Science, Technology and Research Scholars
STARS

Annual STARS II Research Symposium

Yale

Rosenfeld Hall
109 Grove St., Room 101

April 15, 2019
4:30-8:30 PM

Annual STARS II Research Symposium
Monday, April 15th, 2019

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4:30 p.m. **Welcome Reception and & Poster Presentations**

5.45 p.m. Opening Remarks
Yale College Associate Dean, Sandy Chang

Featured Speakers

5.50 p.m. **Creation and Characterization of PEG Hydrogels to Observe Neural Stem Cell Chemotaxis**
Jannet Rivera
Department of Biomedical Engineering

6.02 p.m. **Measuring Radioactive Contamination in CUORE Crystals**
Byron Daniel
Department of Physics

6.14 p.m. **Investigating a Role for N-Glycosylation in Zebrafish Hematopoietic Stem Cell Formation**
Kevin Salinas
Department of Molecular, Cellular, and Developmental Biology

6.26 p.m. **Understanding the autoimmune mechanisms in myasthenia gravis**
Pablo Suarez
Department of Molecular, Cellular, and Developmental Biology

6.38 p.m. **Activation of Hypocretin Neurons in Endometriosis Model**
Tran Dang
Department of Molecular, Cellular, and Developmental Biology

6.50 p.m. **Adaptive variation in the odorant receptor protein Obp59a in *Glossina f. fuscipes*, an obligate vector of *African trypanosomiasis***
Alanna Pyke
Department of Molecular, Cellular, and Developmental Biology

7.02 p.m. **Physical and Chemical Properties of First Hydrostatic Core Candidates**
Stephanie Spear
Department of Astronomy

7.14 p.m. **Reducing Filamin A levels prevents cortical malformations and decreases seizure activity in Tuberous Sclerosis Complex**
Shannon Teaw
Department of Molecular, Cellular, and Developmental Biology

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7.26 p.m. **Role of Toll-Like Receptors (TLRs) in Regulating the Removal of Developing Autoreactive B Cells During Establishment of Human Central B Cell Tolerance**
Mindy Le
Department of Molecular, Cellular, and Developmental Biology

7.38 p.m. **π-Facial Selectivities in Hydride Reductions of Hindered Endocyclic Iminium Ions**
Amy Chan
Department of Chemistry

7.50 p.m. **BCL-2 Ovarian Killer Promotes Erythropoiesis in a Mouse Model of Myelodysplastic Syndrome**
Oscar Perales
Department of Molecular, Cellular, and Developmental Biology

8.02 p.m. **Mechanisms of ER-associated degradation by the E3 ubiquitin ligase Doa10**
Carlos Rivera
Department of Molecular Biophysics & Biochemistry

8.14 p.m. **Analysis of RNA Structural Elements in β-globin mRNA Stabilization**
Jared Peralta
Department of Molecular Biophysics & Biochemistry

8.25 p.m. Closing Remarks
STARS II Coordinator, Robert W. Fernandez
STARS Program Director, Dr. Kenneth Nelson
Yale College Associate Dean, Sandy Chang

Creation and Characterization of PEG Hydrogels to Observe Neural Stem Cell Chemotaxis

Jannet Rivera[1], Rita Matta[2], and Anjelica Gonzalez[2]

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The subventricular zone (SVZ) is the largest area of the adult brain where neurogenesis, the creation of new neurons, occurs. From the SVZ activated neural stem cells (NSCs) travel through the rostral migratory stream (RMS) where they differentiate to respond to brain injury. Interactions between NSCs, and the microvasculature, particularly endothelial cells (EC) and pericytes (PC), suggest that vascular cells play a role in NSC maintenance. However, there is a lack of evidence regarding the specific roles that ECs and PCs play in NSC migration, proliferation, and survival. Therefore, we have developed a porous poly-ethylene glycol (PEG) hydrogel system to investigate vascular cues directing NSC migration. PEG, an inert and chemically tunable material, has been modified via conjugation of the cell-adhesive protein fibronectin (Fn). Pores are created through the leaching of zinc oxide microparticles to render a templated hydrogel. Preliminary chemotaxis studies demonstrate that NSCs cluster and migrate throughout the porated, chemically modified hydrogel towards an EC monolayer. NSC clustered migration through the hydrogel is not seen in the absence of ECs, suggesting ECs regulate NSC chemotaxis. Therefore, this system can be used to further investigate chemotactic pathways, in particular the role of PCs on NSCs. Future studies will incorporate oxygen-glucose deprivation to understand how vascular cells probe NSC migration during brain injury *in vitro*, in particular post ischemic stroke.

