Clustering MS Associated Risk Loci and Understanding Their Functional Effects in an Evolutionary Context

Lab: Dr. Philip de Jager

Multiple sclerosis (MS) is a neurodegenerative inflammatory disease with genetic basis. Ongoing genome-wide association studies (GWASs) have identified at least 194 genome-wide (GW) single nucleotide polymorphisms (SNPs). Although a single individual is not expected to be a carrier of all risk alleles, it is more probable that a specific combination of loci predisposes an individual to MS. Our objective is to determine whether MS GW SNPs are organized in particular patterns in a subset of MS subjects, and whether these are enriched for specific biological pathways.

To understand how the MS GW SNPs may group together, we analyzed SNPs from a large cohort (n=821) of MS patients from Brigham and Women's Hospital (BWH). By applying a biclustering algorithm, that allows clustering in 2 dimensions (SNPs and individuals), we identified 3 clusters of SNPs and 3 subsets MS patients, which we replicated in an independent cohort (n=1,815). Then, we used prioritized genes, through cis-eQTL analysis, in immune cells and brain tissue to test whether the 3 clusters are enriched for different pathways. At a false discovery rate (FDR) of 5% only the first cluster (n=56 SNPs) had statistically enriched KEGG pathways (n=4), driven by mostly immune loci, e.g. NFKB1. The dorso-ventral axis formation pathway was the only enriched for both immune and brain prioritized genes.

We observed that GW SNPs could be grouped together in subsets of patients in a reproducible fashion, however the biological implications of these clusters cannot be elucidated using the current knowledge of pathways.

Computer Vision System for Mouse Sniff Detection

Lab: Dr. Venki Murthy

In order to analyze eletrophysiological recordings made during olfaction tasks accurately, it is necessary to identify when the animal from which the recordings were made sniffs. Otherwise, it is impossible to associate neuronal responses with perceptual events. Unfortunately, the current state-of-the-art in sniff detection involves a moderately risky surgery and daily maintenance, which is costly and inconvenient for the researcher. This project therefore seeks to utilize cutting-edge techniques in computer vision and artificial intelligence to design a system that is capable of identifying when a mouse sniffs from video data. As a system that might actually prove usable in diverse laboratory settings must have a high degree of context independence—that is, it must work regardless of parameters such as camera angle, lighting conditions, and mouse strain—this system is primarily based around AlexNet, a previously designed convolutional neural network that achieves a high degree of tolerance. Additionally, the most successful system tested makes use of phase-based video magnification, a video processing technique that allows motions of a particular frequency, such as sniffs, to be selectively magnified. Although more development is needed in order to produce a system that can completely displace surgical methods for sniff identification, the results obtained thus far indicate that a system that combines phase-based video magnification and convolutional neural networks has high potential for non-invasive, successful sniff detection.

Cortical Development and Preferential Looking in Face-Deprived Juvenile Macaques

Lab: Dr. Marge Livingstone

While the existence of face-selective regions in the inferotemporal cortex is well established, less is known about the factors driving its development. Specifically, the role of experience is unclear. In this study, we use macaques to study the development of the face recognition system, as it pertains to the organization of the inferotemporal cortex, and behavior as measured through viewing preferences. We deprived two juvenile macaques of exposure to faces from birth and explored the effects of this grossly abnormal developmental environment through weekly fMRI and behavioral testing, using a series of images consisting of natural scenes and side-by-side comparisons. We compared the results of the face-deprived juveniles with two control juveniles shown the same images. As expected, controls exhibited a preference for faces over other objects and image regions, beginning early in development. In contrast, the face-deprived juveniles did not exhibit the same preference for faces, instead, we observed a preference for hands. At one year of age, we exposed the deprived monkeys to faces. At this point they developed a preference for faces, roughly equivalent to the controls, despite little prior evidence of this preference. The results suggest that preferential viewing of faces depends on exposure to faces, but the critical period during which this preference first emerges, can be extended by delaying the first exposure. It is unclear whether this delayed-emergence of face selectivity could be suppressed by extending the deprivation period; this is an area for further investigation. Additionally, future studies should probe the interesting and unexpected result of hand-selective viewing in the deprived subjects.

Determining the Presence of a Residual Motor Memory in Songbird Vocal Learning

Lab: Dr. Bence Ölveczky

Elucidating the mechanism of human vocal learning and control remains a challenge, as it involves a complex interplay between audition and motor functions. The zebra finch, a songbird that learns a complex stereotyped song, offers a tractable model that shares broad neurophysiological similarities with humans. Known to rely on auditory feedback during the song learning process, these birds continue to utilize auditory input to maintain their learned song in adulthood. Given the fine motor control and repeated practice necessary to produce a consistent song, it is hypothesized that a consolidated motor memory may also play a role in such song maintenance. To test this, adult zebra finches were trained using conditional auditory feedback (CAF) to reliably increase the duration of a specific song syllable. After the termination of CAF and spontaneous recovery to their baseline-length songs, these birds underwent CAF training again. After this CAF training, the birds were immediately deafened and observed to determine the rate and magnitude of spontaneous recovery to baseline levels. Even in the absence of auditory feedback, the duration of the succeeding bird songs consistently displayed a downward progression towards the baseline duration levels. These results suggest the presence of an independent motor memory strong enough to correct the changes brought on by the CAF training.

