



THE KLARMAN FAMILY FOUNDATION

Eating Disorders Research Grants Program 2017 Grant Cycle

Grant Recipients

Three-Year Grants

Mark Andermann, Ph.D.

Beth Israel Deaconess Medical Center

“Focus on the positive: pathways for biasing responses to mixed-valence food cues”

Fasting increases positive reactions to food signals, and decreases defensive reactions to danger signals that inhibit food-seeking. Patients with anorexia nervosa (AN) exhibit a selective behavioral bias to danger signals, and an associated neural response bias insular cortex, an area essential for food-seeking under threat. We hypothesize that habitual fasting in AN patients serves to partially attenuate the flow of anxiety-promoting signals to the basolateral amygdala (BLA), and that the behavioral and insular cortex hypersensitivity to danger signals in AN is mediated by excessive flow of anxiety-promoting signals from the paraventricular thalamus (PVT) to BLA, thereby modifying the perceived valence and salience of learned cues. To test this hypothesis, we will use a new method to repeatedly image the activity of hundreds of identified neurons in the insular cortex of healthy and AN model mice exposed to visual food cues, danger cues, or mixed cues, combined with specific manipulations of the pathway from PVT to BLA. We will then test whether the excessive behavioral and neural responses to danger cues in AN mouse model can be rescued by directly tamping down the pathway from PVT to BLA, thereby providing a sensitive framework for development of pathway-specific therapies.

Scott Crow, M.D.

University of Minnesota

“Goal based learning and habit in Anorexia Nervosa”

Anorexia Nervosa (AN) symptoms are difficult to treat and often last for years or even decades. This may be because they serve a purpose by making people feel better or helping to eliminate bad feelings (goal directed learning), or because overtime they become ingrained and second-nature (habit learning). This study will follow people with AN for 12 months. At the beginning, goal directed and habit learning will be measured using brain scans and questions that people

answer on their cell phones. We predict that evidence of goal directed learning will be stronger in people whose AN has recently started and evidence of habit learning will be stronger in people who have had AN longer. We also predict that evidence of habit learning will predict having more eating disorder problems after 12 months. Understanding the how the brain makes decisions at different time points during AN can help us to develop better treatments that will be well suited to different stages of illness.

Daphna Shohamy, Ph.D.

Columbia University

“Mechanisms of decision-making in Anorexia Nervosa: a computational psychiatry approach”

Anorexia Nervosa is a devastating illness with substantial morbidity and a mortality rate among the highest of any psychiatric illness, characterized by persistent, rigid dietary restriction leading to low body weight and complications of starvation. We propose to examine the cognitive and neural mechanisms that contribute to choice of foods in patients with Anorexia Nervosa, using state-of-the-art tools and computational modeling, to determine whether and how these processes differ from healthy controls, what the underlying brain differences are, and whether and how behavioral training can modify choice. Our study will develop a new pathway for understanding and treating the salient behavior of restrictive eating that contributes to the significant disease burden of Anorexia Nervosa.

Kellie Tamashiro, Ph.D. and Angela Guarda, M.D.

Johns Hopkins School of Medicine

“Neural Mechanisms of Appetite Dysregulation in Anorexia Nervosa: The Role of AgRP”

Anorexia Nervosa (AN) is a severe eating disorder for which there is no effective treatment. AgRP is a brain neuropeptide that increases appetite and is involved in addiction, anxiety-related behaviors and response to stress. Individual differences in AgRP and its brain circuits may explain why some patients develop severe and enduring AN and others recover. The rodent model of AN known as “activity-based anorexia” (ABA) results in self-starvation, excessive exercise and weight loss. Our preliminary data found that a subset of ABA animals (those with a passive coping style and exposed to early life stress) had lower AgRP levels and lost more weight. This proposal examines AgRP in adult inpatients with AN before and after weight gain. We will measure blood levels of AgRP and their relationship to brain imaging in women with AN compared to healthy women. Results will provide new information about brain changes in AN and how these predict differing weight restoration trajectories and treatment response. This information could help clinicians identify what therapy will be most effective for which patients. Furthermore, results could identify novel targets in the brain that may help develop more effective medications or treatment interventions for AN and related eating disorders.

