The START@Yale program is grateful for the generous support of Nancy J. Brown, MD, Dean of Yale School of Medicine; Alan Graham, M.D., Yale School of Medicine, Class of 1971; and Paul Rothman, M.D., Yale School of Medicine, Class of 1984.

We would also like to thank the START@Yale mentors for providing our students with outstanding research experiences.
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OVERVIEW OF THE START@Yale PROGRAM

START@Yale is a program to provide incoming M.D. and M.D-Ph.D. students with a mentored research rotation and associated educational and social activities during the summer before the first year.

Dr. Laura Ment, Associate Dean of Admissions & Financial Aid, YSM Student Affairs, Professor Pediatrics & Neurology – 2021 START@Yale Director

Dr. Peter Aronson, CNH Long Professor of Internal Medicine and Physiology – 2010 START@Yale Co-Director

Dr. Anees Chagpar, Professor of Surgery (Oncology) – 2021 START@Yale Associate Director

Student Mentors: Nikkita Khattar, Haleigh Larson and Amy Rushing
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<th>Time</th>
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<td>8:30 a.m.</td>
<td>Daniel Jovin, Alan Dardik, MD, PhD, FACS, DFSVS, FAHA</td>
<td>“Tenascin C Controls Coagulation and Arteriovenous Fistula Patency”</td>
<td>Surgery</td>
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<tr>
<td>8:45 a.m.</td>
<td>Zachary Pickell, Albert Sinusas, MD</td>
<td>“Computed Tomography and SPECT Imaging for In Vivo Measurement of 3D Lagrangian Strains in the Left Ventricle After Myocardial Infarction in Swine”</td>
<td>Internal Medicine</td>
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<td>9:00 a.m.</td>
<td>Caroline Echeandia-Francis, Keith Choate, MD, PhD</td>
<td>“Failure to Thrive in Children with Ichthyosis”</td>
<td>Dermatology</td>
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<td>9:15 a.m.</td>
<td>Roselyn Terrazos-Moreno, Hugh Taylor, MD</td>
<td>“Effects of LPS on Uterine Gene Expression in Mice”</td>
<td>Obstetrics, Gynecology and Reproductive Sciences</td>
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<td>9:30 a.m.</td>
<td>Cory Wu, Gerald Shulman, MD, PhD, MACP, MACE, FRCP</td>
<td>“Uncovering the Role of the Mitochondrial Calcium Uniporter in Hepatic Mitochondrial Metabolism”</td>
<td>Internal Medicine</td>
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<td>9:45 a.m.</td>
<td>Justice Hansen, Todd Constable, PhD</td>
<td>“Dynamic Node Reconfiguration in Forrest Gump”</td>
<td>Radiology</td>
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<td>10:00 a.m.</td>
<td>Nana Ekua Entsiwa Adenu-Mensah, Eyiymesi Damisah, MD</td>
<td>“Investigating Statistical Learning Impairment in Patients with Dementia”</td>
<td>Neurosurgery</td>
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<td>10:15 a.m.</td>
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<td><strong>15 minute break</strong></td>
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<td>10:30 a.m.</td>
<td>Jaspreet Kohli, Noah Palm, PhD</td>
<td>“Identifying and Characterizing TRP-Modulating Gut Microbial Metabolites”</td>
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<td>10:45 a.m.</td>
<td>Jacob Howard, Francis Lee, MD, PhD, FAAOS, MBA</td>
<td>“Desperate Times Call for Precision Measures: Investigating AORIF’s Role as a Response to Femur Fractures for Poor Surgical Candidates”</td>
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<td>11:00 a.m.</td>
<td>Serina Applebaum, Kieran O'Donnell, PhD</td>
<td>“Maternal Prenatal Mood Entropy: How Unpredictability Shapes Brain Development”</td>
<td>Child Study Center</td>
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<td>11:15 a.m.</td>
<td>Matthew Yuen, Hal Blumenfeld, MD, PhD</td>
<td>“Accelerating Research on Consciousness: An Adversarial Collaboration to Test Contradictory Predictions of Global Neuronal Workspace and Integrated Information Theory”</td>
<td>Neurology</td>
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11:30 a.m.  Christina Jayaraj, Sanjay Aneja, MD, “Cautious Deep Learning”, Therapeutic Radiology

11:45 a.m.  15 minute break

12:00 p.m.  Closing Symposium Guest Speaker: Elijah Paintsil, FAAP, MBChB Professor; Professor of Pediatrics (Infectious Diseases), Pediatrics; Professor of Public Health, Yale School of Public Health; Professor of Pharmacology, Molecular Medicine, Pharmacology, and Physiology; Professor of Management, School of Management

12:45 p.m.  10 minute break


1:10 p.m.  Forest Mahoney, Mustafa Khokha, MD, “Spliceosomal Factor SMU1 Likely Plays a Role in Normal Cilia Function”, Pediatrics