Discovering an action perception and comprehension impairment linked to cerebral palsy

Lab: Dr. Alfonso Caramazza

This research study aims to investigate if and how the motor abilities differentially impacted by cerebral palsy affects body movement and action perception. This will help shed light on how an individuals' own motor abilities, limitations, and motor system contributes to his movement perception. We aim to study a small group of adults, ages 18-64, living with cerebral palsy. In this experiment, the participants are asked to complete eight different computerized tasks in order to assess both their action and movement perception as well as their physical limitations of simple movements. In these tasks, participants will be asked to make conceptual answers based on the perceived movements and actions of the videos and pictures on screen. The participants will also be interviewed in order to establish the limitations that cerebral palsy has placed on their limbs. Our preliminary results show that people living with cerebral palsy are impaired at action perception tasks in comparison to control subjects. Thus, according to these results, this suggests that cerebral palsy negatively impacts one's ability to perceive movement and action. This further suggests that one's motor capabilities is influential in his ability to perceive and understand human body movements.

Dynamic regulation of the cholesterol biosynthetic pathway in glioblastoma multiforme

Lab: Dr. Mark Johnson

Despite research surrounding lipogenesis in various cancers, conclusive evidence has yet to be found concerning the explicit role of a lipogenic phenotype. Tumorigenesis has been linked to activation of sterol regulatory element binding factor 1 (SREBP1), a fatty acid biosynthesis-promoting gene; however, cholesterol toxicity to tumor development suggests harmful consequences of sterol regulatory element binding factor 2 activation (SREBP2), which aids cholesterol biosynthesis. Given the contradictory nature of lipid metabolism in cancer, our research sought to examine the regulatory pathway of cholesterol production in the context of glioblastoma multiforme (GBM).

Upon EGF stimulation, LN229, U251 and U343 GBM cells revealed down- regulation of SREBP2 expression, while western blot analysis demonstrated an increase in overall production of SREBP2 after mTOR inhibition. Immunocytochemistry with mTOR inhibitors further revealed a shift in SREBP2 expression from cytoplasmic to nuclear, supporting EGF/mTOR-dependent SREBP2 cleavage during cholesterol synthesis. Additionally, mTORC1 inhibition up regulated site 1 protease (S1P) expression to expose the mechanism of SREBP2 cleavage regulation in GBM. Nonetheless, growth assays revealed a positive correlation between cell growth and SREBP2 activation, suggesting a vital role of cholesterol biosynthesis for GBM cellular activity.

Given our results, which introduce SREBP2 regulation via the EGF/mTORC1 pathway in GBM despite the importance of SREBP2 for cell growth, we propose the existence of an optimal level of cholesterol production to integrate the competing needs of the cancer cell. Due to this limited range of growth-supporting cholesterol levels, our results demonstrate a novel mechanism for therapeutic efforts that target metabolic networks in GBM.

Effect of developmental activation of serotonergic neurons on adult aggression in Drosophila melanogaster

Lab: Dr. Ed Kravitz

The monoamine serotonin (5HT) is a major neurotransmitter in the central nervous system (CNS) and has been implicated in modulating aggression across species. In Drosophila, acute inactivation of the serotonergic system reduces aggression, while selective activation increases aggression. Moreover, similar effects result from inactivating/activating a symmetrical pair of 5HT neurons located in the posterior lateral protocerebrum (PLP). Despite the importance of these 5HT-PLP neurons in aggression regulation, little is known of their morphogenesis and how they are shaped to affect adult aggression.

Using intersectional genetics, we targeted and manipulated either the entire population of 5HT neurons or the small PLP subset. We showed that activation of both groups of 5HT neurons for the entirety of development heightened aggression in adulthood. Though a similar trend was observed with one of the parental controls, additional analysis identified notable differences in the fight dynamics between the experimental and control flies. Next, we limited the activation window to the final 3 days of development, during which 5HT neurons undergo dramatic reorganization. 3-day activation of the entire set of 5HT neurons yielded heightened adult aggression, but had no effect when restricted to the 5HT-PLP subset. This suggests that other neurons within the serotonergic population might be responsible for the late-development impact on adulthood aggression.

This research will help understand the connection between known phases of 5HT neuron morphogenesis and the long-term consequences of the increased neuronal activity during development. Ultimately, this knowledge will help elucidate the organization of aggression-modulating pathways in the Drosophila CNS.

Functionality Connectivity of Reward Circuitry in Patients with Bipolar Disorder and Cannabis Use Disorder

Lab: Dr. Ann Shinn

Many patients diagnosed with bipolar disorder are also diagnosed with substance use disorder. The goal of this study was to use resting state functional magnetic resonance imaging (fMRI) to study regions of the brain associated with reward circuitry in patients with bipolar disorder and alcohol use disorder. Subjects were identified with bipolar disorder and alcohol use disorder using the Structured Clinical Interview for DSM-IV (SCID). The functional connectivity of bilateral ventral striatum, more specifically the nucleus accumbens, and the anterior cingulate cortex were investigated through resting state fMRI. Data analysis was conducted using FMRIB Software Library (FSL). The data were then compared with healthy controls and subjects with bipolar disorder without alcohol use disorder to find whether differences in reward circuitry may be associated with the co-morbidity of bipolar disorder and alcohol use disorder.