Measuring Radioactive Contamination in CUORE Crystals

Byron Daniel[1,2], Reina Maruyama[2], and Christopher Davis[2]

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The Cryogenic Underground Observatory for Rare Events (CUORE) contains a bolometer-based detector with $Te0_2$ crystals that is used to search for neutrinoless double-beta decay in the isotope ^{130}Te. If we were to find that neutrinoless double beta decay occurs, it would mean that neutrinos are their own antiparticles, and would have implications on why we ended up with a universe that is made up almost entirely of matter and not antimatter. Searching for rare events such as neutrinoless double-beta decay requires a deep understanding of radioactive backgrounds. In particular, this project sought to identify crystal-based radioactive backgrounds by looking for correlations between the activities of certain isotope decay rates and crystal parameters (e.g. when the crystal was created). This identification would allow the CUORE collaboration to be more confident about its understanding of background radiation. In this presentation, I will show the results from studies of the activities of multiple sources on the CUORE crystals in order to identify and track crystal-based contaminations.

Investigating a Role for N-Glycosylation in Zebrafish Hematopoietic Stem Cell Formation

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Many disorders of the blood, such as leukemia, are difficult to treat and pose a major problem for modern medicine. While multipotent hematopoietic stem cells (HSPCs) hold the potential to treat these disorders, no method for mass-producing HSPCs is currently available as the regulators of HSPC formation have not been completely identified. Recently, we have identified microRNA (miR)-223 as an important regulator of HSPC formation in the zebrafish model organism. MicroRNAs inhibit gene expression by targeting mRNAs for degradation or translational silencing. Notably, many putative miR-223 target genes are N-glycogenes, which suggests that regulation of N-glycosylation, a process that has previously been associated with stem cell differentiation states, may be essential for HSPC production. In order test the hypothesis that N-glycosylation modulates HSPC formation, zebrafish embryos were treated with early and late inhibitors of glycosylation acting in the endoplasmic reticulum and Golgi apparatus, respectively. Preliminary results indicate that zebrafish embryos exposed to tunicamycin, an early inhibitor of N-glycosylation, exhibit reduced levels of HSPC formation during embryonic development, as demonstrated by HSPC counts using in situ hybridization methods to label expression of cmyb, a marker for HSPCs. Nevertheless, swainsonine and a sialyltransferase inhibitor, both late inhibitors of N-glycosylation, phenocopy HSPC expansion defects observed in miR-223 mutants. These results suggest that alterations to the N-glycosylation pathway can limit or enhance HSPC formation during zebrafish embryonic development. Further investigation could determine whether N-glycosylation effects on HSPC formation are spatially or temporally regulated, and whether observed defects are also present in more differentiated lineages that originate from HSPCs, such as the myeloid lineage of blood cells. Ultimately, this work aims to shed light on the previously unidentified molecular and cellular regulators of HSPC production and to open new avenues for improving regenerative blood therapies.

Understanding the Autoimmune Mechanisms in Myasthenia Gravis

Pablo Suarez[1], Miriam F.L. Fichtner[2], Kazushiro Takata[2], Panos Stathopoulos[2], Erik S. Benotti[2], Richard J. Nowak[2] and Kevin C. O'Connor[2]

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Myasthenia gravis (MG) is an autoimmune disorder in which autoantibodies target specific proteins on the muscle endplates and disrupt neuromuscular signaling. In a sub-type of the disease, autoantibodies target muscle-specific tyrosine kinase (MuSK), a membrane receptor that is integral for the maintenance of the neuromuscular junction. We isolated the specific cells that produce anti-MuSK autoantibodies and generated MuSK specific monoclonal antibodies (mAbs). These mAbs were characterized by an accumulation of somatic mutations in the complementarity-determining regions (CDRs) and framework regions (FWRs). We hypothesize that MuSK is the antigen that initiates the naïve-B cell immune response and also drives autoantibody hypermutation. To test this hypothesis, we have reverted somatic mutations back to germline in a step-by-step manner using site-directed mutagenesis. Binding capacity of these mAbs was measured in a cell-based assay. Our overall goal is to determine the impact of affinity maturation on binding to MuSK. We anticipate that a decrease in MuSK binding will occur in increments with each reversion step. Preliminary findings showed that reversions of somatic mutations in the light chain did not affect antigen binding. In the heavy chain, reversion of the CDRs showed only a modest decrease in binding. These data suggested that the FWRs could contribute to the binding capacity of the autoantibodies. Recent experiments showed that FWR reversion did not affect binding to MuSK. Determining the driving stimulus of high affinity MuSK autoantibody production and the somatic mutations critical for binding will provide further understanding of the mechanism of autoantibody production in MG and potentially impact treatment.