1:25 p.m.  Michael Nock, Gail D’Onofrio, MD, MS, “The Effectiveness of Emergency Department-Initiated Sublingual Buprenorphine Versus Extended-Release Injectable Buprenorphine for Opioid Use Disorder”, Emergency Medicine

1:40 p.m.  Rafael Maarek, Alexandra Lansky, MD, FACC, FAHA, FSCAI, FESC, “Evaluating the Effects of Pre-operative Statin Therapy on Periprocedural Outcomes in TAVR Patients”, Internal Medicine

1:55 p.m.  Bridget Chen, Lauren Sansing, MD, MS, FAHA, FANA, “Immune Tolerance and Attenuated Pro-inflammatory Response in the Mouse Brain with Stroke-Associated Pneumonia”, Neurology

2:10 p.m.  Warren Carter, Elizabeth Jonas, MD, “Parkinson’s Disease Protein DJ-1 Facilitates the Localization of ATPβ mRNA to Outer Neurites”, Internal Medicine

2: 25 p.m.  Talia Lichtenberg, Jaime Grutzendler, MD, “In Vivo Imaging of Astrocyte Calcium Dynamics During Cell Death and Injury”, Neurology

2:40 p.m.  Rachel Lutz, David Hafler, MD, FANA, “Type 1 Interferon Induces PD-1highCXCR5- Peripheral Helper T Cells”, Neurology

2:55 p.m.  wrap up
Tenascin C Controls Coagulation and Arteriovenous Fistula Patency

Daniel Jovin, Dr. Alan Dardik, MD, PhD

Problem: Tenascin C (TnC), a matricellular glycoprotein, is highly induced with inflammation such as following arteriovenous fistula (AVF) creation, but TnC's role during venous remodeling is not yet understood.

Hypothesis: TnC is induced in the inflammatory response and following tissue injury; therefore, we expect that reducing TnC expression will alter venous remodeling to decrease AVF patency.

Methods: Prior to AVF creation, tail bleeding was performed in adult wild type (WT) and TnC-knockout (TnC-KO) mice to analyze time to initial hemostasis and bleeding volume in 20 minutes. AVFs were created using an established aortocaval AVF model. Patency was assessed using ultrasound, and occluded AVFs were harvested and processed for Martius Scarlett Blue (MSB) staining to detect fibrin and erythrocyte content.

Results: TnC-KO mice had significantly reduced AVF patency 42 days post-operation (p = 0.0001, n=40 vs. 28 for WT vs. TnC-KO), and AVF occlusions in TnC-KO AVF mice trended towards greater fibrin (p = 0.27) and erythrocyte content (p = 0.25) via MSB (n=2). TnC-KO mice had both a significantly increased time to hemostasis (p < 0.001) and total bleed volume (p = 0.01) (n=16).

Conclusions: These results demonstrate TnC’s critical role in hemostasis and venous remodeling. Ablation of TnC inhibits coagulation in normal vessels, but lack of TnC in AVF increases risk of vessel occlusion and may enhance thrombotic content of occlusions. It is important to study how TnC controls vascular remodeling and coagulation to understand its potential as a novel strategy to improve AVF maturation.
Computed Tomography and SPECT Imaging for In Vivo Measurement of 3D Lagrangian Strains in the Left Ventricle After Myocardial Infarction in Swine

Zachary Pickell, Albert Sinusas, MD

Problem: Myocardial infarction (MI) causes regional dysfunction, infarct thinning and expansion, increased wall stress, changes in left ventricular (LV) geometry and dilation, and results in (LV) remodeling and heart failure. Recent work with theranostic hydrogels suggests that intramyocardial injection into the infarct region may have mechanical and molecular effects, leading to reduced remodeling and significant therapeutic benefit. Currently, there is no robust, non-invasive method of tracking and assessing therapeutic efficacy in vivo.

Hypothesis: Cine computed tomography (CT) and single photon emission computed tomography (SPECT) imaging can be used to calculate regional 3D Lagrangian strains, assess changes in LV deformation, and track therapeutic efficacy overtime in a swine model of MI.

Methods: ECG gated high-definition contrast cineCT defined the endocardial and epicardial LV surfaces, and ⁹⁹ᵐTc-Tetrofosmin SPECT imaging mapped the location of infarcted, border, and normal myocardial regions. Hybrid SPECT/CT imaging was performed immediately after reperfusion of a 90-minute percutaneous occlusion of the left anterior descending coronary artery and 8 days post-MI. Custom Matlab programs calculated LV volume and ejection fraction over the cardiac cycle. Vector displacement fields generated using nonlinear image registration in BioImage Suite were imported into Matlab along with LV segmentations from a custom U-Net AI to calculate radial, circumferential, long-axis, maximum principal, and maximum shear strains.