Identification of the releasing stimuli of pup-directed behaviors: biochemical and behavior studies

Lab: Dr. Catherine Dulac

The vomeronasal organ (VNO) plays a key role in mediating the behavioral responses of vertebrates to chemosignals that convey information about sex and species, yet although more than 250 pheromone receptors have been identified in the mouse VNO, the specific signals detected by individual receptors have yet to be fully determined. In particular, it has been discovered that VNO receptors Vmn2r65 and Vmn2r88 are required for pup-directed attack, but the ligands to these receptors, without which it would be difficult to gain a more comprehensive understanding of the specific contexts in which signals for pup-directed aggression are generated and detected, have not been identified. This project consequently investigated the identity of the ligands for Vmn2r65 and Vmn2r88 in an effort to understand their roles in pup recognition. To determine the molecular identity of these ligands, we performed chromatographic fractionation and purification of male B6 salivary glands, followed by exposure of these fractions to CD-1 male mice and processing of the VNO's of these mice for two color RNA in situ hybridization probing Egr1 and Vmn2r88 or Vmn2r88. Taken together, we have successfully established purification schemes for potential Vmn2r65 and Vmn2r88 ligands and have obtained a candidate list of potential ligands by mass spectrometry. This list of proteins highly enriched in these active fractions will serve as a starting point for creating and testing recombinant ligands for neural and behavioral activities. In the second part of the project, I sought to develop a behavioral assay in which the activities of the identified ligands in pup-directed behaviors can be tested. In particular, I have focused on examining whether inanimate objects, such as rubber dummy pups, can reconstitute pup-directed behaviors. Taken together, the identification of molecular cues for pup recognition will not only illuminate the molecular logic behind which pups are specifically recognized, but also shed light on the biophysical nature of these signals — thus allowing us to elucidate how these pheromonal cues regulate pup-directed behaviors.

Identifying downstream effects of truncated DISC1 in human neuronal cells using iPSCs

Lab: Dr. Tracy Young-Pearse

Major mental illnesses such as schizophrenia, bipolar disorder, and depression impose a significantly lower quality of life on patients and a large economic burden on society. Studies that show Disrupted in Schizophrenia 1 (DISC1) mutation increases the risk of major mental illness support the hypothesis that the underlying cause of mental disease is neurodevelopmental, however the molecular mechanism is still unknown. Genetic mutations can be introduced at a specific locus using transcription activator-like effector nucleases (TALENs) or a CRISPR/Cas (clustered regularly interspaced short palindromic repeats) system. Furthermore, using human induced pluripotent stem cell (iPSC)-derived neurons to study mutations introduced through targeted genome editing allowed us to study the role of a mutation in the context of a human genome, which is particularly important for diseases whose manifestation—and possibly mechanism— differs among animal models. We generated isogenic cell lines of iPSC-derived neurons with frameshift mutations in several loci of the DISC1 coding sequence and studied the effects of these mutations on other genes by RNA sequencing. We selected several genes from the dysregulated genes identified through RNA sequencing that were implicated in cell adhesion, neuronal migration, and neuronal maturation in order to further study their dysregulation from DISC1 mutation by qPCR, protein analysis, and immunostaining

Investigating Potential Targets of the Let-7 Complex: Dally-like and Folded Gastrulation

Lab: Dr. Sam Kunes

MicroRNAs (miRNAs) are a small set of noncoding nucleotides that can alter gene expression by impeding mRNA translation. The genes that code for miRNA-100, let-7 miRNA, and miRNA-125 are all closely located within the same 1-kb region of the Drosophila genome, denoted the let-7 miRNA complex. This let-7 miRNA complex is a key developmental and protein regulator in Drosophila. The numerous targets of the let-7 miRNA complex remain largely unknown and under investigation. Using the analytical scores of four miRNA databases and previous experimental research, I identified ten potential let-7 miRNA complex targets: Protein tyrosine phosphatase 52F, Futsch, Abrupt, Dally-like (dly), Rho GTPase activating protein, Folded gastrulation (fog), Mesoderm-expressed 2, Labial, and Retinal degeneration C. After assessing the quality of antibody staining for the ten potential targets, I focused on the targets dly and fog. In the Drosophila brain, dly is expressed in the mushroom body and fog seems to be expressed in the Pars intercerebralis neurons. Immunostaining and Drosophila short-term memory training suggested an increase in the overlap of let-7 expression and dly expression in the calyx, an important brain structure for memory formation. Immunostaining, genetic manipulation by RNA interference of the fog gene to decrease its expression, and the utilization of let-7 sponges to decrease let-7 expression will elucidate the relationship between let-7 and fog in the PI neurons. Further experimentation is recommended to clarify the relationship among these genes and the let-7 miRNA complex.

