA did not impact innate odor preference in mice and had minimal effects on the response profiles of neurons providing input to the OB. Nevertheless, despite a lack of observable effects on OB input, my experiments demonstrate that mice do indeed exhibit a behavioral aversion to the presence of TCA alone. Based on my findings, the effects of TCA on input to the olfactory system are unlikely to result from non-specific interactions with sensory neurons.

Neurobiology of Nesting Behavior in Peromyscus: Striatal Activity during Nesting and Candidate Gene Expression Patterns in Peromyscus polionotus and P. maniculatus Brains

Research Director: Dr. Hopi Hoekstra

Peromyscus polionotus and Peromyscus maniculatus display species-specific thermoregulatory nesting behavior, and the neural basis of this difference remains largely unknown. Understanding this behavior requires knowledge of which brain regions are involved and which genes act in these regions. This study investigated which striatal regions were differentially active during thermoregulatory nesting behavior. We quantified the percentage of neurons expressing Immediate Early Gene cfos mRNA in animals who recently nested and control animals who were not given nesting material. Our results showed significant differences in total striatal activation between the test and control experimental groups (ANOVA: F1 = 67.75, P = 1.9e-08), including significant increases in cfos+ cells in the nucleus accumbens (Student's t-test: t5.39 = 5.15, P=0.0029), caudate putamen (Student's t-test: t4.85= 5.96, P=0.0021), and lateral septum (Student's t-test: t4.25= 2.95, P= 0.039) of nesting animals. These results suggest that striatal regions, especially the nucleus accumbens and caudate putamen, could be thermoregulatory nesting-related brain regions. Next, to identify differences in gene expression that may underlie the species difference in behavior, we performed histology to visualize the spatial expression patterns of nine candidate genes identified in a previous mapping experiment. While three genes had very similar expression patterns across species and data is pending for three more, Dct, Ndfip2, and Slitrk6 had region-specific differences when the two species were compared. Together, these findings implicate the nucleus accumbens and caudate putamen in Peromyscus thermoregulatory nesting behavior and increase the priority of Dct, Ndfip2, and Slitrk6 for future study of species-specific variation in nesting.

Novel biomarkers for visceral and liver fat: Establishing a framework for testing the role of ectopic fat in cognitive impairment

Research Director: Dr. Bruce Kristal

Age-related dementia is among the most individually feared and publicly costly consequences of the aging process. Growing evidence may link obesity to dementia, particularly through inflammation-mediated mechanisms associated with increased storage in visceral fat depots. To address this, this study leverages a unique dataset encompassing adiposity, biomarker, and cognitive data. The MEC-Adiposity Phenotype study follows a group of 1781 healthy women and men, a subset of individuals from the Multiethnic Cohort. In a subset of 1000 people, a series of redox-active, small-molecule blood metabolites were tested to identify visceral and liver fat biomarkers. Three metabolites, only identified chromatographically, are shown to distinguish groups with a cutoff at 5.5% liver fat, with p values of 10-5 to 10-16. Seven metabolites, three not structurally identified, can distinguish upper and lower tertiles for mean visceral fat area with p values of 10-5 to 10-14. Using data from the nested Brain-Gut-Adiposity study, the relationship of these blood metabolites to cognitive impairment was also investigated. These visceral fat-associated molecules showed no association with cognitive impairment assessed using pencil-and-paper cognitive assessments. Within the limits of the experimental design, this places mathematical constraints on the link between obesity and early mild cognitive impairment. However, elevated blood levels of serotonin and guanosine were associated with cognitive impairment and decreased scores in specific cognitive domains such as memory and computational abstraction. This supports previous findings linking guanosine complexes and imbalances in serotonin to cognitive impairment and extends them to a multiethnic cohort.