Conclusions: High-resolution cineCT/SPECT mapping can be used to accurately and non-invasively calculate LV volume, ejection fraction, and 3D strain and was implemented to track the efficacy of therapeutic interventions resulting in alterations of regional myocardial deformation and LV remodeling.
Failure to Thrive in Children with Ichthyosis

Caroline Echeandia-Francis, Keith Choate, MD, PhD

Problem: While observations that children with ichthyosis may be experiencing failure to thrive has long been noted by physicians, a large study providing data regarding the average growth metrics for children with ichthyosis and how these metrics compare to population averages are not present in the current literature.

Hypothesis: Children with more severe types of ichthyoses will experience failure to thrive as indicated by growth metrics that fall standard deviations below population averages.

Methods: Current registrants under the age of 25 years of the Foundation for Ichthyosis and Related Skin Types were asked to consent to pediatricians releasing pediatric growth records. Nonregistrants with ichthyosis under the age of 25 years were also invited to participate. Participants’ pediatricians were contacted, and pediatric growth records were obtained. Height, weight, and head circumference data from participants’ pediatric growth records were compared to child growth standards from the World Health Organization.

Results: We are currently in the data collection phase, but predicted results are that children with severe types of ichthyoses will experience failure to thrive as indicated by height, weight, and head circumference metrics that fall standard deviations below W.H.O. child growth standards.

Conclusions: Predicted conclusions are that children with severe types of ichthyoses experience failure to thrive. Potential early-life interventions including supplemental nutrition and gastrostomy tubes may mitigate growth delay among these children, thus reducing some of the stigmatizing features of ichthyosis surrounding smaller stature.
Effects of LPS on Uterine Gene Expression in Mice
Roselyn Terrazos-Moreno, Hugh Taylor, MD, Ramanaiah Mamillapalli, PhD

**Problem:** Retrograde menstruation is a common theory for the cause of endometriosis. Although 90% of women ages 15-49 experience retrograde menstruation, only 5-10% develop endometriosis. This points to multiple underlying factors involved in the pathophysiology of the disease. A 2010 study demonstrated that levels of E. Coli in women’s menstrual blood were higher in those with endometriosis than those without endometriosis. Vaginal bacterial exposure could play a significant role.

**Hypothesis:** Mice injected with lipopolysaccharide (LPS) will show greater gene expression amongst target genes of the NF-kB pathway, a pathway activated by LPS, than mice injected with phosphate-buffered saline (PBS).

**Methods:** 11 mice received intraperitoneal injection of 30 μL of LPS/200μL PBS. 12 mice received 200μL injection of PBS. Injections were delivered daily for 7 days. Uterine horn and peritoneal tissue were collected on day 8. RNA isolation and RNA sequencing were then performed.

**Results:** It is expected that mice receiving IP injections of LPS will experience higher gene expression amongst target genes of the NF-kB pathway. Some gene examples are CASP4, CXCL2, CXCL10, MADCAM1.

**Conclusions:** These results can help us understand the role of bacterial exposure in uterine gene expression and its relation to endometriosis. Elimination of menstruation is utilized as a therapy for retrograde menstruation and endometriosis, but many women still experience symptoms after elimination. Understanding the other mechanisms behind endometriosis can help us improve upon existing treatments.
Problem: The mitochondrial calcium uniporter (MCU) is a key regulator of mitochondrial calcium, however, the role of MCU in mediating hepatic mitochondrial metabolism has little characterization.

Hypothesis: MCU KO mice will exhibit decreased mitochondrial calcium import, leading to increased activation of CAMKII, inhibition of citric acid cycle dehydrogenases, and increased glucose tolerance.

Methods: Immunoblotting of P-CAMKII compared to non-phosphorylated CAMKII was utilized to measure selective activation of CAMKII in MCU KO mice. MCU KO mice were infused with 13-C glutamine radiotracers. LC/MS processing of homogenized liver samples was performed that quantified levels of key metabolites that measured flux through the gluconeogenic and citric acid cycle flux. To measure glucose tolerance, the lab performed a hyperinsulinemic-euglycemic clamp, in which a steady flow of insulin is provided to MCU KO mice, but glucose infusate rate is varied to maintain a consistent blood glucose concentration.

Results: We observed an increased level of phosphorylated CAMKII in MCU KO mice, which corresponds with existing data of increased lipolysis and acetyl-COA levels. LC/MS metabolite quantification revealed an increase in gluconeogenic flux, which may be a product of acetyl-COA allosteric activation of pyruvate carboxylase. We also found that MCU KO mice exhibit increased glucose tolerance, meaning increased insulin responses to glucose, as well as a muted increase in blood glucose levels in response to infused glucose.

Conclusion: Overall, the results indicate that MCU KO mice exhibit altered levels of cytoplasmic and mitochondrial calcium levels that lead to downstream alterations in metabolic pathways and energy processing. Our data preliminarily suggests MCU inhibition could garner further interest as a potential target for Type-2 diabetes pharmaceuticals, due to its effect on glucose tolerance.
**Dynamic Node Reconfiguration in Forrest Gump**

*Justice Hansen, Todd Constable, PhD*

**Problem:** Recent fMRI work shows that the functional nodes of the brain reconfigure with brain state. This study seeks to understand dynamic node reconfigurations at time scales shorter than the typical 6 minute task.