Investigating the Neural Correlates of Delay Discounting Across Mood Disorders:A High-Density Event Related Potential Study

Lab: Dr. Diego Pizzagalli

Background: Dysfunction in brain reward pathways may underpin symptoms in bipolar disorder (BD), such as increased impulsivity. Emerging evidence indicates that comparing neural correlates of these symptoms in individuals with major depression (MDD) and BD may yield biomarkers that differentiate BD and MDD. This study examined whether neural responses during a delay discounting task (a putative marker of impulsivity) differentiate individuals with BD vs. MDD. We hypothesized that, relative to individuals with MDD or healthy controls, individuals with BD would show greater reductions in neural responses to delayed relative to immediate rewards.

Methods: Participants with BD (n=8), subthreshold depressive symptoms (n=16), MDD (n=21) and healthy controls (n=23) completed a novel delay discounting task while 128-channel event-related potentials (ERPs) were recorded and time-locked to receipt of reward feedback. Analyses focused on the N1, an early component associated with the allocation of attentional resources, and the feedback-related positivity (FRP), which is involved in early outcome evaluation and thought to arise from phasic reward-related signaling that originates in the striatum and projects to the ACC. N1 amplitude has been shown to be larger for more salient and pleasant stimuli, and FRP amplitude is more positive for more rewarding outcomes.

Results: We found that, as expected, N1 amplitude was larger and FRP amplitude was more positive following receipt of immediate versus delayed rewards across the sample (N1: $F_{1,65}=7.317$, $p=.009$, $\eta p2=.101$; FRP: $F_{1,65}=14.110$, $p<.001$, $\eta p2=.178$). Contrary to our hypotheses, there were no differences in N1 or FRP amplitude between the four groups ($ps>.05$). However, across the sample, a greater difference in N1 amplitude following receipt of an immediate versus a delayed reward was correlated with higher scores on a self-report measure of trait impulsivity ($r=-0.25$, $p<.05$; Barratt Impulsivity Scale). This relationship remained significant even after controlling for depressive symptom severity (partial correlation $r=-.27$, $p=.03$). Additionally, increasing self-report scores of general distress were significantly correlated with an increasing difference between FRP amplitude to immediate rewards versus delayed rewards when controlling for impulsivity (partial correlation $r=.45$, $p=.01$; Mood and Anxiety Symptom Questionnaire GDD subscale).
Conclusion: Although no group differences emerged in ERP responses, different ERP components in response to delayed relative to immediate rewards were correlated with different symptomatology. In particular, the N1 was associated with impulsivity, a core symptom of BD, irrespective of depressive symptom severity, while the FRP was associated with depressive symptoms irrespective of impulsivity. Together, these findings suggest that examining early attentional neural processing may provide insights into core symptoms of BD, while later reward-related processing may be more related to MDD.

Language Outcomes in Toddlerhood for Infants at High-Risk for Autism Spectrum Disorder and Language Disorder: The Association Between Brain and Behavior

Lab: Dr. Chuck Nelson

Autism spectrum disorder (ASD) and Specific Language Impairment (SLI) are developmental disorders affecting social and verbal communication. Currently, the neuronal-behavioral risk profiles for ASD and SLI have not been clearly delineated. Thus, we conducted a prospective, longitudinal study of infants from 12 through 36 months, with infants falling into three categories: High Risk Autism (HRA: toddlers who had an older sibling with ASD) (n=135), High Risk Language (HRL: toddlers who had an older sibling with SLI) (n=19), and Low Risk Control (LRC: toddlers who had an older sibling considered typically developing) (n=120). Language skills (VIQ) were assessed using the Mullen Scales of Early Learning (MSEL), and neuronal activity was assessed across all frequency bands using baseline electroencephalography (EEG). Additionally, infants were diagnostically assessed for ASD at 36 months.

Kruskal-Wallis One-Way ANOVA revealed that from 12 through 18 months, HRA and HRL infants had significantly lower VIQ scores compared to LRC infants ($p < .05$). However, by 36 months only HRA infants who developed autism differed significantly from LRC infants in VIQ scores ($p < .05$). Surprisingly, there were no significant group differences in EEG power across any frequency band at any age ($p > .05$). Yet, for HRL infants, higher EEG power in the low alpha and theta bands was associated with improved language outcomes, and for HRA infants, higher gamma band EEG power was associated with poorer language outcomes. These findings suggest familial risk for ASD or SLI leads to different trajectories of early language development and neuronal-behavioral associations.

Locomotor plasticity in Drosophila melanogaster: A role for proprioception in acute response to injury

Lab: Dr. Ben de Bivort

Locomotion requires several sensory modalities, among them proprioception, which allows an organism to sense the position of its body parts in three-dimensional space as well as the amount of force being applied by the body to the environment. Mechanosensory neurons that innervate muscles, skin, exoskeleton and other tissues send proprioceptive information to the brain by transducing mechanical energy into an electrical neural signal. We investigated the role of proprioception in mediating locomotor plasticity after injury in Drosophila melanogaster. Specifically, we used a circling bias behavioral assay to demonstrate the proprioception-dependent recovery in exploratory locomotion turning bias after amputation of a limb. Flies were placed in circular arenas and allowed to explore freely while video recording tracked the centroid of the fly during locomotion. We then quantified the turning bias and characterized both the initial response to amputation and subsequent recovery over a four day period. The robustness and cell-type specificity of the wild-type and proprioceptive mutant phenotypes was tested using the GAL4-UAS system. Interestingly, we observed a larger induced circling bias immediately after amputation in wild-type flies compared to proprioceptive mutants. While the initial response to amputation was robust, the subsequent recovery phenotypes were less predictable. In order to investigate locomotor plasticity on a finer scale, we used high-speed video analysis to demonstrate the absence of gait plasticity following removal of a limb. We developed a neuromechanical model to show that force tuning is the likely mechanism for the observed recovery in the absence of gait recovery.

