

# Klarman Family Foundation Grants Program in Eating Disorders Research 2013 Award Recipients

## Two-Year Awards

- **Mark Andermann, Ph.D.**

Assistant Professor of Medicine

*Beth Israel Deaconess Medical Center, Harvard Medical School*

“Cortical Circuits Underlying Attentional Biases in Binge Eating Disorder”

Key Words: Binge eating, Attention, Reward, Cortex, Two-photon calcium imaging, Awake mouse, Dopamine, Pharmacogenetic inactivation

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Patients with binge eating and associated eating disorders often demonstrate increased attention to cues associated with high-calorie foods. Inappropriate and often uncontrollable attention to high-calorie food cues represents an important target for therapeutic intervention. At the neural level, human functional imaging studies have identified a network of cortical brain areas that is biased to processing of high-calorie food cues, particularly in patients with binge-eating behaviors. The biases in this brain network may reflect its strong sensitivity to reward-related dopaminergic neuromodulation. However, little is known about the precise microcircuitry that guides attention of food cues, or how a 'loss of control' of this circuitry might trigger unbridled cue-reactivity, craving, seeking, and binge eating of high-calorie foods.

The goals of this proposal are to define the sensory cortical circuits and dopaminergic inputs to cortex involved in inducing and sustaining pronounced attention to high-calorie food cues during the development of cue-food associations. We will combine periods of food restriction with associative learning sessions pairing visual cues with high-calorie foods -- a procedure that gradually induces binge-like eating behavior in rodents. Visual cortical responses to these cues will be recorded from the same neurons across interspersed daily sessions prior to and during the development of appetitive conditioning to high-calorie foods, using long-term widefield and two-photon calcium imaging across spatial scales in awake, head-fixed mice. Specifically, we will image simultaneous responses to food-associated and neutral visual cues across multiple cortical areas (Aim 1), across hundreds of dopamine-sensitive and neighboring neurons in all cortical layers within a cortical area (Aim 2), and in dopaminergic axonal inputs to that area (Aim 3).

The proposed experiments will establish a unique mouse model system that will propel translational research efforts to dissect the cortical microcircuits driving the inappropriate attention to high-calorie foods in binge eating and associated disorders.

- **David Anderson, Ph.D.**

Seymour Benzer Professor of Biology; Investigator, Howard Hughes Medical Institute  
*California Institute of Technology*

“Dissecting the Central Amygdala Neural Circuits of Anorexia”

Key Words: Anorexia, Eating disorders, Central amygdala, Neural circuits, Protein kinase C delta, Neuron, Optogenetics

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The neuronal cause of anorexia nervosa and other eating disorders is unknown, but it is increasingly acknowledged that neural circuits for appetite, body weight and emotions contribute significantly to eating disorders. Our long term goal is to determine the specific neural circuits, abnormality of which cause eating disorders, and identify druggable targets in the brain.

Using novel genetic methods, we have identified a specific subpopulation of neurons in the lateral part of central amygdala (CEl), marked by the expression of protein kinase C delta. Preliminary results have revealed that optogenetic activation of CEI protein kinase C delta neurons induces a severe anorexic effect. We hypothesize that the CEI protein kinase C delta neurons regulate anorexic behavior through a circuit that involves their connections with the lateral parabrachial nucleus (LPB) and other input or output brain regions.

To dissect the underlying neural circuits, we will (1) determine the output pathway of CEI protein kinase C delta neurons for anorexia using optogenetic and behavioral assays, and characterize the downstream neurons; (2) systematically screen the upstream brain regions that send input to CEI protein kinase C delta neurons using the Cre-dependent monosynaptic retrograde rabies system, and optogenetically manipulating the upstream neurons to test their role in feeding behavior; (3) finally, we will use chemogenetic methods to ablate the neural activity of CEI protein kinase C delta neurons, and test if this would attenuate the anorexic effect in mice models of anorexia.

This work will help establish the neural circuits that regulate anorexia, and help understand the neuronal cause of eating disorders.