**Hypothesis:** First, if a state-specific atlas is generated from 40-second intervals in 15 subjects watching a 2-hour film, the data will significantly cluster by brain state across subjects. Second, node size vectors can predict brain state.

**Methods:** All fMRI data were obtained from the StudyForrest Project. Brain states were defined by which of four characters was shown interacting with Forrest Gump. Frames were grouped together by brain state and divided into 20-second intervals. To account for the inherent sluggishness of the BOLD signal, 6 seconds were added to each interval, the vector of intervals was randomized twice, and the new vectors were each combined with the original vector to produce two sets of 40-second intervals per brain state. Next steps are to generate a state-specific atlas from each interval for a total of 6180 atlases, apply clustering algorithms to the atlases, and use node size to predict brain state in the 15th subject. Analysis was performed using custom Matlab scripts and BioImage Suite command line tools.

**Results:** Atlas generation is pending. We anticipate that in spite of the complexity of the stimulus, atlases will cluster meaningfully and node size can predict brain state.

**Conclusions:** To be discussed at the START@Yale closing symposium.
Investigating Statistical Learning Impairment in Patients with Dementia

Nana Adenu-Mensah, Ayman Aljishi, Eyiyemisi Damisah, MD

Problem: Memory loss is the most debilitating consequence of the Dementias (e.g., Alzheimer’s Disease (AD)) as it severely affects an individual’s sense of self and quality of life. Studies have shown that patients with Dementia have hippocampal-dependent explicit memory deficits. However, it is less clear if implicit memory is impaired in the Dementias and whether the hippocampus is critical for implicit memory processing. Statistical learning (SL), a form of implicit memory, is a fundamental mechanism of cognition that affects the way we predict and attend to our environment. It is unclear if SL is impaired in the Dementias.

Hypotheses: SL is impaired in AD. SL performance is correlated with hippocampal volume (HPC) and phospho-Tau, and can predict clinical neurocognitive assessment scores.

Methods: We recruited seven AD (A+ T+ N-) patients, 11 non-AD dementia (A-T- N+) patients, and 15 healthy controls to complete a validated SL task. We collated results from cerebrospinal fluid (CSF) analysis, structural MR brain volumes, and Montreal Cognitive Assessment (MoCA) scores.

Results: Patients with AD scored at chance in the SL and item memory tasks, and also scored significantly lower than healthy controls in the item memory task (p<0.006). Multivariate linear regression showed male sex (p<0.001), SL (p<0.07), and HPC (p<0.02) were associated with higher MoCA scores, while total intracranial volumes (p<0.001) were associated with worse MoCA scores (R² = 0.93).

Conclusion: Preliminary findings suggest that SL is impaired in AD, and may be used to identify AD patients with implicit memory dysfunction early in the disease process.
Immune Tolerance and Attenuated Pro-inflammatory Response in the Mouse Brain with Stroke-Associated Pneumonia

Bridget J. Chen, Lauren H. Sansing, MD

**Problem:** Stroke-associated pneumonia (SAP) is a challenging complication for which patients experience higher mortality and lower functional independence at discharge. We aim to identify biological mechanisms contributing to worsened outcomes in SAP patients, by examining how infection affects mortality, the brain’s immune response, and neurobehavioral outcomes in the murine model.

**Hypothesis:** Stroke and infected mice (stroke-infected) may do more poorly than mice with strokes alone (stroke-sham), due to immune training, where a prior injury amplifies the proinflammatory response upon the second insult, through an increase in brain leukocytes and proinflammatory cytokines.

**Methods:** Twelve C57BL/6J mice underwent middle cerebral artery occlusion stroke on day 0. On day 3, six animals were inoculated intra-tracheally with *Klebsiella pneumoniae*, while six received sham inoculation. 24 hours later, Gentamicin was initiated. On day 7, brains were harvested for flow cytometry. Cells were stained for identity (CD45, CD11b, CD4, CD8, CD25, CD19, Ly6G, Ly6C) and intracellular cytokines (IFN-gamma, TNF, and IL-6). A neurologic deficit score was conducted at day 3 pre-inoculation and on day 7 pre-sacrifice.

**Results:** Compared to stroke-sham, stroke-infected mice have increased brain leukocytes that produced more TNF. Between groups there were similar levels of cytotoxic T-cells and neutrophils but in the setting of concomitant infection, T-cells made less IL-6 and IFN-gamma, and neutrophils made less IFN-gamma and TNF. There were no differences in behavioral deficits. One stroke-infected mouse died pre-harvest.

