Caltech
Student-Faculty Programs

Session B Abstracts

Investigating the role of cortical structures in spatial learning and navigation using acortical mice
Katelyn A. Sadorf
Mentors: Markus Meister and Jieyu Zheng

This project investigates how mice navigate complex environments in the absence of cortical structures. Acortical mice– genetically engineered to lack neocortex and hippocampus– were trained in the Manhattan Maze, a modular two-layer system requiring goal-directed navigation through masked corridors. Using Bpod behavioral software, we precisely recorded reward acquisition and port entry latencies to assess learning progress. While wild-type mice rapidly learned single-decision paths, acortical mice required extended exposure and sometimes manual intervention. Despite this, they reliably acquired simpler masks over time. We introduced intermediate-level maps to test for generalization and found limited transfer to more complex configurations, suggesting a lack of flexible learning compared to wild-type mice. However, acortical mice retained maze proficiency after rest periods, indicating durable memory. Ongoing work involves retraining, expanding mask complexity, and comparing behavioral data to wild-type performance. Future analysis will include DeepLabCut-based trajectory modeling to explore subcortical decision-making strategies. These findings contribute to understanding the extent to which spatial learning can occur without cortical input.

Mechanistic insights into the binding of Girdin and Daple to dynein transport machinery
Aurelia H. Kuester
Mentors: Aga Kendrick, Rustem F. Ismagilov, Álvaro de la Gándara, and Delaney Sanders

Cytoplasmic dynein-1 (dynein) is a motor protein that is responsible for retrograde intracellular transport and requires both dynactin and an activating adaptor adaptor protein for motility. Girdin and Daple, two signaling proteins linked to cancer progression and cell migration, contain conserved motifs found in dynein activating adaptors, including a Hook domain and Spindly motif. Their oncogenic potential is closely tied to their subcellular localization, a process which may be influenced by dynein-mediated transport. This project investigates how phosphorylation and disease-associated mutations in Girdin and Daple affect their ability to interact with dynein transport machinery. Specifically, we assess whether phospho-mimetic or phospho-deficient mutations at conserved serine residues modulate binding to the dynein-dynactin complex. Preliminary data from biochemical assays suggest that select mutations alters binding of Girdin and Daple to dynein and dynactin, supporting a model in which adaptor autoinhibition and phosphorylation state modulate motor recruitment. These findings support a model in which adaptor phosphorylation and autoinhibition regulates binding with dynein transport machinery.

Investigating neural and physiological synchronization during cooperative gameplay
Zeynep Goktepe
Mentors: Shinsuke Shimojo and Katelyn Haly

This project explores the connection between team flow and synchronization in brain and body activity. Team flow is a shared state of deep focus that occurs when people work together smoothly. We studied this by recording brain signals (EEG) and heart rate from pairs of players as they played cooperative video games. In the first phase, participants played a rhythm game called ePlegona, but the gameplay was too complex and unfamiliar to reliably trigger flow. To address this, we designed a simpler game, Hop Harmony, inspired by Flappy Bird. This game promotes faster learning and coordinated actions. Using a 37-pin interface, we linked in-game events to external trigger signals for precise alignment with EEG data. We plan to compare the results from both games to test whether higher flow states lead to stronger neural and physiological synchronization between players. Our goal is to understand whether known brain patterns linked to flow extend across different cooperative settings.

Building towards predictive modeling of team flow based on solo flow across cognitive abilities

Ridah S. Shanavas
Mentors: Shinsuke Shimojo and Mohammad Shehata

Team flow is a highly immersive, synchronized collective cognitive state linked to enhanced engagement and group performance but its relationship to the cognitive abilities profiles of team members remains largely unexplored. Towards this, we developed a platform supporting individual and dual-player gameplay with four cognitive card-matching mini-games. Each mini-game is based on the Cattell-Horn-Carroll (CHC) framework and targets a specific ability: visual processing, general knowledge, quantitative reasoning, or lexical knowledge. Each mini-game has a preview phase, when cards are visible but not selectable, followed by an execution phase where participants select cards from memory. This allows separate analysis of planning and decision-making. Eye-tracking measurements, including gaze, pupil size, and saccades, are collected using NeonGlasses by Pupil Labs, with custom calibration steps for accurate mapping. Subjective flow experience is assessed through questionnaires administered during each session. Initial testing has refined the data analysis pipeline processing both game and eye-tracking data. With large-scale participant testing planned for the future, this platform provides the foundation to investigate how cognitive ability profiles shape team flow and inform predictive modeling of team formation. Ultimately, this research aims to guide strategies for maximizing team flow, with applications in education, sports, and collaborative work.

Investigating the contribution of the zfh1 protein to the polarity in the collective cell migration of the caudal visceral mesoderm in *Drosophila*

Olivia M. Hatcher
Mentors: Angelike Stathopoulos and Jingjing Sun

The caudal visceral mesoderm (CVM) cells, specified at the beginning of embryogenesis, undergo collective cell migration and populate the visceral muscle lining of the *Drosophila* midgut. One aspect of the overall research goal of the Stathopoulos lab is to understand how the front-back polarity is established in the migrating CVM cells during organogenesis. Initial data suggests that zinc finger homeodomain 1 (zfh1, ortholog of human ZEB1), which is transcribed evenly throughout CVM cells, is post-transcriptionally regulated, and Zfh1 protein localization exhibits front-back polarity. Therefore, it is hypothesized that Zfh1 plays a critical role in breaking the symmetry within the migrating CVM cells. To investigate this further, truncated and full-length forms of the Zfh1 protein were expressed, and the polarized gene expression in CVM cells were examined in developing embryos using in situ hybridization chain reactions.