- **Tamas Horvath, D.V.M., Ph.D.**

Chairman, Section of Comparative Medicine; Professor of Comparative Medicine  
*Yale School of Medicine*

“AgRP-driven Mal-adaptations of Higher Brain Functions in Anorexia Nervosa”

Key Words: Anorexia Nervosa, OCD, Animal Model, Hypothalamus, Midbrain, Hippocampus, Cortex

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Eating disorders associated with chronic negative energy balance, such as anorexia nervosa and bulimia nervosa, show clear sex differences with a significantly higher number of women suffering from these disorders than men (Rolls et al., 1992; Schousboe et al., 2003; Zigman and Elmquist, 2003). While the initial trigger to the development of these diseases may vary between individuals, we suggest that the onset of the disease results from altered cortical functions triggered by hypothalamic circuitry expressing Agouti-related peptide (AgRP). Based on our preliminary data, we hypothesize that there are both developmental and adult components of the action of the AgRP system on altered higher brain functions, each of which alone as well as together can bring about disease development. We showed that during early postnatal period, AgRP circuit regulates the development of adult excitability of the midbrain reward circuitry, an event that we believe is an important determinant of the vulnerability to anorexia nervosa in adult/adolescent females. We also propose that activation of AgRP neurons by chronic negative energy balance in the adult/adolescent female can propagate the onset of anorexia nervosa symptoms. We derive this assertion from our preliminary studies showing that remote activation of AgRP neurons in adult female mice robustly induces altered cortical oscillations and behaviors similar to obsessive-compulsive disorder (OCD). We hypothesize that when the developmental and adult effects of AgRP neuronal system are combined, the most severe promotion of anorexia-nervosa-like and OCD symptoms will emerge. We will test these hypotheses through 2 specific aims utilizing our newly developed animal models of AgRP neuronal control in anorexia nervosa and OCD paradigms.

- **Timothy Moran, Ph.D.**

Paul R. McHugh Professor

*Johns Hopkins University School of Medicine*

“Role of Corticotropin Releasing Factor in Exercise Induced Avoidance of High Fat Diet”

Key Words: Exercise, Diet preference, Corticotropin releasing factor, siRNA

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Anorexia nervosa (AN) is an eating disorder of unknown etiology that can have a chronic course with frequent relapses. Patients with AN have abnormal eating behavior and are obsessed with losing weight. Typically AN sets in around puberty and up to 60% of patients report that involvements in sports or regular exercise preceded the dieting in the development of the disorder. In addition, 75% of patients become obsessed with exercise during the acute phase of the disorder and there is an inverse relationship between food intake and physical activity. Despite the apparent relationship between exercise and dieting in AN, we do not have a clear understanding of the mechanisms underlying the effects of exercise on food intake or diet choice. Our preliminary data demonstrate that wheel running in a rat model significantly suppresses the intake of highly palatable, energy dense foods (e.g. a high fat or high sucrose diets), in favor of consuming less dense, less palatable diets. The experiments in this proposal will investigate the mechanisms underlying WR induced decreases in preferences for highly palatable diets. Prior data have indicated a role for corticotropin releasing factor (CRF) signaling in the effects of exercise on energy balance. Thus, it is hypothesized that central CRF signaling is involved in exercise-induced shifts in palatable diet preference. The hypothesis will be tested using behavioral, physiological, pharmacological and genetic approaches. Specifically, viral-mediated knockdown of CRF signaling will be used to elucidate neural circuits that are critically involved in the shift of palatable diet preference induced by exercise. The overall results will contribute significantly to our understanding of how exercise affects food intake and food choice and may lead to new conceptualizations of the etiology and maintenance of eating disorders.

- **Garret Stuber, Ph.D.**

Assistant Professor of Psychiatry

*University of North Carolina at Chapel Hill*

“Extended Amygdala–hypothalamic Circuits that Regulate Feeding”

Key Words: Extended amygdala, BNST, Hypothalamus, Reward, Optogenetics, Electrophysiology, Binge eating, Food intake, Obesity

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Eating disorders, characterized by insufficient or excessive food intake, exact a tremendous toll on society, as well as the mental and physical health of those who suffer from them. While seeking out and consuming caloric substances is essential for survival, specific brain circuits that control adaptive food seeking, likely become dysregulated to promote binge–eating behavior. Thus, experiments designed to further understand the specific neural circuit components that control excessive food consumption are of critical importance for identifying important novel neurocircuit targets for the development of future treatments for eating disorders. Lesions of the lateral hypothalamus (LH) suppress food intake, while stimulation of this structure can elicit feeding, however the precise neural circuit afferents that regulated LH circuits to control excessive food intake, as well as the precise orchestration of behavior by genetically defined postsynaptic neurons in the LH is still poorly understood. One of the densest inputs to the LH comes from the bed nucleus of the stria terminalis (BNST) an area of the extended amygdala critical for integrating states ranging from motivation, emotion, stress, and reproduction. The goal of the experiments proposed here are to dissect the neural circuit connectivity between BNST GABAergic projections to genetically distinct LH neurons, and to delineate the contribution of this pathway in regulating food intake. Overall, these experiments will provide novel and important information into the neural circuit mechanisms that regulate LH circuits to control feeding behavior.