Activation of Hypocretin Neurons in Endometriosis Model

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Endometriosis is a gynecological disease impacting 10% of women of reproductive age. It is characterized by the growth of endometrial-like tissue outside of the uterus. The symptoms include chronic pelvic pain and infertility, with treatment options including hormonal therapy and surgical excision. In addition to gynecologic symptoms, endometriosis has been shown to have various systemic effects, inflammation, altered metabolism/body weight, and behavioral changes. Previous mice studies show that endometriosis is linked to increased pain sensitization, anxiety and depression. One possible cellular target that may be affected by endometriosis is the hypocretin/orexin neuron. This neuronal system plays a role in regulating wakefulness/sleep cycles, arousal, reward system, and pain perception. We hypothesize that endometriosis alters activity level of the hypocretin/orexin neuronal system. Mice underwent endometriosis induction surgeries (n = 8) or sham surgeries (n = 8) for the development of the experimental model. Immunocytochemistry was performed on harvested samples of the lateral hypothalamus, and activation level was quantified by cell counting. Mice with endometriosis had a statistically significantly greater ratio of activated hypocretin/orexin neurons did sham mice. These results demonstrate that endometriosis is associated with increased hypocretin/orexin activity, which could be implicated in the behavioral changes seen in the disease.

Adaptive Variation in the Odorant Receptor Protein *Obp59a* in *Glossina fuscipes fuscipes*, an Obligate Vector of African Trypanosomiasis

Alanna Pyke[1], Norah Saarman[2], Kirstin Dion[2], and Adalgisa Caccone[2]

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The obligate vector of African trypanosomiasis responsible for the majority of disease transmission in Uganda is a species of tsetse fly, *Glossina fuscipes fuscipes*. This species has a large geographic range with patchy distributions across habitats that vary greatly in many aspects, including humidity. However, the genes controlling tsetse fly success in the broad variety of habitats they inhabit is largely unknown. The Carlson Lab's recent findings indicate that the protein, Obp59a, is involved in detection of humidity levels in *Drosophila*, can influence survival of flies during desiccation events and is functionally conserved in the tsetse fly *Glossina morsitans morsitans*. It remains to be confirmed that the gene *Obp59a* plays an important role in tsetse fly desiccation tolerance in the wild. This study aims to investigate adaptive variation in *Obp59a* across extreme environments in *Glossina fuscipes fuscipes*. Specifically, we aim to test the hypothesis that there is genetic adaptive variation of *Obp59a* sourced from environments with differences in humidity levels. To test if there is genetic adaptive variation, the Obp59a gene was sequenced in order to identify haplotypes associated with different (high/low) humidity environments. Three pairs of high/low humidity sites that are each from the same major genetic cluster identified in previous studies were sampled. The *Obp59a* gene was amplified with PCR amplification and Sanger sequencing using newly designed primers that target the ~4,000 bp region of the published *G. f. fuscipes* genome. Sequences will then be aligned and edited in the GENEIOUS program to identify haplotypes, and will be exported in FASTA format for downstream analysis. Preliminary results indicate that there is both inter- and intra-population variation in *Obp59a* that can have adaptive significance for *G. f. fuscipes* across environments in Uganda. Statistical analyses to test this hypothesis is ongoing. Future investigations could include sampling from more sites, individuals from different genetic backgrounds, and other tsetse fly species, such as the *G. pallidipes* in Kenya.

Physical and Chemical Properties of First Hydrostatic Core Candidates

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The First Hydrostatic Core (FHSC) is a theoretical stage between the prestellar and protostellar phases of star formation. Several candidate FHSC have been identified observationally but none have been definitively proven to be true first cores. This is due to a combination of factors such as the short duration of the FHSC phase and low-resolution images, which make it difficult to identify the particular molecular lines associated with the FHSC spectral energy distribution. The observational detection and confirmation of the FHSC is of prime importance for understanding of the evolution of dense cores and star formation. Radio observations of the FHSC candidates can be used to probe the physical properties (temperature, density and kinematics) and chemistry of the surrounding gas. Multi-line observations of four FHSC candidates located in or near the Perseus star-forming region were conducted using the Very Large Array (VLA). I will present maps of $NH_3(1,1)$ and $NH_3(2,2)$, along with maps of velocity, excitation temperature, column density and line width for each of these cores. These maps show the distribution and kinematics of the dense gas surrounding the core and are discussed in the context of the evolutionary state of these sources. In the future, these maps will be combined with other molecular line observations to provide further specificity regarding the evolutionary state of the sources.