Lori Zeltser, Ph.D.
Columbia University

“Stress and Eating Behavior in Anorexia Nervosa”

Structured inpatient behavioral treatment programs can increase food intake and restore body weight in individuals with anorexia nervosa (AN), but there is a very high rate of relapse. The Eating Disorders Research Unit at the New York State Psychiatric Institute has used objective measurements in laboratory-based meal tests to assess eating behaviors in AN. We demonstrated that there is a strong link between anxiety and the avoidance of high calorie foods, even after body weight restoration. Moreover, consumption of a lower fraction of calories from fat at discharge is associated with poor outcomes one year later. Since anti-anxiety medication fails to impact anxiety or food intake in AN, there is a need to identify new therapeutic targets. The Zeltser lab at Columbia University has developed a mouse model to study how social stress and dieting can lead to anorexia-like behavior in genetically-susceptible individuals. We used this model to identify a novel target for AN treatment. The proposed studies will validate assays to evaluate the relationship between anxiety and avoidance of fattening foods in mice and human subjects and thereby provide a critical foundation to assess the potential clinical utility of candidate pharmaceutical compounds in AN.

One-Year Pilot Studies

Scott Bunce, Ph.D. and Fauzia Mahr, M.D.
Pennsylvania State University

“Evaluating the Role of the Bed Nucleus of the Stria Terminalis in the Neurocircuitry of Anxiety in Patients with Anorexia Nervosa Restricting Type”

Anxiety contributes to the development and maintenance of anorexia nervosa (AN) symptoms, and high trait anxiety and anxiety disorders are common in AN patients. A very small nucleus, the bed nucleus of stria terminalis (BNST), plays a critical role in organizing how we respond to anxiety-producing (versus acute fear) situations. Elevated resting BNST metabolism mediates anxious temperament in primates. The BNST is also smaller in females, and, in animal models, this change occurs around or after puberty through cell death in females and cell growth in males. However, no studies have reported examining the structure or function of BNST in AN. In this study, we use a novel form of a well-known neuroimaging technique (fMRI) to image both size and function of BNST while participants engage in an anxiety-eliciting task. Uncertain, versus certain, expectation of seeing unpleasant images is expected to elicit greater BNST activation in both AN and high anxiety adolescent females relative to healthy controls. When the task "threat" is changed to the receipt of a sweet taste, we expect differential BNST activations in AN relative

to both high anxiety and healthy control groups. This research is expected to help design new treatment targets for patients with AN.

Jeffrey Friedman, M.D., Ph.D.

Rockefeller University

“Dissecting a Septal Circuit Involved in the Crosstalk between Stress, Anxiety and Anorexia”

Anorexia Nervosa (AN) is a clinically important syndrome that tends to affect young women and that is characterized by restrictive eating. The National Association of Anorexia Nervosa and Associated Disorders (ANAD) estimates that 0.9% of American women suffer from AN in their lifetime and 25% of deaths from AN patients are from suicide. About half of patients with AN also present depression and anxiety as a common comorbidity. However, it remains unclear how these psychiatric alterations influence the neurobiology of AN. Neurotensin is a peptide that regulates mood by interacting with the dopaminergic system in mammals and its levels in plasma are altered in patients with affective disorders.

We propose that neurotensin plays a role in the neurobiology of AN by providing a biological link between mood alterations and restrictive eating. By using state-of-art techniques in imaging, neuroscience and behavioral science we will identify the neural circuitry involved in the cross talk between anxiety and restrictive eating and further manipulate these circuits in order to prevent AN symptoms. Our proposal will provide a novel target for treatments for AN and will shed light on the biology underlying AN comorbidities.

Joel E. Kleinman, M.D.