Exploring single- and multi-modal embedding spaces through whole-image and component representation analysis

Amrita Pasupathy
Mentors: Matthew W. Thomson and Surya Hari

Images are often represented as high-dimensional vectors within embedding spaces, enabling quantitative similarity assessment based on spatial proximity. While single-modal models can embed only image data, multi-modal models also embed additional input types, such as text captions, within the same space. Our work aims to identify the strengths and limitations of various single- and multi-modal embedding spaces in order to inform improvements in representation quality. Specifically, we analyzed semantic and geometric similarities between neighboring embeddings, examined the influence of individual embedding dimensions on image features, and compared whole-image embeddings with those of their components. The findings will guide the construction of component graphs inspired by Hippocampal Indexing Theory, representing the first step toward advancing existing graph-conditioned diffusion approaches.

Optimizing machine learning-enabled spatial barcodes for pooled optical screens
Benjamin Y. Yang
Mentors: David A. Van Valen and Sam Holtzen

In order to probe cellular dynamics of multiple pathways, a pooled screen subjects cells with several simultaneously expressed biosensors to a given treatment. However, pooled screens are currently limited by the availability of reliable and fast barcoding methods to identify each expressed construct in a given cell. Therefore, we are developing a barcoding method where guide RNAs (gRNAs) generate spatially-resolved barcode patterns to enable multiplexed phenotypic screens. In this method, an enzymatically-deactivated Cas9 (dCas9) and the gRNAs bind to genomic repeat regions. The subsequent nuclear patterns are visualized by fluorescent in-situ hybridization and classified by a machine learning model. Here, we make several improvements to this method. We have collected new training data for the machine learning model to improve the signal-to-noise ratio. We show that a tetracycline-inducible gRNA plasmid design can be used to prevent epigenetic silencing of the gRNA system. Lastly, we aim to demonstrate the utility of spatial barcodes using a model system of nuclear-translocating biosensors to concurrently probe the activation of three mitogen-activated protein kinases: ERK, p38, and JNK.

Measuring MTCH2-dependent protein insertion into the outer mitochondrial membrane using a split-luciferase system and targeted AAVS1 integration
Diego Alfaro Carcoba
Mentor: Rebecca M. Voorhees

Proper localization of proteins to the outer mitochondrial membrane (OMM) is essential for maintaining mitochondrial integrity and function. MTCH2, an OMM insertase, mediates the insertion of a subset of mitochondrial membrane proteins. This project develops a reporter system to measure the efficiency of MTCH2-dependent protein insertion. Using a split-luciferase reporter system, one half is fused to Metaxin2, an OMM protein independent of MTCH2, while the other half is fused to test certain proteins, such as CYB5B, that are known to rely on MTCH2 for insertion. These constructs are integrated into the AAVS1 safe harbor locus via transfection into both wild-type and Metaxin2-knockout HEK293T cells. Genomic DNA extraction and PCR confirmed genomic integration, and luminescence assays show CYB5B properly inserts into the OMM and reconstitutes the reporter, resulting in luminescence. Ongoing work expands the system to include more MTCH2-interacting proteins and introduces MTCH2 knockout via lentiviral transduction to further validate its role by comparing luminescence before and after knockout.

***In vitro* translation and aminoacylation of Bpa-tRNA(UAG) for cotranslational incorporation of a site-specific photo-crosslinker**
Marton Szabo
Mentors: Rebecca M. Voorhees and Lena Boegeholz

The quality control of multisubunit membrane protein complexes in the endoplasmic reticulum (ER) requires coordinated folding, membrane insertion, and subunit assembly. In the absence of interaction partners, orphan subunits could be targeted for degradation, a process linked the ER-located protein TXNDC15, hitherto identified through experiments conducted at the Voorhees group. TXNDC15 was established as a factor interacting with MARCH6 ubiquitin ligase, promoting degradation of certain ER-related membrane proteins, including GET1, a protein responsible for the post-translational delivery of tail-anchored proteins. To validate physical interactions between TXNDC15, MARCH6, and the orphan subunit GET1, a system is being developed for site-specific incorporation of the UV-inducible crosslinker, p-benzoyl-L-phenylalanine (Bpa). This project establishes an optimized in vitro transcription system based on purified T7 RNA polymerase for producing full-length Bpa-tRNA(UAG), and an in vitro translation platform for the incorporation of the aminoacylated tRNA by the purified Bpa-tRNA synthetase, ultimately incorporating the non-canonical amino acid into the target protein GET1 at multiple probing sites. Preliminary results confirm the success of the IVT platform and the incorporation of Bpa into GET1 constructs at defined sites. This experimental pipeline could support targeted crosslinking experiments to map transient ER protein interactions site specifically during membrane protein quality control.