Reducing Filamin A levels prevents cortical malformations and decreases seizure activity in Tuberous Sclerosis Complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that causes brain malformation and tumors in other organs. Phenotypes of TSC included seizures, developmental delay, and cognitive and psychiatric deficits. Cellular phenotypes include cell misplacement and altered morphogenesis (e.g., increased dendritic complexity) of cortical pyramidal neurons, which may contribute to epilepsy and neurocognitive deficits in TSC. TSC is caused by mutations in the *Tsc1* or *Tsc2* genes causing the hyperactivity of the mechanistic target of rapamycin (mTOR), a major converging point in cell signaling. Previous studies conducted by Zhang et al. 2014, have identified Filamin A (FLNA), an actin binding protein, as a key player in the disrupted cellular morphology associated with TSC. However, the link between FLNA and cortical malformations and seizures characteristic of TSC is unknown, thus prompting this investigation. A plasmid encoding a constitutively active Rheb ($Rheb^{CA}$), a mTOR activator, was electroporated into wildtype mice to drive mTOR hyperactivation. To reduce FLNA levels, short hairpin RNA (shRNA) against FLNA was co-electroporated to decrease FLNA levels. In addition, PTI treatment, a blood-brain barrier penetrant that binds FLNA, was administered to reduce FLNA function. Seizure activity was monitored in animals using video-EEG recordings, and cell migration and dendrite complexity were analyzed in the cortical sections in each condition. Cell morphology data and EEG recordings suggest that normalizing FLNA level in TSC prevents cortical malformations and reduces seizure activity. These data suggest that targeting FLNA offers a novel therapeutic option to treat epilepsy in TSC.

Role of Toll-Like Receptors (TLRs) in Regulating the Removal of Developing Autoreactive B Cells During Establishment of Human Central B Cell Tolerance

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Understanding the mechanisms that regulate the production of autoreactive B cells during central B cell tolerance establishment may improve the design of specific therapies for patients with autoimmune diseases. One protein important to this process is TACI, the transmembrane activator and calcium-modulating ligand that acts as a regulator in the immune response by inhibiting B cell expansion and promoting plasma cell differentiation. Previously, two genetic mutations in the TNFRSF13B gene that encodes TACI have been identified, associated with the development of common variable immunodeficiency disease (CVID). These mutations were found to impact B cell tolerance through interference with the removal of developing autoreactive B cells in bone marrow, and induction of anti-nuclear antibodies (ANAs). However, it is unclear why TACI is required for B cell tolerance, including how TACI contributes to self-antigen sensing during central counter-selection of developing autoreactive B cells and to ANA secretion in the periphery. The working hypothesis is that TACI is required for B cell tolerance because it mediates the tolerogenic function of Toll-like receptors (TLRs) 7 and 9 in B cells.

To determine if TLRs regulate the removal of developing human autoreactive B cells, we study humanized mice where TLR7 or TLR9 expression is inhibited in human hematopoietic stem cells transduced with GFP-tagged lentiviruses expressing shRNA that inhibits the expression of these receptors. Three months post-transplant when lymphocytes have developed, the frequencies of B cells expressing ANAs and polyreactive antibodies from the humanized mice are studied to determine if there is a break in central B cell tolerance following diminished TLR7 or TLR9 expression. Currently, the inhibition of TLR7 expression does not seem to affect the elimination of autoreactive B cells, whereas the abrogation of TLR9 appears to impair central B cell tolerance. We conclude that TLR9 plays an essential role in preventing the production of autoreactive B cells in the bone marrow.

π-Facial Selectivities in Hydride Reductions of Hindered Endocyclic Iminium Ions

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The origins of π-facial selectivities in the borohydride reduction of endocyclic iminium ions have been elucidated by density functional theory calculations. In reductions of conjugated ("thermodynamic") iminium ions, the π-facial preference of the hydride attack was found to be due to torsional steering. Attack at the favored π-face leads to a lower-energy "half-chair"-like conformation of the tetrahydropyridine product, whereas attack at the other π-face results in an unfavorable "twist-boat" conformation. In reductions of nonconjugated ("kinetic") iminium ions, torsional distinction is small between the top- and bottom-face attacks, and the π-facial selectivity of the hydride approach is primarily due to steric hindrance.

BCL-2 Ovarian Killer Promotes Erythropoiesis in a Mouse Model of Myelodysplastic Syndrome

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Myelodysplastic syndromes are clonal hematopoietic stem cell disorders characterized by cytopenia and intramedullary apoptosis. BCL-2 Ovarian Killer (BOK) is a pro-apoptotic member of the BCL-2 family of proteins which, when stabilized from Endoplasmic Reticulum-associated degradation (ERAD), induces apoptosis in response to ER stress. Although ER stress appropriately activates the unfolded protein response (UPR) in BOK-disrupted cells, the downstream effector signaling that includes ATF4 is defective. We used Nup98-HoxD13 (NHD13) transgenic mice to evaluate the consequences of BOK loss on hematopoiesis and leukemogenesis. Acute Myeloid Leukemia developed in 36.7% of NHD13 mice with a Bok gene knockout between the age of 8 and 13 months and presented a similar overall survival to the NHD13 mice. The loss of BOK exacerbated anemia in NHD13 mice, and NHD13/BOK-deficient mice exhibited significantly lower hemoglobin, lower mean cell hemoglobin concentration, and higher mean cell volume than NHD13 mice. Hematopoietic progenitor cell assays revealed a decreased amount of erythroid progenitor stem cells (BFU-E) in the bone marrow of NHD13-transgenic/BOK-deficient mice. RT-QPCR analysis demonstrated decreased mean value of ATF4 in the erythroid progenitors of NHD13/BOK-deficient mice. Our results suggest that in addition to induction of apoptosis in response to ER stress, BOK may regulate erythropoiesis when certain erythroid progenitors experience cell stress.