Johns Hopkins University

“Molecular Biology of Anorexia Nervosa”

Anorexia nervosa (AN) is a complex psychiatric and human brain disorder with a high rate of morbidity and mortality. Current treatment options thus far have mixed success in achieving remission in AN. In order to understand the underpinnings of AN, our lab has a long-term objective to elucidate the molecular biology of AN with the goal of identifying potential novel targets for treatment. Two specific aims are required to achieve this goal. First, is to determine the molecular biological mechanism of the genetic risk. Second, is to determine if that mechanism is abnormal in the brains of patients with AN/ED. This will be accomplished with molecular biological methods (RNA sequencing and genotyping) routinely used at Lieber Institute for Brain Development (LIBD) and applied to a unique collection of AN/ED brains and controls collected and characterized by LIBD.

Carrie J. McAdams, M.D., Ph.D.

University of Texas Southwestern Medical Center

“Biological Factors Related to Treatment-Refractory Anorexia Nervosa”

Anorexia nervosa is defined as a mental illness because many of its symptoms include cognitive distortions and behavioral changes related to feeding. However, in healthy people, physiological changes, such as occur during physical illness, also systematically alter cognitive perception and behaviors. We have identified differences in brain activations in anorexia nervosa related to course of disease, by comparing recently-ill women to long-term recovered women; interestingly, these differences are in the same brain regions that change during physical illness. Anorexia nervosa involves severe physical changes, which include elevated levels of inflammation and changes to the types of symbiotic bacteria that inhabit the gastrointestinal track. This proposal will examine whether physiological changes related to inflammation correlate with neural activations in women with anorexia nervosa, by simultaneously measuring brain function, levels of inflammation in the blood and types of bacteria in the gut. Second, this pilot study will recruit subjects in two specialized cohorts: those in sustained weight-recovery following anorexia nervosa and those that have been treatment-refractory. These two groups will be compared to determine if factors related to inflammation perpetuate the disease by changing the brain to alter cognition and behaviors.

Carol B Peterson, Ph.D.

University of Minnesota

“Investigating the Impact of Oxytocin on the Neurobiological Underpinnings of Socioemotional Deficits in Anorexia Nervosa”

This investigation will examine how oxytocin (OT), a hormone that impacts social behavior, can change the brain functioning of individuals with anorexia nervosa (AN). Brain scans of adults with AN will be compared to brain scans of individuals without AN after receiving OT (nasal spray) and viewing pictures of faces with different emotions and completing a task related to social acceptance and rejection. Study participants will also eat a test meal after receiving OT to examine the impact on eating behavior. Brain scans and eating behaviors of individuals with AN who receive OT will also be compared with scans of the same individuals who, on a different day, receive saline. These comparisons will determine whether brain changes and eating behaviors are directly related to the OT rather than "placebo". This study will provide important information about how OT changes the brain functioning and eating behaviors of individuals with AN. Given the importance of social perception in AN, OT may provide a powerful method of changing brain regions associated with social relationships as well as increasing food intake among individuals with AN.

2014 Klarman Award Recipients

- **Benjamin Arenkiel, Ph.D.**

Assistant Professor of Molecular & Human Genetics

Baylor College of Medicine

“Dissecting Cholinergic Circuits in Feeding Behavior”

Key Words: Acetylcholine, Optogenetics, Circuits, Hypothalamus, Obesity,

Neuroscience

Eating disorders such as anorexia and bulimia nervosa, and obesity together result in significant morbidity and mortality. Interestingly, numerous neuropsychiatric disorders have been found to be associated with obesity and metabolic dysfunction. Feeding behavior is largely regulated in the brain, and perturbations to feeding circuits contribute to the pathogenesis of eating, metabolic, and mood disorders. We have recently discovered that loss of cholinergic neurons from the basal forebrain leads to increased food intake and severe obesity in adult mice. Furthermore, we have shown that these neurons project to the hypothalamus, which has been identified as a major feeding control center. Using multifaceted metabolic profiling, electrophysiological, and optogenetic experimentation, we propose the following specific Aims 1) determine how cholinergic signaling affects food intake, body weight, and metabolism, and 2) map the cholinergic brain circuits that influence body weight control. Together these studies will help elucidate the critical, and yet unknown mechanisms by which cholinergic drive influences control of feeding behaviors and body weight. Importantly, the proposed research will help reveal previously unidentified components of feeding circuits, and provide new insight into the convergent mechanisms of how cholinergic circuits intersect to influence body weight, metabolism, and mood control.