**Conclusions:** Contrary to our hypothesis, infection after stroke attenuates the immune response by pushing neutrophils and cytotoxic T-cells towards an anti-inflammatory phenotype, reflected in their reduced production of pro-inflammatory cytokines, protecting against brain injury—consistent with no observable difference in behavioral deficit—but weakening mice’s ability to fight the infection, resulting in increased mortality.
Problem: Patients suffering from cancer or immune-related diseases that inhibit bone healing or even actively destroy bone experience poor outcomes from conventional responses to fractures. Additionally, cancer patients experiencing osteolysis may require post-operative care that interferes with oncological care, highlighting the importance of minimally invasive surgical alternatives.

Hypothesis: AORIF will significantly reduce pain and improve functional status for patients suffering from fractures of the proximal femur.

Methods: A single center prospective cohort investigation of 16 patients and 10 different underlying malignancies who underwent image-guided Ablation, balloon Osteoplasty, cement Reinforcement, and Internal Fixation (AORIF) for impending or minimally displaced pathological fractures of the proximal femur. Patients were recruited between September 2018 and June 2020 with a mean follow-up of 9.6 months. Primary outcomes (pain and function) were evaluated postoperatively, along with secondary outcomes such as surgical site infection, length of stay, and conversion to open surgery. Visual analogue, Musculoskeletal Tumor Society (MSTS), and combined pain and ambulatory function (PAF) scores were utilized to compare pre- and postoperative pain and functional status. Actual survival within the cohort was compared with that of a predictive survival model derived from the machine-learning tool, PATHFx3.0.

Results: Significant differences in assessed pain and functional status was observed within the first four weeks post-surgery. By the first postoperative encounter, VAS score improved from $8.6 \pm 2.5$ to $1.9 \pm 2.3$ ($p<0.001$), MSTS scores improved from $8.7 \pm 8.4$ to $24.0 \pm 7.6$ ($p<0.001$), and PAF scores improved from $4.5 \pm 2.5$ to $8.7 \pm 1.6$ ($p<0.001$).

Conclusions: AORIF provides a promising surgical route for patients likely to experience prolonged recovery due to invasive surgery, specifically patients suffering from cancer that may induce osteolysis.
Problem: Rodent models have shown that unpredictability of maternal signals early in life produces lasting cognitive dysfunction, but the influence of unpredictability of maternal mental health in the human prenatal environment is less established.

Hypothesis: The current study hypothesized that unpredictability of prenatal maternal mood influences the developing fetus independent of level of mood.

Methods: Depression and anxiety symptom questionnaires collected on two occasions during pregnancy from a prospective, longitudinal cohort study (N = 13,202) were used to calculate prenatal maternal mood unpredictability, operationalized using Shannon’s entropy. Child cognitive function and school performance were assessed at 8 and 5-7 years of age using age-appropriate exams. Maternally reported socio-emotional and behavioral problems were obtained using a validated questionnaire at 6 years of age. Potential confounders included obstetric risks, socioeconomic status, maternal pre and postnatal mood level, and postnatal mood entropy.

Results: Bivariate correlations and multiple linear regressions indicated that higher prenatal maternal anxiety entropy predicted lower cognitive function (Est. = -0.062, SE=0.019, p=0.001), lower standardized test scores (Est. = -0.012, SE=0.004, p=0.002), and increased externalizing behavioral problems (Est. = 0.021, SE=0.005, p<0.001), with no sex-specific effects. Prenatal maternal depression entropy was significantly associated only with the standardized writing test (Est = -0.003, SE=0.001, p=0.039), after accounting for covariates.

Conclusions: These findings indicate that the predictability of maternal mood in utero has a lasting influence on the developing brain. Entropy derived from standardized screening assessments of maternal antenatal anxiety/depression may be of use for understanding individual differences in child mental health.
Accelerating Research on Consciousness: An Adversarial Collaboration to Test Contradictory Predictions of Global Neuronal Workspace and Integrated Information Theory

Matthew M. Yuen, Hal Blumenfeld, MD, PhD

**Problem:** Two competing theories of consciousness – the Global Neuronal Workspace (GNW) and Integrated Information Theory (IIT) – have arisen without significant crosstalk. One of the main differences is that GNW asserts that the neural correlates of consciousness (NCCs) involve the prefrontal cortex (PFC), while IIT posits that NCCs are in the posterior areas of the brain.

**Hypothesis:** We hypothesize that differential activation of the PFC versus posterior areas during a conscious perception task will facilitate meaningful arbitration between the GNW and IIT.

**Methods:** Functional magnetic resonance imaging (fMRI) will be used to measure brain activity in participants as they complete a visual conscious perception task. Subjects will be presented with two target stimuli that will be either pictorial (faces and objects) or symbolic (letters and false-fonts). A sequence of suprathreshold stimuli of any type will be presented, and the subject will report when a target stimulus is shown.

Machine-learning classifiers will be constructed to decode conscious contents from the brain. Specifically, the classifier will predict whether a participant consciously perceived certain types of stimuli (e.g., face, object) based on patterns of activity in specific brain regions (e.g., PFC or posterior areas).

**Results:** GNW predicts that NCCs should be decodable from prefrontal areas. In contrast, IIT predicts that NCCs will be maximally decodable from posterior regions of the brain.