Lynx1 Expression Mediates Attentional State

Lab: Dr. Takao Hensch

Attention is a neurobiological process fundamental for higher cognitive function, and deficiencies in attentional function are implicated in disorders such as schizophrenia, Alzheimer's disease, and attention-deficit hyperactivity disorder. Previous studies have demonstrated the importance of acetylcholine acting within the prefrontal cortex as an attentional regulator and have also explored the nicotinic acetylcholine receptor [nAChR] subtypes involved in mediating attention. However, the research on the receptor subtypes involved in attention has been conflicting, and no research prior to this study had explored the attention-regulating effects of proteins that interact with nAChRs. This study investigates the role of Lynx1, an endogenous prototoxin that desensitizes nAChRs, in regulating attention and also explores the subtypes of nAChRs involved in Lynx1's moderation of attention. Attentional performance in wild-type and Lynx1 knockout mice was measured using a 2-choice visual attention task. Mice completed the task firstly under drug-free conditions and then after intraperitoneal injections of both non-specific and specific nAchR antagonists. Performance measures included percentages of correct and error responses, as well as correct response latency. Lynx1 knockout mice showed higher percentages of correct responses and lower percentages of error responses as compared to wild-type, and this difference in performance was restored when mice received injections of non-specific and α7 nAChR antagonists. These results indicate that Lynx1 is involved in mediation of attention, acting specifically to reduce over-attention, and provide evidence for the involvement of α7 nAChRs in Lynx1's control over attention.

Maternal Anxiety and Infant Face-Processing: an event-related potential study

Lab: Dr. Chuck Nelson

Some of our first introductions to social relationships come through exposure to faces and facial emotions in infancy. The face-processing capabilities that develop during the first year underlie vital, later-emerging socio-cognitive skills. This development is shaped by numerous factors; in particular, maternal characteristics have been shown to significantly impact the infant's social environment and influence emotion-processing development. One maternal characteristic, maternal anxiety, has already been linked to various social and emotional disorders in childhood, but its relationship to emotion-processing in infancy has yet to be explored. The present study seeks to investigate this relationship between maternal anxiety and infant neural response to facial emotions. Event-related potential (ERP) recordings were collected from five-, seven-, and twelve-month-old infants (n= 116) as they viewed happy, angry, and fearful face images in a passive viewing paradigm. Concurrently, mothers of participants self-reported anxiety using the Spielberger State Trait Anxiety Inventory- Trait Form. The study finds a statistically significant relationship between the face-sensitive N290 ERP component amplitude in 7-month-old infants (n= 39) and maternal anxiety, indicating that higher levels of maternal anxiety are associated with a reduction in infant ERP component amplitude ($p=0.004$, $R=0.456$). These results suggest that maternal anxiety may impact the critical stabilization and pruning of face-processing neural circuitry thought to occur between five and seven months of age. Mechanisms for this effect could be experience-dependent, as anxiety in the mother may impact the emotional range and tone of faces the infant is exposed to, or biological in nature, as maternal stress hormones have already been shown to impact the proliferation and migration of neurons in utero.

MicroRNA-34 and its Targets Mediate Disease Phenotypes of the Synapse During Development

Lab: Dr. David Van Vactor

Many major neurological and psychiatric disorders, including Autism, Schizophrenia, and Alzheimer's Disease, have been tied to misregulation during synaptic development and maintenance. Considerable evidence has shown that numerous microRNAs play significant regulatory roles during development at the synapse. Here, we misexpressed of a subset of microRNAs in Drosophila and examined the effect on synaptic morphology during the wandering third instar larval stage, identifying those miRNAs which significantly alter bouton number at the neuromuscular junction. Interestingly, when underexpressed in motor neurons, miR-34 disrupts both postsynaptic and presynaptic phenotype, causing an increase in bouton number and a decrease in area of the sub synaptic reticulum. Furthermore, when miR-34 is depleted in muscle, the opposite effect is observed; bouton number is significantly decreased. We went on to characterize the mechanism through which miR-34 regulates synaptic morphology, using qPCR and immunohistochemistry to identify genes differentially expressed in motor neurons between miR-34 nulls and wildtype flies. NrxIV and Dok were overexpressed in the motor neurons of null animals, suggesting that these genes are targeted by miR-34. We then overexpressed Nrx-IV during development, observing an overgrowth phenotype similar to that of miR-34 depleted flies. Additionally, we knocked out Dok in miR-34 null animals, and observed a partial rescue of normal synaptic phenotype. These results suggest that misregulation of both Dok and Nrx-IV underlies the disruption of synaptic development that we see in miR-34 null animals.