Mechanisms of ER-Associated Degradation by the E3 Ubiquitin Ligase Doa10

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The ubiquitin-proteasome system (UPS) is a cellular machinery that can selectively target misfolded proteins for degradation. In this system, proteins are degraded by the proteasome after undergoing ubiquitylation, the covalent attachment of ubiquitin (Ub) to a protein. Endoplasmic reticulum-associated degradation (ERAD), a branch of the UPS, is the process by which proteins are ubiquitylated at the ER and subsequently degraded by the proteasome. Luminal and membrane ERAD substrates require membrane extraction prior to proteasomal degradation in a process called retrotranslocation. The ERAD machinery is highly conserved and enables the turnover of short-lived regulatory proteins and damaged proteins. Defects in ERAD have been linked to numerous diseases, such as diabetes and Parkinson's disease. A greater understanding of the molecular mechanisms governing ERAD is crucial for developing strategies to maintain protein homeostasis in these diseases. We aim to uncover the molecular mechanisms regulating Doa10, a conserved transmembrane E3 ubiquitin (Ub) ligase that senses and ubiquitylates ERAD substrates containing cytosolic degradation signals. This project focuses on understanding the nature of Doa10 substrate recognition and retrotranslocation. We assessed Doa10 interaction with a known substrate using an *in vivo* site-specific photocrosslinking assay. Furthermore, cycloheximide chase analysis suggested the de-ubiquitylating enzyme (DUB) Otu1 is involved in the Doa10 degradation pathway, potentially during the retrotranslocation of membrane substrates. Continued investigation of these basic ERAD mechanisms will be useful for developing therapeutics to treat the many diseases affected by ERAD deficiency.

Analysis of RNA Structural Elements in β-globin mRNA Stabilization

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The element for nuclear expression (ENE) is a *cis*-acting structural element that stabilizes RNAs via formation of a triple helix with the poly(A) tail. It is composed of a U-rich internal loop flanked by GC-rich stems. Various forms of the ENE have been identified and their stabilizing effects were studied using an intronless β-globin reporter gene. However, the role that the distance between ENEs and the poly(A) tail has on mRNA stabilization has not been investigated. It is not known whether reduced ENEs containing only the U-rich loop and one of the stems are sufficient to confer mRNA stabilization. Moreover, some gammaherpesviruses have ENEs with a semi-conserved GU-rich sequence upstream of the triplex structure which may be involved in ENE function. We aim to investigate the effects of ENE location and ENE structure on mRNA stabilization. The Rhesus Rhadinovirus (RRV) canonical ENE or a putative Anopheles C virus (ACV) reduced ENE was inserted into the 3' UTR of β-globin reporter at various distances relative to the poly(A) tail and tested for the stabilization of β-globin mRNA in human tissue culture cells. Mutant RRV ENEs carrying mutations in the GU-rich sequence were also tested for stabilization. Increased accumulation of reporter transcripts was observed for the RRV ENE whereas no significant increase was seen for ACV ENE. Moreover, accumulation of reporter transcripts carrying mutant RRV ENEs significantly decreased. Our results demonstrate that insertion of RRV ENE closer to the poly(A) tail enhances mRNA stabilization and that the semi-conserved GU-rich motif of the ENE also has significant influence on mRNA accumulation.

The In-Between: Analyzing Loosely Bound Dwarf Galaxies

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Most galaxies are either too far for us to detect distinctive stellar spectra or too close to have retained their gas after interactions with other systems. However, IC-10, LGS 3, NGC 6822, and the Pegasus Dwarf Spheroidal are three dwarf galaxies orbiting at distances that range from 769 to 795 kpc away from the Milky Way's nearest neighbor, M31. These three galaxies have not been extensively studied, yet are uniquely situated because they are close enough to detect individual stellar velocities and orbital motions but are far enough that their gas has not yet been stripped from interactions with M31. This facilitates insight into them that is typically difficult to glean from stellar or gaseous observations alone. Our aim is to survey these galaxies and learn more about their mass, kinematics, and other general properties from their stellar and gaseous emission and absorption spectra. We used recently obtained DEep Imaging Multi-Object Spectrograph (DEIMOS) 2D spectral data to do so and are currently in the process of extracting stellar velocities and other kinematic information to determine the masses of these galaxies. We will then begin to extrapolate about their other properties.