Novel microdialysis reporter illuminates genetic expression of the dopamine transporter in the brain of awake, free-moving rats

Research Director: Dr. Barak Caine

Measurements of gene expression in the brain are widely limited to the use of DNA, RNA, and protein quantification techniques that require access to brain tissue for nucleotide isolation. Until now, no in-vivo reporter techniques had been developed to analyze gene expression in a time-dependent manner in the brain of awake rats or in any other living organisms. In this paper, we used a microdialysis technique to show such time-dependent increases in the expression of the dopamine transporter (DAT) protein in-vivo in the brain of awake rats following psychiatric drug valproate (VPA) administration. A rat model was developed to contain a gene construct that produces the luminescent protein Gaussia Luciferase (GLuc) driven by the same mechanisms that drive DAT expression, resulting in GLuc synthesis in dopaminergic neurons. These neurons in turn secrete the reporter protein into the cerebrospinal fluid (CSF). Thus, using VPA to increase cellular production of DAT in the experimental group and utilizing a microdialysis method to sample secreted GLuc in the striatum, we correlated DAT expression with the results from GLuc enzymatic activities. In order to confirm that GLuc enzymatic activity corresponded to the actual levels of expression for the dopamine transporter, we purified and measured the quantity of DAT mRNA in the sample tissue through qPCR and saw expression profiles that paralleled that of GLuc activity. Most importantly, this method can be extended as an in-vivo reporter for the expression profile of any protein of interest.

Parkinson's Disease-Linked Gene Products Alter Mitochondrial Function

Research Director: Dr. Matt LaVoie

Parkinson's disease (PD) is a devastating neurodegenerative disorder that results in the loss of motor control and cognitive function. PD affects over 10 million people worldwide making it the second most prevalent neurodegenerative disease thus developing therapeutic drugs is becoming more essential. PD is hallmarked by the aggregation of the protein alpha-synuclein in both familial and sporadic PD. This leads to the formation of Lewy Bodies and eventual neuronal cell death. In addition to the accumulation of synuclein protein, mitochondrial dysfunction is thought to play a prominent role in disease etiology. Autosomal dominant missense mutations in the genes encoding alpha-synuclein or LRRK2 are causal for PD. There is a growing body of interest in LRRK2 for its capability of phosphorylating certain Rab GTPases, specifically Rab8a and Rab10. Rab proteins are responsible for the regulation of trafficking of proteins and organelles throughout the cell. Therefore, we investigated the effects of a synthetic, disease-based mutant of synuclein and modulated expression of Rab8a and Rab10 in A549 and HEK cells on activities of the mitochondrial electron transport chain. We performed both cellular and mitochondrial characterizations of these cells. We observed by Western blot (WB) that the A549 KO cells show decreased levels of synuclein intracellularly and increased levels extracellularly compared to the wild-type (WT) control. However, we observed no change in the HEK KO cells. Subsequently, we performed mitochondria Complex 1 and 2 assays and found that the Rab8a KO in both the A549 and HEK cells showed decreased levels of Complex 1 activity, but no significant difference in Complex 2 activity. These effects were consistent with the consequences of mutant synuclein expression: selective reductions in Complex-1 activity. These data suggest that multiple PD-linked proteins may play a role in mitochondrial Complex 1 activity, which could provide potential greater insight into the mechanisms underlying mitochondrial dysfunction in PD and novel therapeutic opportunities to halt disease progression.

Patterns of cortical metabolism and their association with longitudinal neurodegeneration in the three clinical variants of Primary Progressive Aphasia

Research Director: Dr. Brad Dickerson

Frontotemporal dementia (FTD) is a neurodegenerative disease that primarily affects the frontal and temporal lobes of older adults. FTD can be further divided into a primary progressive aphasia (PPA) category, which causes language deficits. PPA currently has three known variants that have unique combinations of atrophy and behavioral effects, however these variants have significant overlapping characteristics, which can make diagnoses and further research difficult. In this paper we analyze the metabolic rates in the cortex of PPA patients with each variant to try to describe the differences in metabolic activity between the variants as well as explore the correlation between metabolic rates and longitudinal atrophy in PPA generally. We found that there is a significant association between hypometabolic rates in brain regions and subsequent atrophy in those areas longitudinally, regardless of variant. We also found regional hypometabolic differences between variants that significantly mapped onto known connectivity networks in major language centers of the brain that each variant has been previously thought to affect.