Molecular correlates of tumor size and MRI contrast enhancement in a cohort of lower-grade glioma patients

Lab: Dr. Daniel Cahill

Lower-grade gliomas (LGGs) are classified by recurrent mutations in the IDH1/2 and TP53/ATRX genes, the TERT promoter, and chromosome 1p/19q loss. These alterations cluster tumors into three molecular groups with distinct prognoses: the "molecular astrocytic" (group A) defined by co-presence of IDH1/2 and TP53-and/or-ATRX mutation, "molecular oligodendroglial" (group O) defined by presence of IDH1/2 mutation and absence of TP53/ATRX mutations, and "molecular glioblastoma-like"(group G) defined by absence of IDH1/2 mutation. Here, we examined the traditional clinical heuristic of tumor size, assessed by T2/FLAIR and T1 post-gadolinium MRI contrast enhancement (both measured by the product of two orthogonal dimensions on the axial MRI image with the greatest tumor area), within the three molecular groups from cases in the TGCA LGG cohort with available image data in TCIA (n=172). Upon examination of preoperative MRIs, median tumor measurement by T2/FLAIR was 31.96 cm2 in group A, 19.14 cm2 in group O, and 17.45 cm in group G. Notwithstanding this larger size, only 19/85 group A tumors had enhancing area > 10% of the overall tumor size, while 11/47 group O and 24/40 group G gliomas showed similar extent-of-enhancement. Thus, group G gliomas were more frequently enhancing (p=0.0001 and p=0.0009 for the comparisons between group A and O respectively, Fisher's exact test). The prognostic impact of contrast enhancement may therefore reflect, in part, this differing distribution between molecular groups. Indeed, WHO grade was also partially linked to enhancement, with 10 of 83 grade II tumors displaying enhancing areas >10% of the overall tumor size, while 44 of 89 grade III tumors were similarly enhancing. Given the inability to identify molecular categories of low-risk tumors from clinical and radiographic factors alone, we conclude that surgical resection or biopsy is indicated in all suspected LGGs, to facilitate molecular group assignment and guide further therapy.

Neuronal Basis of Burrowing Behavior in Peromyscus
Lab: Dr. Hopi Hoekstra

The evolution of complex behaviors is poorly understood, and examining the neuroanatomical substrate of adaptive behaviors may enable researcher to explain how genetic diversity accounts for behavioral diversity in mammalian brains. With the overarching goal of contributing to our understanding of the genetic basis of behavior, the principal objective of this thesis is to determine what areas of the brain of Peromyscus, a genus of wild mice, are particularly active during burrowing behavior.

To determine which areas of the brain are most active during burrowing behavior, I used expression of the immediate-early gene c-fos as proxy for neural activity. I designed a novel behavioral assay to elicit consistent burrowing from two sister species with distinct burrowing behaviors: Peromyscus polionotus and Peromyscus maniculatus. This acute burrowing assay permitted me to look for burrowing-driven c-fos expression throughout the brain using fluorescent in situ hybridization.

So far, we have analyzed data from a pilot study in Peromyscus polionotus. We found that the somatosensory cortex, striatum, and nucleus accumbens showed greater c-fos expression in burrowing animals than in non-burrowing controls. We plan to incorporate data from larger numbers of Peromyscus polionotus and Peromyscus maniculatus in the final iteration of this thesis. This work supports the Hoekstra lab's overarching goal of understanding how genetic diversity in Peromyscus accounts for behavioral differences—areas active during burrowing can be targeted for measuring candidate gene expression and manipulation of gene expression using viral vectors.

Organization of Second-Order Afferent Neurons in the Larval Zebrafish Posterior Lateral Line Circuit

Lab: Dr. Florian Engert

The mechanosensory lateral line system in fishes and amphibians allows for these organisms to detect changes in water displacement and current, and is necessary for behaviors such as navigation and hunting. The posterior lateral line (PLL) forms the posterior component of the lateral line system, and is comprised of the peripheral sensory organs along the tail, called neuromasts, and the posterior lateral line ganglion (PLLg) that contains the sensory neurons that innervates them. Previous studies have traditionally used lipophilic carbocyanine dyes to label these primary afferent neurons. However, the technological limitations of such methods have prevented characterization of higher-order neuronal connections. The present study aimed to use a novel transsynaptic viral tracing technique in vivo to characterize the afferent circuitry of the posterior lateral line in full. We infected PLL neuromasts with vesicular stomatitis virus, and imaged fish with evidence of neuronal labeling in the brain. We identified second-order afferent projections located within the medial octavolateralis nucleus (MON). These neurons appear to be divided into a “double fan” of two discrete cell populations: a fan of smaller, ventral cell bodies, and a fan of larger, dorsal cell bodies. We are currently working to determine the organization of the MON cell populations by infecting different neuromasts along the tail. We seek to characterize their identity, through the usage of transgenics and antibody staining. These results lay the groundwork for understanding how the lateral line translates sensory input, water flow, into behavioral output at the single cell level.