- **Joel Elmquist, D.V.M., Ph.D.**

Maclin Family Professor in Medical Science

Department of Internal Medicine

University of Texas Southwestern Medical Center

“Hypothalamic Htr2c Circuits and the Regulation of Feeding and Emotional Behaviors”

Key Words: Serotonin receptor, Anorexia, Hypothalamus, Paraventricular nucleus, Anxiety, Mood

Given the multi-symptom nature of Anorexia Nervosa (AN), the pathophysiology likely involves disruptions in complex neural systems that affect both feeding and emotional behaviors. The brain serotonin (5-HT) system is a key regulator of diverse physiological processes and behaviors. Importantly, perturbations of this system have been shown to cause both feeding and emotional deficits that are characteristic of AN. The 5-HT receptor, Htr2c, has been implicated as the primary target that mediates the anorexigenic actions of 5-HT compounds. Moreover, Htr2c is highly expressed in several brain sites that are important for emotional processing, and has been implicated in the regulation of mood.

We have recently identified two key regions within the hypothalamus where activation of Htr2c has opposite effects on food intake. We found that activation of Htr2c in pro-opiomelanocortin (POMC) neurons in the arcuate nucleus suppresses food intake. In contrast, we found that the activity of this anorexigenic circuit is opposed by a previously unrecognized orexigenic circuit that is regulated by Htr2c in the paraventricular nucleus (PVH) of the hypothalamus. Interestingly, Htr2c expression in the PVH includes a population of parvocellular neurons that express corticotropin-releasing hormone (CRH). CRH is a major regulator of stress responses and hypothalamic--pituitary--adrenal (HPA) axis activity. Our findings lead us to hypothesize that the PVH is a key integration site where Htr2c mediates the neuromodulatory actions of 5-HT on both feeding and emotional behaviors. Furthermore, our model predicts that dysfunction of this circuit leads to overt anorexia and additional associated perturbations in stress responses.

We will use advanced mouse genetic and circuitry mapping tools to test components of our hypothesis. Results from these studies will not only reveal the fundamental mechanisms underlying the pathophysiology of AN, but may provide rational targets for the development of novel treatments of AN and other eating disorders.

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

Assistant Professor of Neurobiology

Yale School of Medicine 1, 2014

As of 8/1/14, Associate Professor of Biology

American University

“Functional Connectomics of Cortico–striatal–hypothalamic Circuits and the Motivational Control of Feeding”

Key Words: Optogenetics, Multi–electrode recording, Lateral hypothalamus, Medial prefrontal cortex, Ventral striatum

This project will determine if the medial prefrontal cortex and ventral striatum control eating behavior by competing to control neural activity in the lateral hypothalamus. Dysfunction in these brain areas might underlie the tendency of patients with eating disorders to show apathy and/or anxiety about eating and encountering food–related stimuli. Recent studies from my laboratory, supported by a one–year award from the Klarman Family Foundation, have established that the medial prefrontal cortex and ventral striatum have opposing roles in the control over eating. If we turn off the medial prefrontal cortex, using optogenetic inactivation methods, rats show reduced consumption of sucrose rewards. By contrast, if we inactivate the ventral striatum, rats consume excessive amounts of food. Here, we propose two studies to further examine these neural circuits. In the first study, we propose to use recently developed optogenetic methods to reversibly inactivate the medial prefrontal cortex and ventral striatum as rats consume liquid sucrose and measure effects of manipulating each brain area on the animals' consummatory behavior. Then, as the optogenetic viruses also serve as fluorescent neuronal tracers, we will use anatomical methods to trace connections from the medial prefrontal cortex and ventral striatum to the lateral hypothalamus. Using this approach, we will create functional connectivity maps based on the extent to which silencing the medial prefrontal cortex and ventral striatum alters the animals' consummatory behavior. In the second study, we will record neuronal activity in the lateral hypothalamus while reversibly inactivating the medial prefrontal cortex and ventral striatum as rats consume liquid sucrose. We will determine if the rate or timing of feeding–related neuronal activity in the hypothalamus is sensitive to neural processing in the mPFC and vStr. The results from these two studies will contribute to an emerging literature on the neuronal basis of motivational control of eating.