Resting EEG Activity as Predictor of Treatment Response in Depressed Adolescents

Research Director: Dr. Randy Auerbach

Although numerous studies have examined the relationship between frontal alpha asymmetry and depression, the findings have been mixed. Additionally, few studies have examined associations between posterior alpha asymmetry and adolescent depression. Our goal was to examine the differences in frontal and posterior alpha asymmetry between depressed adolescents and healthy controls. Given the heterogeneity of major depressive disorder, we also aimed to identify correlations between higher alpha asymmetry and individual depressive symptoms. Our goal was also to examine any potential normalization of frontal alpha asymmetry in depressed adolescents following 12 weeks of individualized cognitive behavioral therapy. Upon beginning the study, healthy (n = 43) and depressed (n = 34) female adolescents ages 13-18 completed a clinical interview and self-report questionnaires measuring lifetime mental health disorders and symptomatology. In addition, 128 – channel resting EEG was recorded to facilitate analysis of alpha asymmetry. Depressed adolescents then completed 12 weeks of cognitive behavioral therapy. Following this time period, healthy controls and the treatment group completed the same self-report questionnaires completed at baseline. Resting EEG was also recorded post-treatment. In accordance with previous studies, we found that the depressed adolescents had greater frontal alpha asymmetry than the healthy control group, and that higher scores for depressive symptoms were correlated with greater left versus right alpha asymmetry. However, no significant differences emerged between pre- and post- treatment alpha asymmetry in the depressed adolescents. These findings suggest that greater alpha asymmetry is correlated with depression in adolescents, but is not remedied by CBT alone.

Role of Childhood Onset Schizophrenia and Epilepsy Genes in the Brain and Behavior

Research Director: Dr. Alex Schier

Schizophrenia is a chronic neurological disorder afflicting about one percent of the population. It is characterized by degeneration of thinking, motor and emotional processes. While its symptoms such as hallucinations and behavioral regression are widely recognized, its causes are not well understood. Although schizophrenia has been diagnosed in children, early onset schizophrenia has received little attention in research literature. Furthermore, recent epidemiological and genetic studies have revealed a bidirectional link between epilepsy and schizophrenia. Epilepsy, a disorder defined by recurrent seizures, also affects one percent of the population. By examining the genetic overlap between the two diseases, we hoped to better understand phenotypic abnormalities in both disorders.

We used CRISPR/Cas technology to create genetic knockouts of genes associated with either or both diseases in zebrafish. We focused on examining genes implicated in early onset schizophrenia (EOS): pdxdc1, ntan1; ptprg, kcnq3, and in epilepsy: pcdh19. The genes involved in EOS have also been associated with epilepsy. In order to localize the neural circuits involved in generating schizophrenic behavior, we examined the neural activity of these knockout zebrafish through phosphorylated extracellular-regulated kinase (pERK) brain imaging. In addition, we conducted behavioral tests, to examine the difference in their response compared to wild type fish. Our goal is to shed light in the biological pathways and mechanisms involved in these diseases, so that we can identify suitable treatments to target these mechanisms.

Role of Nociceptors in Driving Antibody Class Switching in Allergic Inflammation

Research Director: Dr. Clifford Woolf

During allergic inflammation, B cells secrete antibodies that amplify immune responses. Antibody genes can re-arrange through immunoglobin class switching, allowing B cells to produce diverse antigen responses. The antibody IgE in particular is tightly regulated, as excess antibody production can lead to anaphylaxis and death. Nociceptors, high-threshold sensory neurons that detect noxious stimuli and elicit pain, are known to interact with immune cells to modulate inflammatory response and in particular allergic immunity. However, the interaction between B cells and nociceptors has not been studied. We created two in vivo models of inflammation (calcipotriol, a skin irritant, and house dust mite, an airway allergen) to study this interaction in wild type mice or mice with ablated or silenced nociceptors. Our goal is to determine the role of nociceptors in modulating B cell responses in allergic inflammation. Interestingly, in both of our models of inflammation we found that in the absence of nociceptors, serum IgE levels were significantly reduced. However, naïve B cells taken from nociceptor-ablated mice produced similar levels of IgE in vitro, implying that the decrease in IgE may not be an intrinsic defect in B cells. We then cultured naïve B cells with nociceptors activated with a TRPV1 channel agonist. We found that IgE production could be stimulated by nociceptor activation alone. However, activating nociceptors in the presence of a known IgE class switch stimulus (LPS+IL-4) decreased IgE production. These findings suggest that nociceptors may modulate the production of IgE.