Role of CACNA1C gene variant in abnormal behavioral phenotypes and potential serotonergic neuropathology

Lab: Dr. Kathryn Commons

Bipolar disorder and major depressive disorder are severe psychiatric disorders that affect approximately 1% to 3% and 15% of the world's population respectively (Dao et al., 2010), yet little remains understood about the specific causal, or functional, differences in the circuitry of these disorders. Animal models that assess therapeutic effects amidst the influence of pharmacological, genetic, and environmental manipulations provide a desperately needed route towards greater understanding and more targeted therapeutics (Soares, 2003). Because there is a well-established, consistent heritable risk with many neuropsychiatric disorders, one particularly promising area of manipulations originates in genetics. Large-scale, genome-wide association studies have revealed strong associations between bipolar disorder, schizophrenia, and major depression susceptibility and a haplotype of an intronic region of the L-type voltage gated calcium channel (VGCC) subunit gene CACNA1C (Yoshimizu et al., 2015). Mutations of the CACNA1C gene can have significant effects because the Cav1.2 channel accounts for 85% of LTCCs expressed in the mammalian brain (Bhat et al., 2012). Research thus far leans towards a gain of function model as the pathology of the CACNA1C risk genotype, in that the risk variant possibly increases Cav1.2 channel expression and hence calcium signaling, which then leads to disrupted behavior. This study seeks to elucidate the pathology of the CACNA1C risk genotype by 1) assessing the depressive behaviors and antidepressant responses of a mouse model of the CACNA1C risk genotype 2) analyzing how these results correlate with imaging results of the serotonergic system, a neurotransmitter network in a region of the brain called the dorsal raphe commonly implicated in neuropsychiatric disorders.

Role of the Pedunculopontine Tegmentum in Signaling Reward Prediction Error to Dopamine Neurons of the Ventral Tegmental Area

Lab: Dr. Nao Uchida

The ventral tegmental area, located in the midbrain, has been identified as a region that encodes signals essential to the learning process. Dopamine neurons in the region encode reward-prediction error, or the difference between expected reward and actual reward. This error signal can be used to alter future expectations. Though the dopamine neurons are well studied, their input signals have not been as well-documented. Studying inputs is essential, as it reveals how learning signals are constructed in dopamine neurons in the first place. The pedunculopontine tegmentum (PPTg) stands out as an input region due to its responsiveness to sensory cues and value-dependent signaling. In this experiment, we label and record from PPTg neurons that directly synapse on to dopamine neurons in mice. We accomplish this by tracing monosynaptic inputs to dopamine neurons with a modified rabies virus and marking input neurons with channelrhodpsin-2, a light-sensitive cation channel. We also record from cell-type specific neurons (GABAergic and glutamatergic) in the PPTg to serve as a point of comparison to the input neurons. We find that input neurons tend to produce phasic signals (short excitatory bursts) in response to reward predicting cues and rewards. We also find that a large portion of glutamatergic neurons in the PPTg exhibit phasic signaling whereas other cell-types exhibit more sustained signaling, suggesting that those input neurons in the PPTg showing phasic responses are likely to be glutamatergic.

Role of Twist-1 in cerebrovenous angiogenesis and neurodevelopment

Lab: Dr. Elizabeth Engle

Twist -1 is a basic helix-loop-helix transcription factor essential for mesoderm specification and subdivision into different tissue types. As early as E11.5, mice lacking Twist-1 in cells in the developing mesoderm and a subset of the neural crest exhibit defects in the development of the cerebrovenous system. Though the growth and remodeling of the major head veins is perturbed in these mutants, arterial sprouting is normal, suggesting that Twist-1 works in a pathway that regulates venous angiogenesis independently of arterial angiogenesis. This mutant could be a particularly important tool for the study of cerebrovenous development, a field that is drastically understudied despite high clinical relevance. Here, I develop and validate quantitative methods for analyzing the cerebrovenous system, characterizing it by its density, branch points, and lacunarity Qualitative and quantitative analysis and comparison of mutant embryos to controls reveal the specific events in the development of the head veins that are genetically controlled by Twist-1. These findings concerning the cerebrovenous system and others concerning the development of the skull have led to my investigation of previously uncharacterized brain phenotypes caused by the deletion of Twist-1, which include hyperproliferation and hyperplasia of the capillaries, abnormalities glial patterning and choroid plexus morphology, and corpus callosum agenesis. The findings of my thesis represent the first attempts to characterize changes to brain morphology due to non-cell autonomous loss of Twist-1 in the surrounding neural-crest derived meninges, suggesting that Twist-1 is necessary not only for cerebrovenous angiogenesis, but also for the development of the brain.

Targeting inhibitory interneuron maturation in a mouse model of Rett Syndrome

Lab: Dr. Michaela Fagiolini

Rett Syndrome is a neurodevelopmental disorder that commonly causes serious intellectual disability among girls. The disorder, for which there is no known cure, is characterized by a seemingly normal period of development in young patients, followed by rapid regression of social, motor, and cognitive skills by two years of age.