Investigating the Normal Function of α-Synuclein, a Parkinson's Disease Gene

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α-Synuclein is a presynaptic protein known to be involved in the pathogenesis of Parkinson's Disease (PD). When mutated α-synuclein aggregates in Lewy Bodies, an intracellular structure defined as the hallmark of PD. However, the function of α-Synuclein is still unclear. Other proteins in the synuclein family are known to be capable of sensing and generating membrane curvature, properties associated with the synaptic vesicle cycle. A previous study with αβγ-synuclein triple knockout mice (αßγ-/-), showed that loss of synuclein function leads to deficits in synaptic vesicle endocytosis (SVE). The Chandra lab conducted a membrane recruitment assay and recruited endocytosis in cells, which revealed α-synuclein recruitment to synaptic membranes upon excitation and modulation of α-synuclein early in endocytic protein recruitment and clathrin pit dynamics. These results allow us to hypothesize that α-synuclein is a regulatory endocytic protein. To test whether this SVE activity is solely a α-synuclein characteristics or a general synuclein property, we will expand this experiment to conduct a comparative analysis of α-synuclein and other synuclein proteins in reconstitution experiments. For this we must first purify endocytic proteins as well as ß- and γ-synuclein. Protein synthesis and optimization was conducted for three different proteins, but the focus was on the β-synuclein. For future direction, we propose using the purified synuclein proteins in electron microscopy experiments to observe the localization of synuclein protein on membranes in conjunction with other endocytic proteins.

A Visual Pavlovian Conditioned Hallucinations Task, Using Adaptive Bayesian Psychometric Method QUEST

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The predictive coding framework for perception postulates that we automatically infer what is around us by combining our sensory input with our prior beliefs about the world. Mathematical models based in Bayesian statistics have been used to describe this process, elucidating both typical as well as atypical brain processes, such as emergence of hallucinations. A previous study using a Pavlovian conditioning task showed that this phenomenon could be a result of over-weighting of prior beliefs about sensory information versus immediate sensory input. Individuals with Auditory Verbal Hallucinations (AVH) were more susceptible to conditioned hallucinations than individuals without AVH, regardless of whether or not they had a diagnosable psychotic illness. This suggests a common underlying mechanism for the emergence of hallucinations irrespective of functional status. Our current work is focused on developing a complementary task, exploring conditioning effects for the visual modality. The study aims to test that visual hallucinations are easier to induce in individuals with visual hallucinations compared to those with different sensory hallucinations, or with no hallucinations. The methodology starts with estimating an individual's psychometric curve, which models the relationship between the contrast level of the visual stimulus, and the responses to a forced-choice task gauging the detection of the visual stimulus. This curve is estimated using QUEST, an adaptive procedure provided by Psychtoolbox package in MATLAB. These individual contrast values are then used to train subjects on the pairing of the salient auditory stimulus (tone) and a visual stimulus presented at threshold. This is followed by testing the conditioning for the visual stimulus with trials in which the target visual stimulus is absent, but the paired tone is present. Preliminary data from neurotypical controls show an observable conditioned hallucinations effect in this novel task, comparable to the effect produced by the earlier auditory-based study. To ensure the most robust performance possible, the psychometric modeling requires further optimization. Further steps include developing an online version of the task, to increase the speed and the scope of data acquisition, crucial for task optimization. Online deployment also allows for access to remote, specialized populations, such as those with specific hallucinatory experiences.

Versatile Laser Monitoring System for Ultracold Atom Experiments

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Lasers are central tools in atomic, molecular, and optical physics. They have many applications, from optical clocks to optical control of atoms. One important use is laser cooling, a technique that has been used to cool atoms to nanokelvin. These ultracold atoms have drawn considerable attention as platforms for quantum simulation and quantum computing. The Navon group makes use of ultracold atoms to study quantum many-body physics by controlling ultracold quantum matter. The laser systems used to achieve the ultracold atoms in such a setup however, tend to be very complex, owing to the many laser beams used of various frequency and intensities. The intensity of each laser can vary over time. For example, local temperature fluctuations and heat dissipation in the laser systems can change laser power, enough to considerably degrade the operation of the apparatus. For this project, we designed and assembled a remote monitoring system using the Raspberry Pi platform. Using open-source Adafruit libraries, Adafruit sensors, and the commercial Cloud4RPi platform, we have achieved power monitoring that is precise to the order of 10^{-2} mW. Current work is directed towards installing more sensors using the platform; currently, two sensors have been successfully installed and used in the operation of the experiment.