**Conclusions:** The results of the study will evaluate competing predictions of the GNW and IIT to assess which theory better explains the neural underpinnings of consciousness.
Cautious Deep Learning

Christina Jayaraj, Sanjay Aneja, MD

**Problem:** Machine learning algorithms provide clinicians the opportunity to achieve more accurate diagnoses and reduce medical errors. However, overconfidence in the generalizability of these models can lead to adverse clinical outcomes. As a means to combat this, the method of cautious deep learning acts as an overlaying layer that can be applied to any algorithm. This allows the model to mitigate variability in confidence levels across individual predictions and classify inputs with a null operator.

**Hypothesis:** Cautious Deep Learning will produce more accurate results compared to deep learning predictions in a more computationally efficient manner.

**Methods:** Cautious Deep Learning will be tested against Deep learning algorithms with an imaging data set with in three scenarios: 1) Comparison of algorithmic accuracy 2) Comparison of computational efficiency 3) Interpretation of out-of-distribution data sets

**Results:** The results of this experiment are ongoing

**Conclusions:** The overall goal for this project is to determine if cautious deep learning can be effectively employed in order to improve the accuracy of medical diagnosis, namely in medical oncology. As this is a relatively new application in the clinic, the specific scenarios in which this process can be applied is relatively unknown. In medical imaging, it can be difficult to apply machine learning algorithms due to limited data sets, epigenetic and genetic variability, and diverse imaging practices across institutions. In light of this, if machine learning algorithms are to be applied in clinic, it is necessary to employ a method which is highly accurate and specific.
Investigating T Cell-Mediated Immunity Using Sheep Red Blood Cells in Mice

Paul Serrato, Joseph Craft, MD

Problem: Follicular helper T (Tfh) cells are essential for the formation of germinal centers in secondary lymphoid organs and the processes that occur within them that help develop immunity. The goal is to introduce an antigen that can induce the proliferation of Tfh cells and germinal center B cells (expected) in mice.

Hypothesis: Immunizing mice with sheep red blood cells will increase the number of Tfh cells and germinal center B cells (expected).

Methods: We immunized mice via intraperitoneal injection using sheep red blood cells (SRBC) for the experimental group and compared them to a control group immunized with phosphatebuffered saline (PBS). We evaluated the splenocytes in both groups 7 days post-immunization using flow cytometry. We developed a gating strategy to identify follicular helper T cells and germinal center B cells (expected) based on known cell-surface markers.

Results: Two mice were immunized with SRBCs, and two were immunized with PBS. Follicular helper T cells increased in SRBC-immunized mice in terms of frequency (p=0.29) and absolute count (p=0.08). Germinal center B cells also increased in SRBC-immunized mice in terms of frequency and absolute count (expected).

Conclusions: There is an increase in follicular helper T cells and germinal center B cells (expected) in SBRC-immunized mice, but it was not statistically significant. Further studies should consider greater sample sizes (expected).
Spliceosomal Factor SMU1 Likely Plays a Role in Normal Cilia Function

Forest Seely Mahoney, Mustafa K. Khokha, MD

**Problem:** A six-month old male infant presented with a double inlet left ventricle, L-looping heart, and significant motor and developmental delays. Whole exome sequencing of the proband and both parents indicated a deleterious de novo variant in SMU1, a known spliceosomal factor.

**Hypothesis:** We hypothesize that the patient phenotype has a genetic mechanism and that SMU1 plays a role in proper cardiac, neural, and motor development.

**Methods:** Xenopus embryos with reduced SMU1 expression (SMU1-KOs) were generated via CRISPR-Cas9 gene editing. These embryos were compared with a set of unmanipulated controls (UCs), selected from the same clutch of fertilized eggs. SMU1-KOs and UCs were assayed for edema, length, pitx2 expression, ependymal cilia function, and epidermal multi-ciliated cell (MCC) density.

**Results:** SMU1-KOs demonstrated a greater ($p < 0.0001$) rate of severe edema at stage 43 (62.2%, $n = 222$) than did UCs (5.3%, $n = 246$). Further, SMU1 KO also decreased ($p < 0.0001$) the length of Xenopus embryos (18.1%). The assays for pitx2 expression, ependymal cilia function, and epidermal MCC density are in progress.

**Conclusions:** The increased rate of edema among SMU1-KOs indicate that SMU1 may cause a ciliopathy. Given the importance of cilia in determining internal asymmetry, these results are consistent with disorders that produce phenotypes similar to that of the patient discussed herein. Early edema precludes the ability to score tadpole hearts for laterality defects. Assessing molecular markers in the left-right patterning signaling cascade will allow the role of SMU1 in internal asymmetry to be properly evaluated. Further, a titrated SMU1 knock-down via morpholino oligonucleotides may better phenocopy the patient CHD.
The Effectiveness of Emergency Department-Initiated Sublingual Buprenorphine Versus Extended-Release Injectable Buprenorphine for Opioid Use Disorder

Michael Nock, Gail D’Onofrio, MD

Problem: The Emergency Department (ED) serves as a key point of care for patients with opioid use disorder (OUD). The effectiveness of ED-initiated sublingual (SL) buprenorphine for helping patients with OUD transition to addiction treatment was established by D’Onofrio et al. (2015). The effectiveness of an ED-initiated extended-release (XR) injectable formulation of buprenorphine is not yet known.