## One-Year Awards

- **Nicholas Bello, Ph.D.**

Assistant Professor

*Rutgers University*

“Dietary-Induced Binge Eating Effects on the Noradrenergic Controls of Stress and Feeding”

Key Words: Alpha 2A adrenergic, Bulimia nervosa, Binge eating, Prefrontal cortex, Corticotropin-releasing factor, Guanfacine, Treatment

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Binge eating is a prominent feature of bulimia nervosa (BN) and binge eating disorder (BED). Treatment is often difficult for BN and BED because binge eating is accompanied by a sense of a "loss of control". Because stress or perceived stress is an often cited reason for binge eating, one notion is that the neural pathways that overlap with stress reactivity and feeding behavior are altered by recurrent binge eating to promote a sense of a "loss of control". A critical brain structure for attention and impulsivity is the medial prefrontal cortex (mPFC). Norepinephrine (NE) in the mPFC indirectly influences corticotrophin-releasing factor (CRF) in the hypothalamic paraventricular nucleus (PVN) and CRF neurons directly project to caudal hindbrain feeding structures. The hypothesis of this pilot project is that dietary conditions that promote binge eating in female rats reduce NE in the dorsal medial prefrontal cortex (mPFC) to attenuate the neuroendocrine stress responses and decrease hindbrain satiety. Aim 1 will use in vivo microdialysis to determine NE efflux in the dorsal mPFC during binge-like eating and to a standardized binge food. Because alpha 2A adrenergic receptor activation strengthens prefrontal cortical networks, Aim 2 will determine whether chronic peripheral infusion of an alpha 2A adrenergic receptor agonist, guanfacine, can alter the binge-like feeding and stress reactivity in animals exposed to dietary-induced binge eating. Aim 3 will use double-labeled immunohistochemistry to determine whether dietary-induced binge eating alters the neural activation of CRF neurons in PVN and hindbrain feeding areas. This proposal would be the first to explore whether dietary conditions that promote binge eating alter a NE-modulated descending neural pathway that impacts hindbrain feeding structures. These findings would provide experimental evidence for examining the feasibility of using NE-targeted strategies in the treatment of BN and BED.

- **Mark Laubach, Ph.D.**

Associate Fellow, The John B. Pierce Laboratory

Associate Professor of Neurobiology,

*Yale School of Medicine*

“Apathy versus Exuberance: Optogenetic Control of Food Seeking in Corticostriatal Systems”

Key Words: Nucleus accumbens, Medial prefrontal cortex, Multi-electrode recording, Optogenetics, Progressive ratio procedure

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The goal of this project is to test the hypothesis that the medial prefrontal cortex and nucleus accumbens have opposing roles in the control of food seeking behavior. The prefrontal cortex is proposed to mediate positive motivational control over food seeking. Dysfunction in this part of the brain results in apathy and/or anxiety about eating and encountering food-related stimuli. By contrast, the nucleus accumbens is proposed to mediate a negative motivational control over food seeking, with dysfunction leading to uncontrolled, overexuberant engagement in food-related behaviors. In Aim 1, we will test this hypothesis using cutting-edge optogenetic methods for reversibly inactivating the medial prefrontal cortex and striatum in animals performing an effort-based decision-making task. This experiment will directly compare effects of silencing the two brain regions during effort-based decision-making and will determine if they have distinct functions in the control of eating. In Aim 2, we will record neuronal activity in the nucleus accumbens while optogenetically inactivating the medial prefrontal cortex. This experiment will determine the influences of prefrontal processing on instrumental and consummatory related neuronal activity in the nucleus accumbens. In Aim 3, we will record neuronal activity in the medial prefrontal cortex while optogenetically inactivating the nucleus accumbens. This experiment will determine how blocking cortico-basal ganglia loops alters instrumental and consummatory related neuronal activity in the frontal cortex. Our results will shed light on the neuronal circuits that mediate motivational control of eating, will contribute to understanding how dysfunction in these circuits may lead to eating disorders, and will provide neuronal signatures of cortical and accumbens processing that could be used to help develop therapeutic treatments for eating disorders.