Investigating the Role of BOK on the Inflammatory Response through Mitochondrial Changes

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The BCL-2 family member BOK (BCL-2 related Ovarian Killer) is well-studied for its role in inducing apoptosis, but our recent research suggests another function: controlling the inflammatory response at a safe and healthy level. However, we do not know what exactly triggers the BOK expression after an infection, and we do not know exactly which signaling pathways BOK subsequently activates. Since the mitochondria is a major site for inflammatory response mechanisms—such as the immune signaling mediated by RIG-I and MAVS—we have focused our study on BOK's role on the mitochondrial dynamics. We have seen that BOK knock-out in MEFs (mouse embryonic fibroblasts) results in a more fissed mitochondrial network, disrupting normal mitochondrial fusion-and-fission equilibrium. In addition, BOK expression safely curbs the levels of antiviral responses. Specifically, after an infection, the interferon (IFN) levels are reduced within wildtype BOK expressing MEFs compared to the BOK knock-outs. Understanding these specific relationships between antiviral responses, mitochondrial dynamics, and signaling proteins is important in further studying the molecular mechanisms of BOK expression. In doing so, we are able to investigate the previously unknown significance of BOK in infectious diseases and in the immune responses.

Characterization of Vasoactive Intestinal Peptide – Expressing Interneurons Using Chronic Manipulations

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Inhibitory interneurons function to maintain stable activity within local neural circuits and have been hypothesized to play a key role in shaping the processing of sensory information. Despite their sparseness, comprising approximately 1% of all cortical neurons, vasoactive intestinal peptide (VIP) expressing interneurons have been hypothesized to play a key role in shaping cortical activity. They are positioned at an interesting intersection between long-range inputs, including neuromodulatory inputs such as acetylcholine and serotonin afferents, and the local cortical circuit. The primary goal of this project is to examine how cortical activity is shaped by VIP interneurons through chronic manipulations of these cell populations. To determine how chronic VIP interneuron manipulations affect cortical population activity, we will compare the efficacy of a virally injected genetically targeted toxin, Caspase3, in mice in which Ca2+ transients have been imaged *in vivo* using two-photon laser scanning microscopy (2PLSM). We will quantify how many cells have been successfully ablated, and will compare this result with *in vivo* Ca2+ imaging data. Additionally, almost nothing is known about the distribution of excitatory and inhibitory inputs along the VIP interneuron dendrites. This project aims to further characterize VIP interneurons by identifying the distribution of inhibitory and excitatory postsynaptic inputs VIP interneurons. This will require viral injections to specifically target PSD-95 and Gephyrin, scaffolding proteins of excitatory and inhibitory postsynaptic densities, with a fluorophore, followed by confocal microscopy. Disruptions of inhibitory interneurons have been shown to lead to perturbations of primary cortical functions, such as the encoding of sensory information and higher-order cognition. This disruption has been suggested as a strong candidate mechanism underlying several neuropsychiatric and developmental diseases, such as schizophrenia and autism. This project, therefore, can provide insight not only on the distinct roles of inhibitory interneuron populations in shaping visual cortical activity and their distribution in the healthy brain but also on the effects their disruption on primary cortical functions in disease.

The Effects of Fyn Inhibition in Tauopathy

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Alzheimer's disease (AD) is the sixth leading cause of death among adults in the United States. This progressive neurodegenerative disease is characterized by the presence of insoluble, extracellular Amyloid-beta plaques and intracellular tau tangles. Recent studies have revealed that the presence of soluble Amyloid-beta oligomers (rather than the insoluble Amyloid-beta plaques) are neurotoxic to synapses and lead to impaired memory. A key signaling molecule that connects neurotoxic Amyloid-beta oligomers with tauopathy is the non-receptor tyrosine kinase Fyn. Fyn phosphorylates tau and the two are mis-localized to dendrites together, where Fyn can phosphorylate NMDA receptor 2B and result in increased NMDAR activity and excitotoxicity. To better understand the therapeutic utility of Fyn kinase inhibition in AD and other diseases in which tauopathy is present, we treated transgenic mice expressing mutant human tau and wild-type littermates with a drug that serves as a Fyn kinase inhibitor. Treatment began at two months of age, prior to the onset of any tau pathology or memory impairment, and continued until eight months of age. Following treatment, learning and memory were assessed in the Morris water maze. To investigate the histological changes that underlie this phenotype, immunohistochemistry was employed to monitor total and phosphorylated tau accumulations, as well as synaptic density. To the extent that these measures are reduced by Fyn kinase inhibition, the intervention may be of benefit in Alzheimer's disease. Drugs that demonstrate utility in this model of tauopathy may also prove useful in the treatment of other tauopathies, such as Frontotemporal lobe dementia and Progressive Supranuclear Palsy.