Hypothesis: A greater percentage of patients who are initiated with XR-buprenorphine will be engaged in addiction treatment at 7 days and 30 days following their initial ED visit than those patients who are initiated with SL-buprenorphine.

Methods: A randomized controlled trial (RCT) comparing the efficacy of SL-buprenorphine versus XR-buprenorphine initiation is being conducted in ~30 EDs across the nation. Patients at participating EDs are universally screened for eligibility in the study. ~2,000 ED patients will be enrolled in the study, and ~1,000 will be randomized to receive SL-buprenorphine initiation and ~1,000 will be randomized to receive XR-buprenorphine initiation. Study participants will be followed up with at 7 days and 30 days following the initial ED visit to determine their engagement in addiction treatment.

Results: The RCT is currently in progress; addiction treatment engagement data will be analyzed at the conclusion of the study.

Conclusions: If the results of the RCT are consistent with the hypothesis presented here, XR-buprenorphine initiation should serve as the standard of care for patients who present to the ED with untreated OUD.
Evaluating The Effects of Pre-operative Statin Therapy on Periprocedural Outcomes in TAVR Patients

Rafael Maarek, Alexandra Lansky, MD

Problem: Transcatheter aortic valve replacement (TAVR) patients suffer from increased risk of various periprocedural adverse events. While traditionally prescribed to lower cholesterol, statins have anti-inflammatory effects and have been shown to reduce the risk of atrial fibrillation in patients following vascular surgery. Whether pre-operative statin therapy can reduce the risk of periprocedural adverse events in TAVR patients remains unclear.

Hypothesis: Pre-operative statin therapy reduces the risk of periprocedural adverse events post-TAVR.

Methods: Baseline characteristics, statin usage data, and outcome data from 497 consecutive TAVR patients enrolled in the Yale TAVR registry were extracted from the electronic medical record. Patients taking statins pre-TAVR were compared against patients not taking statins pre-TAVR. The primary endpoint was permanent pacemaker placement at 30 days post-TAVR. Other endpoints included new-onset atrial fibrillation/flutter, other conduction abnormalities, and 30-day mortality.

Results: 371 patients (74.6%) underwent pre-operative statin therapy. Patients on statins were more likely to be male (61.5% vs. 39.7%, p<0.001) and had lower STS-risk scores (4.95 vs. 6.37, p<0.001). Pre-operative statin therapy was associated with a nearly significant reduction in permanent pacemaker placement at 30 days (OR = 0.70, p = 0.057). There were no significant differences between the two groups with regards to new-onset atrial fibrillation/flutter, other conduction abnormalities, or 30-day mortality (all p’s>0.1).

Conclusions: Our study suggests that pre-operative statin therapy may be associated with reduced incidence of permanent pacemaker placement post-TAVR.
Identifying and Characterizing TRP-Modulating Gut Microbial Metabolites

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**Problem:** The gut microbiome is colonized by trillions of microbes producing unique chemical compounds that can potently impact human biology. Transient receptor potential (TRP) channels, involved in diverse physiological processes, are expressed across tissues comprising the gut barrier. We aim to elucidate the network of vastly unknown microbe-TRP channel interactions to better understand related mechanisms of disease.

**Hypothesis:** We have previously displayed using GCaMP that microbial metabolites can activate the nociceptive TrpV1 channel. We hypothesize that adapting a molecular calcium integrator to a sequencing platform will facilitate multiplex high-throughput screening of microbial metabolites against all TRP channels

**Methods:** A previously identified interaction between Acidaminococcus intestini and TrpV1 was validated through GCaMP. We utilized the FLiCRE system as a model to design a protease circuit. This circuit was adapted into piggyBac vectors to facilitate generation of a stable HEK 293 line. Known TRP channels were linked to unique nucleic acid barcodes to facilitate sequencing.

**Results:** TrpV1 HEK 293s exposed to isolate A. intestini supernatant showed significantly greater fold change activation of TrpV1 than PBS controls (5.52 vs. 1, p = 0.0002). TrpV1 activation induced by A. intestini supernatant was not significantly changed upon introduction of a TrpV1 antagonist (p > 0.05). TEV-circuit transfected HEK cells showed significantly greater fluorescence activity upon calcium induction than in controls (94962 vs. -246.8 RFU, p = 0.0076).