Rett Syndrome is caused by mutation in the X-linked methyl-CpG-binding protein 2 gene. Identification of the gene whose mutation causes Rett Syndrome allowed for creation of a Mecp2-knockout mouse model that closely recapitulates symptoms of the human disease. Research in mouse models suggests that absence of Mecp2 from the central nervous system alone causes many of the most severe symptoms of Rett Syndrome. Specifically in the mouse visual cortex, evidence has shown that lack of Mecp2 disrupts development of inhibitory interneuron systems, leading to compromised balance of inhibitory and excitatory cortical neuronal function.

In this thesis, I characterized the development of inhibitory/excitatory imbalance Mecp2-knockout mouse visual cortex using quantitative real-time polymerase chain reaction. When these assays confirmed that inhibitory interneurons are disrupted in Mecp2-knockout visual cortex, I tested two approaches to restoring interneuron development in these animals. First, I tested if reduction of the transcription factor Orthodenticle homeobox 2, a known trigger of parvalbumin interneuron maturation, via viral and genetic knockdown in Mecp2-knockout mice could restore inhibitory interneuron development and reduce severity of Rett-like symptoms. Second, I tested if a genetic disruption of the circadian transcription factor Circadian locomotor output cycles kaput, also known to delay parvalbumin interneuron maturation, could achieve a similar outcome. Using biochemical and behavioral assays, I determined that some interventions that normalize parvalbumin interneuron development in Mecp2-knockout mice produce promising alleviation of their Rett-like symptoms.

Thalamic Reticular Nucleus and Sleep
Lab: Dr. Bob Stickgold

Cognitive symptoms of schizophrenia greatly contribute to a lower quality of life, although they are not as obvious as the unusual behaviors or 'positive' symptoms. Sleep disturbances, including decreased sleep spindle density (spindles per minutes), are common with schizophrenia. Sleep spindles, 12-14Hz oscillations seen on EEG recordings during sleep, are correlated with measures of intelligence and schizotypy like the WASI IQ and Chapman Scales. People with schizophrenia also have a higher sensory gating ratio, indicating a diminished ability to filter out irrelevant stimuli. Finally, they have difficulty on divided attention tasks, which require simultaneously paying attention to two competing streams of information. We believe that these impaired abilities are related to the thalamic reticular nucleus (TRn), a thin sheet of neurons covering the thalamus. Previous research has shown that the TRn is responsible for generating sleep spindles, implicated in sensory gating and divided attention, and genetically linked to schizophrenia. This evidence has inspired our interest in the relationship between the TRn and schizophrenia.

We recorded high-density EEG data from healthy controls during two naps and one overnight sleep session on three separate visits. Subjects also completed the sensory gating task, divided attention task, WASI IQ test, and Chapman Scales. We are in the process of analyzing this data and will be looking for any correlations. We hope these subjects will serve as controls for the larger study, in which these tasks will be administered to people with schizophrenia.

Utilizing an In-Vitro Based Cell Model to Characterize the Pathogenic Mechanisms of C9ORF72-induced ALS-FTD

Lab: Dr. Kevin Eggan

An expanded mutation in the C9ORF72 gene is linked to pathological symptoms found in the most common form of familial amyolateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the mechanism by which the mutation leads to disease is relatively unknown, and current research in the field aims to characterize the mutation as a loss of function, a gain of function, or both. As such, we created an in-vitro stem cell model to study the downstream effects of a C9ORF72 null mutation. Neonatal cortical neural cells were dissected from transgenic mice that carried SOD1 mutant (positive control), wildtype C9ORF72 (+/+), heterozygous C9ORF72 (+/-) and knockout C9ORF72 alleles. These cells were cultured and sorted by flow cytometry to quantify expression for various genes implicated in ALS. Subsequently, the cortical cultures were characterized with staining assays used to observe morphology across neuronal and glial subtypes. We found that C9 knockout samples had abnormal gene expression patterns associated with ALS disease, thus suggesting that C9ORF72 haplosufficiency could contribute to the onset of ALS-FTD.

Volitional (In)significance of Neuroscience: What Libetian Investigations Can and Cannot Do for Free Will

Lab: Dr. Gabriel Kreiman

Volition and self-initiated behavior is a critical component of cognitive control, and the extent to which humans possess free will has implications in moral, legal, and clinical settings. While some progress has been made in variations of the Libet paradigm, in which brain activity is temporally compared to subjects' reports of conscious decisions, such neural correlates have been historically limited to extracranial metrics and, recently, spiking. Therefore, the relative roles of subthreshold neuromodulatory activity, such as neural oscillations across the various frequency bands characteristic of local field potentials, remain poorly understood. To examine the neural dynamics underlying volition at this level of granularity, we exploited the spatiotemporal resolution of intracranial recordings in patients with pharmacologically intractable epilepsy during Libet task performance. We observed significant differences in spectral activity between the baseline period and the period leading up to the conscious decision, particularly across the different beta oscillation frequencies, in the frontal lobe, namely the anterior cingulate cortex (ACC) and the supplementary motor area (SMA). We report the first ever LFP coherence studies during the Libet task, and construct a statistical classifier to discriminate baseline from conscious activity on an individual trial basis. After flagging several conceptual shortcomings of Libet's paradigm, we dissociate the relative roles such experiments could play in the free will debate, and propose that while such experiments could theoretically cast doubt upon some versions of free will, even the best neuroscience leaves much of the dialectic untouched.