- **Andres Lozano, M.D., Ph.D.**

Professor of Surgery

*University Health Network, Toronto*

“Phase II Trial of Deep Brain Stimulation of the Subcallosal Cingulum for Treatment-Refractory Anorexia Nervosa”

Key Words: Deep Brain Stimulation, Anorexia Nervosa, Subcallosal Cingulate, Depression, Affective Regulation, Phase II Trial

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Anorexia Nervosa (AN) has the highest mortality rate of any psychiatric illness, with current treatment options largely ineffective in altering the natural history of the most seriously ill, and treatment-refractory patients. We have previously proposed that Deep Brain Stimulation (DBS), a minimally invasive and non-ablative surgical procedure commonly used in movement disorders, may be effective in modulating activity in circuits driving and maintaining AN. We have now completed the world's first phase I trial of DBS in treatment-refractory AN, and have shown that DBS of a critical structure in mood circuitry, the subcallosal cingulate (SCC), is: i) safe in AN patients, and ii) leads to significant improvements in mood, anxiety and affective regulation that translate into a potentiation of previously ineffective treatments. Here, we propose to expand our pilot project, and conduct a phase II clinical trial of SCC DBS in treatment-refractory AN. Our broad objectives are to establish the efficacy of DBS in a larger patient population, as well as determine characteristics of response based on patient profiles, disease subtype and neuroimaging characteristics. Psychometric evaluations, exploring mood, anxiety, affective regulation and eating attitudes, will be performed at baseline and at 1-, 3-, 6- and 12-months after stimulation, with structural (MRI) and functional neuroimaging (PET) performed at 6- and 12-months. The open-label, prospective trial will study 20 patients (in addition to our initial cohort of 12), who will undergo bilateral stimulation of the subcallosal cingulate. Primary clinical outcomes will focus on improvements in psychiatric comorbidity and improved weight outcomes, with imaging outcomes defining the circuitry of AN and establishing differential metabolic effects in responders and non-responders, as well as possible predictors of response. The results of this trial may substantially improve our understanding of refractory AN, and help to establish a novel treatment option in this challenging patient population.



- **D. Blake Woodside, M.D.**

Professor of Psychiatry

*University of Toronto*

“Dorsomedial Prefrontal Cortex r-TMS in Anorexia Nervosa and Bulimia Nervosa– a Pilot Study”

Key Words: DMPFC–rTMS, Anorexia Nervosa, Bulimia Nervosa

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This project proposes to perform a pilot study of fMRI targeted Dorsomedial Prefrontal Cortex repetitive transcranial magnetic stimulation (DMPFC–rTMS) on a sample of 20 individuals with Bulimia Nervosa (BN) at a normal weight, and 20 individuals with Anorexia Nervosa (AN). The DMPFC is a novel target for rTMS, is heavily implicated in the regulation of mood and affect, and has been suggested to be involved in AN, BN, major depression, OCD, and PTSD. Improved function in this area might lead to reduced AN and BN symptoms, such as bingeing, purging, and over–activity, perhaps by improved regulation of mood and affect. Improvements in important areas of comorbidity might allow for better response to intensive treatment for AN and BN, and reduce relapse rates after such treatment. The DMPFC may be a more appropriate target for compared to the Dorsolateral prefrontal cortex (DLPFC), which has typically been the focus of stimulation in the past. Our initial preliminary pilot work has noted unexpected and significant improvements in some core ED symptoms (bingeing and purging) and in important areas of comorbidity (OCD and PTSD), along with expected improvements in mood. These changes have allowed some treatment resistant patients to either complete intensive treatment or be successful in maintaining their progress post intensive treatment. Subjects will receive up to 30 sessions of bilateral DMPFC–rTMS. Response will be evaluated clinically, via psychometric measures, and pre and post fMRI.

- **Lori Zeltser, Ph.D.**  
Assistant Professor  
*Columbia University*

“Multi-Factorial Mouse Model to Study the Onset of AN-like Behavior”

Key Words: Anorexia nervosa, Mouse model, BDNF

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As anorexia nervosa (AN) is a multi-factorial disease involving interactions between genetic, environmental and psychological factors, developing an appropriate mouse model is challenging. By combining factors that are consistently associated with increased risk of AN – adolescent females, genetic predisposition to anxiety, social stress and dieting – we defined a set of conditions that can elicit AN-like behavior. More than 50% of the mice subjected to this protocol exhibit at least one “aphagic episode”, defined as consumption of <0.5g food over a 24hr period. Aphagic episodes are often associated with >15% body weight loss, satisfying a major diagnostic criterion for AN. Although these episodes are usually followed by a rapid rebound to the initial body weight, several mice have died when they refused to eat for several consecutive days.

An important feature of this model is that the timing and number of aphagic episodes are uniquely determined in each animal, affording the opportunity to study physiological and neuronal processes associated with the onset of AN-like behavior, an issue that cannot be addressed in patients with an established AN diagnosis. The studies outlined in this proposal are designed to further define how dieting and genetic predisposition to anxiety influence the onset and severity of AN-like behaviors in our model. In the short term, the proposed experiments will better define risk factors and triggers for AN-like behavior. In the long term, novel insights into changes in neuronal circuits accompanying aphagia-associated weight loss could lead to the development of new strategies to diagnose and treat the early stages of AN.