Role of the Anterior Dorsal Striatum, Dorsolateral Striatum, and Posterior Tail of the Striatum in the Execution and Memory of Learned Motor Skills

Research Director: Dr. Nao Uchida

Located within the basal ganglia of the forebrain, the striatum is essential for maintaining general motor function and memory of learned motor skills. Thus, deficiencies in striatal function can be detrimental to both memory and execution of motor skills. Importantly, current research shows that the anterior and posterior striatum have different functions — the anterior striatum is crucial for habitual behavior, whereas the posterior striatum is critical for goal-directed behavior. Importantly, a universal definition for the term "habitual behavior" currently does not exist, and the term has been loosely used to describe the function of the anterior striatum. Thus, our experiment breaks down this vague terminology into measurable, quantitative parameters to more clearly define the roles of the anterior and posterior striatum. Our experiment is a sequential motor task using AAV-KORD-cre mice. We temporally inactivate the anterior and posterior striatal areas via subcutaneous injections of the active ligand salvinorin-B. Then, we examine quantitative differences in behavior by comparing the data collected under salvinorin-B conditions to those of saline controls. As a result, we found that with inactivation of the anterior striatum, mice had less errors in conducting the sequence motor task, suggesting that that the anterior striatum is important for sequence memory. Conversely, we found that inactivation of the posterior striatum resulted in longer reaction times to the task and an increased number in task trials where mice showed no reaction to the motor task, suggesting that posterior striatum is important for smooth motor execution and quick decision-making.

Skilled manipulandum-based task for motor learning in mice

Research Director: Dr. Nao Uchida

Understanding how we learn new motor skills is crucial for developing therapeutic interventions for patients with motor learning and control deficits. A well-established animal model for studying motor learning involves training rodents on a skilled forelimb reaching task: the animal learns to reach for and grab a food reward. However, due to the difficulty of quantifying this behavior computationally, it remains hand-scored for success rate only, which is tedious and inefficient with regard to the data collected. Furthermore, knowledge of the motor learning circuitry and brain regions of interest remains to be fully explored. To address these shortcomings, we developed a novel manipulandum-based task in Mus musculus—the Precision Pull Task—to study the neural landscape of motor learning. In this task, water-deprived mice were trained to reach for, grab, and pull a joystick manipulandum above a speed threshold to receive a water reward. Movements of the joystick were recorded and analyzed as a proxy for forelimb kinematics, providing high precision behavioral data. In Chapter 1, I describe the rapid acquisition of the Precision Pull Task and analysis of mouse learning behavior. In Chapter 2, I discuss the generation of whole-brain activity maps and the identification of task-responsive brain regions. Finally, in Chapter 3, I offer a hypothesis on the functional roles of several promising regions of interest that warrant further experimentation. Ultimately, I aim to provide evidence for a novel mouse model with which to study the motor learning circuitry.

Transcriptomic Analysis of 3D Human Neural Cell Culture Model of Alzheimer's Disease

Research Director: Dr. Doo Yeon Kim

Alzheimer's disease (AD) is the most common form of dementia. The pathologic hallmarks of AD include amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs). Current AD mouse models develop Aβ plaques but do not progress to the later stage including Aβ-driven NFTs and robust neurodegeneration. Our work focuses on understanding pathogenic cascades using the 3D culture model of AD.