Targeting Survivin as Therapy for Cancer by Varying Substituent Specificity of PROTACs

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Survivin is the smallest member of the inhibitor of apoptosis protein (IAP) family. Previous studies have shown that survivin expression is required for cancer cell survival. Knocking-down survivin expression in K-RAS mutated cells results in spontaneous apoptosis, making it a good target for degrading K-RAS indirectly. Survivin's enzymatic activities remain unknown, making the development of inhibitors difficult. PROteolysis-TArgeting Chimeras (PROTACs) are hetero-bifunctional molecules that recruit an E3 ubiquitin ligase to a given substrate protein resulting in its targeted degradation. PROTAC technology can help overcome limitations in targeting survivin. Preliminary results from our lab indicate that PROTACs with an attachment point at the 5-position of a specific E3 ligase ligand have induced some degradation of survivin. This current study focuses on the synthesis of PROTACs with an attachment group at the 4-position and 5-position of this E3 ligase ligand. The PROTACs were also synthesized with varying linker lengths. In the future, Western Blot analysis will be done in order to determine if the PROTACs that have an attachment group at the 4-position of the E3 ligase ligand are more effective at inducing degradation at lower concentrations than PROTACs that have an attachment group at the 5-position of the E3 ligase ligand. Similarly, Western Blot analysis will be used to determine the impact of linker length on the efficacy of the PROTACs.

Enhancing diabetic wound healing with Platelet-Derived Growth Factor and Vascular Endothelial Growth Factor bound ECM

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Wound healing presents a significant challenge in medicine and has been a focal point of regenerative tissue research especially in the context of diabetes. Extensive research has shown impaired wound healing to be implicated in diabetes and play a large role in the development of non-healing foot ulcers- a condition diabetic patients have a 12-25% lifetime risk of developing. Many therapies have been developed to encourage dermal regeneration in diabetic wound environments. Dermal regeneration and wound healing in diabetic patients is important for decreasing the rate of infections and incidences of death. Research has also shown impaired wound healing in diabetes to be caused by mechanically ineffective extracellular matrix (ECM) as well as a lack of growth factor production in the wound bed. Therapies trying to reverse this phenotype have either tried to improve wound healing mechanically by introducing normal ECM or biologically by applying topical growth factor treatment. However, these therapies have not achieved true dermal regeneration. In the present work, we propose a new therapy to enhance wound healing in the diabetic wound environment. This work aims to enhance wound healing by introducing ECM bound with Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor (PDGF) to the wound site. Current *in vitro* assays have aimed to demonstrate that ECM bound to VEGF and/or ECM bound to PDGF can enhance the proliferation of diabetic fibroblasts and normal endothelial cells. We project that assays will show that growth factor bound ECM will enhance the proliferation of diabetic fibroblasts and WT endothelial cells.

The Role of $G\alpha_o$-coupled Neurotransmitter GPCRs in the *C. elegans* Egg-Laying Circuit

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The *C. elegans* egg-laying circuit is among the best-studied model neural circuits, as the function of each cell and the neurotransmitters it releases have been characterized. Our work has shown there are 20 neurotransmitter GPCRs detectably expressed in this circuit, yet their functions remain largely unstudied. We screened knockout mutants for every one of these neurotransmitter GPCRs to look for egg-laying defects. The single receptor knockouts showed at most mild egg-laying defects. We hypothesized that the weakness of these defects could be due to functional redundancy among the receptors. For example, knocking out $G\alpha_O$, a G protein known to inhibit neurotransmitter release in neurons of the egg-laying circuit, causes a very strong hyperactive egg-laying phenotype, and we found seven $G\alpha_O$- coupled neurotransmitter GPCRs are expressed in the egg-laying circuit that might redundantly activate this G protein. To test this idea, we generated various knockout combinations of five of the $G\alpha_O$-coupled neurotransmitter GPCRs. However, our results showed that even the quintuple knockout resulted in only a weak egg-laying defect. As a strategy to identify the functions of these GPCRs despite their apparent very high functional redundancy, we examined transgenic overexpressors of the GPCRs, as overexpression of this type of receptor can result in a gain-of-function effect. We found that the GABA receptor GPCR, GBB-1, when overexpressed from a high-copy transgene, showed a strong hyperactive egg laying phenotype. GBB-1 is thought to form a functional GABA receptor by forming an obligate heterodimer with a second GPCR called GBB-2. Knocking out *gbb-2* suppressed most but not all of the effect of the GBB-1 overexpressor on egg laying. The residual effect of GBB-1 overexpression in the absence of GBB-2 suggests that GBB-1 may have additional dimerization partners besides GBB-2. These other partners may be other members of the family of class C GPCRs, which are known to form heterodimers with each other.

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