**Conclusions:** Validation of the A. intestini-Trpv1 interaction suggests that microbes are involved in TRP channel activation and modulation. Our initial circuit design shows functionality as a calcium reporter, setting the stage for future nucleic acid barcoding and stable cell line generation.
Parkinson’s Disease Protein DJ-1 Facilitates the Localization of ATPβ mRNA to Outer Neurites

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Problem: Parkinson’s disease is the second most common neurodegenerative disease. Many genes have been linked to development of Parkinson’s, including Park7 which encodes DJ-1, a neuroprotective protein. DJ-1’s functions are not fully understood, however it has been shown to bind to ATP synthase β protein and mRNA promoting ATPβ expression, as well as increase ATP synthase activity, and maintain the integrity of the inner mitochondrial membrane. The mechanism by which DJ-1 exerts this effect still needs to be fully elucidated in order to contribute to our knowledge of Parkinson’s disease and uncover new treatments.

Hypothesis: DJ-1 is affecting ATP synthase β subunit through facilitating ATPβ mRNA localization to outer neurites.

Methods: RNAscope Technology was used to visualize ATPβ mRNA in dopaminergic neurons with immunofluorescent labels in mice with or without DJ-1 expression. Confocal microscopy imaging and analysis enabled us to investigate DJ-1’s on ATP mRNA localization.

Results: DJ-1-/- mice expressed less ATPβ mRNA outside of the soma compared to WT. Following the same culture over multiple weeks revealed that ATPβ mRNA in the soma of the neuron was not different between WT vs DJ-/- mice until the 4th week, in which DJ-1-/- mice had lower expression.

Conclusion: Our results show that without DJ-1, ATPβ mRNA is not being localized to the outer neurites. This suggests the DJ-1 has a role in targeting ATPβ mRNA to neurites in order to facilitate proper mitochondrial function.
In Vivo Imaging of Astrocyte Calcium Dynamics During Cell Death and Injury

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Problem: Astrocytes are abundant, multifunctional glial cells with unique membrane morphology composed of fine dynamic processes. As one of few gap-junction coupled cell types in the brain, astrocytes are thought to convey environmental changes sensed by these processes through astrocytic networks. Several lines of evidence suggest astrocyte signaling is primarily mediated by transient changes in intracellular calcium concentration, particularly in their processes, and subsequent calcium wave propagation. While a recent study demonstrated a phagocytic role for astrocytes in neuronal cell corpse clearance, in vivo astrocytic response to astrocyte death and other forms of injury are not well understood.

Hypothesis: We hypothesize that astrocytes respond to such insults through modulating calcium signaling.

Methods: Previously our lab has developed a technique to induce cell death using two-photon chemical apoptotic targeted ablation (2Phatal) in the living mouse brain. The 2Phatal strategy, along with two-photon thermal injury, allows us to capture potential changes to calcium transients with high spatiotemporal precision. To visualize calcium transients in vivo, we labeled astrocytes with a membrane-localized genetically encoded calcium indicator GCaMP6 through either adeno-associated virus injection into the subarachnoid space or in utero electroporation.

Results: We have performed both methodologies on a group of animals and have begun acquiring astrocyte process calcium imaging. We plan to monitor calcium transients through short time-series prior to ablation and after cell clearance.

Conclusions: Observing in vivo astrocytic responses to targeted astrocyte cell death and injury will allow further insight into local astrocyte-astrocyte interactions, as well as the astrocytic network more globally.
**Type 1 Interferon Induces PD-1highCXCR5- Peripheral Helper T Cells**

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**Problem:** T cell–B cell interactions are fundamental for the generation of protective antibody responses, as well as for the development of harmful autoimmune diseases. Recent studies in patients with autoimmune diseases such as rheumatoid arthritis have established another T cell population separate from follicular helper T cells named PD-1highCXCR5– peripheral helper T (Tph) cells. While they play a role in extra-follicular B cell differentiation, the factors that drive the differentiation of human CD4+ T cells into Tph cells remain largely unexplored.

**Hypothesis:** We recently demonstrated the increase of Tph cells in the acute phase of COVID-19, which led us to hypothesize that type 1 interferon (IFN-I) is important for their induction.

**Methods:** To characterize PD-1highCXCR5-Tph cells six subsets of memory CD4+ T cells, categorized by PD-1 and CXCR5 expressions, were sorted from patients with COVID-19 and gene expression profiles were examined by bulk RNA-seq. CD4+T cells were stimulated with anti-CD3/CD28 antibodies for 4 days in the presence of IFN-I in vitro and analyzed several molecules expressed by Tph cells.

**Results:** Compared to the other five subsets in memory CD4+ T cells, CXCR6, LAG3, and PRR5L were upregulated, and CHD7, ZBTB20, ZNF251, GRK25, and GPRASP1 were downregulated in Tph cells. Most of these genes had the same directions for their expression levels after IFN-I stimulation in vitro.

**Conclusions:** IFN-I plays a significant role in the induction of gene signatures with regard to PD-1highCXCR5- Tph cells. Further research may provide insight on a variety of immune-mediated diseases with possible contribution of aberrant T-B interaction.