Previously, we have identified 790 differentially regulated genes in AD 3D cultures by whole RNA sequencing analyses (n=4 for control and 3 for AD; FDR < 0.05, |logFC|>1.0). To further understand the pathogenic cascade, we took RNA sequencing data from our 3D models and compared it with previously published human AD brain microarray data. Our analysis centered on documenting where genes overlapped in expression in the same direction across samples. Using Ingenuity Pathway Analysis (IPA, Qiagen), we have identified multiple cellular pathways and upstream regulators that are altered both in our 3D AD culture model and human AD brain samples. We are planning to test if chemical/genetic manipulations of these pathways can modulate pathogenic cascades.

Our work also includes continued development of new AD cell lines based on selected mutations, which can specifically alter the ratio of pathogenic Aβ species. We are constructing multiple lentiviral vectors that can deliver these mutations into human neural stem cells. These would provide valuable information regarding AD pathogenic cascades, triggered by different Aβ species.

Our study will assist both in understanding the pathogenic mechanism behind AD and finding therapeutic targets for AD patients.

Viewpoint invariance of macaque inferotemporal cortex: continuity versus spontaneity in object recognition

Research Director: Dr. Marge Livingstone

The ability to see helps to provide connection, dignity, and opportunities to learn. Visual processing largely or exclusively comprises roughly a fourth of the human cerebral cortex and a half of the Macaca mulatta, more commonly known as Rhesus macaque, so it is unsurprising that vision has had the most dedicated literature of the five senses. Previous studies have shown that higher visual cortex representations allow for accurate object recognition from variable orientations. The ability to correctly recognize an object from a different angle promotes continuity in understanding rather than forcing the subject to see a novel image with each slight alteration. While, it is well understood that primates can recognize objects, scenes, and faces from various orientations, it is still unknown how this phenomenon occurs. Here, we identify change over time of cellular response in macaque inferotemporal cortex (IT) when looking at 333 millisecond revolving images of faces, scenes, places, bodies, hands, feet, and food. The diverse subjects of the movies help to mitigate researcher bias in regard to IT function, while also providing variable shapes for analysis. I hypothesize that there are a set of invariant neurons that maintain response throughout a rotation regardless of presented viewpoint which helps to promote object recognition. The results of this project will illuminate whether each neuron in IT continuously decodes an image regardless of orientation or the neuron temporarily locks to a certain angle of the image and must cease firing from one viewpoint before it can respond to a different orientation. The underlying mechanism is still under investigation. Future studies, should compare deep neural networks that build off previous information perhaps through convolutional networks rather than rerun their recognition algorithm from scratch when orientation changes.

ZAP ZAP! An investigation into the capacities and neural loci of visual short term memory and visual manipulation through the use of transcranial direct current stimulation

Research Director: Dr. George Alvarez

In our day to day lives we encounter a constant stream of visual stimuli, which are processed, remembered, manipulated, and responded to. Despite being highly visual animals, humans' visual short term memory (VSTM) is surprisingly limited to only around 3 to 4 items. These limits drop even lower when people are forced to manipulate their stored visual representations. Whether or not these limits represent fundamental capacities of brain function, or merely functioning capacities based on human experience is an open question. Recent technological advances in non-invasive brain stimulation (NIBS), such as trans-cranial electrical stimulation (tES), have made possible more elaborate investigations of neural limitations such as these. We utilized trans-cranial direct current stimulation (tDCS) and trans-cranial random noise stimulation (tRNS) to investigate both the capacities of VSTM and the neural locus of visual memory and manipulation. These stimulation techniques involve the application of a weak, direct current or a weak, randomly fluctuating, alternating current, respectively, to a brain region of interest in order to modulate the underlying neuronal activity. We stimulated the posterior parietal cortex (PPC), an area previously implicated in VSTM, and investigated whether or not the stimulation generated an increase in performance on a test of visual memory and manipulation. Furthermore, we analyzed if visual manipulation increased in the same manner as visual memory, which would indicate a direct connection, and possibly a common neural locus, between the underlying cognitive mechanisms.