- **Michael Lutter, M.D., Ph.D.**

Assistant Professor, Department of Psychiatry
University of Texas Southwestern Medical Center

“The Role of ESRRA in Eating Disorder–related Behaviors”

Key Words: Rare variant, Glutamate, Estrogen–related receptor, Synaptic plasticity, Animal behavior

Eating disorders (EDs) are debilitating mental illnesses in which individuals are plagued by marked disturbances in body image coupled with disordered eating behaviors. While several studies indicate that genetic factors contribute to the development of EDs, the molecular pathways that mediate this dysfunction are not well understood. We recently have identified mutations in two separate genes that increase the risk of developing EDs. The long–term objective of this proposal is to determine how disruption in the estrogen–related receptor alpha (ESRRA) gene affects behaviors related to the development of EDs. The central hypothesis of this proposal is that loss of ESRRA activity increases ED– related behaviors by impairing synthesis of pre–synaptic glutamate and synaptic plasticity. The rationale for this proposed research is that understanding how ESRRA activity contributes to ED–related behaviors in mice will provide a basis for the development of novel approaches to treat patients with EDs. In order to directly test components of this hypothesis, the following Specific Aims have been generated: 1) determine if loss of ESRRA affects behaviors relevant to the development of eating disorders; and 2) examine the electrophysiological and structural deficits in corticostriatal glutamatergic synapses in ESRRA–null mice that predict behavioral abnormalities. Mice genetically deficient in ESRRA will be used to determine if loss of ESRRA activity affects behaviors relevant to EDs in mice. Electrophysiological and structural measures will determine the role of ESRRA activity on synaptic plasticity and to correlate behavioral abnormalities with deficits in functional connectivity. The proposed research is innovative because it examines a distinct signaling pathway, the ESRRA pathway, to define a novel cellular and molecular pathway that contributes to ED–relevant behaviors. The proposed research is significant because it will improve our understanding of the cellular and molecular basis of EDs and identify novel therapeutic opportunities to treat patients with EDs.

- **Alexandros Makriyannis, Ph.D.**

Director, Center for Drug Discovery
Northeastern University

“Cannabinoid Medications for Anorexia Nervosa”

Key Words: Anorexia nervosa, ABA animal model, CB1 agonist, Drug discovery, Endocannabinoid system, Activity based anorexia

Anorexia nervosa (AN) is a devastating illness that primarily affects young women during adolescence and early adulthood. Current treatment options are largely ineffective, relapse is common, and full recovery occurs in fewer than half of AN patients. These findings, together with the high rates of mortality (12.8%) and suicide (6%), highlight a critical need for more effective treatments. The endocannabinoid system (ECS) represents an important therapeutic target for AN, based on its role in modulating energy homeostasis and reward processing, both of which are affected in AN. Moreover, clinical studies have linked impaired endocannabinoid signaling to AN, and treatment with the cannabinoid receptor agonist Delta9-THC improves appetite and modestly attenuates weight loss in a pre-clinical, animal model of AN. While these and other studies support the use of cannabinoid-based drugs in treating AN, Delta9-THC and related compounds possess poor pharmacodynamic properties and promote undesirable side effects. To overcome these limitations, our group is engaged in a novel drug discovery program to develop a new generation of cannabinoid receptor agonists with predictable time courses of pharmacological action and "built-in" detoxification mechanisms. Using a "soft drug" methodology, we will develop a small number of novel cannabinoid receptor agonists with superior pharmacokinetic profiles, fewer side-effects, and enhanced safety over that of Delta9-THC. These compounds will then be pre-screened for their onset and duration of behavioral action in female rats, followed by an evaluation of their efficacy in improving appetite, reducing exercise, and preventing weight loss in an animal model of AN. The overall results of our work will contribute to our understanding of the role of the ECS in AN, and bring us closer to achieving our long-term goal of developing a novel endocannabinoid-based pharmacotherapy that will ultimately improve the lives of individuals suffering from AN.

- **Saleem Nicola, Ph.D.**

Assistant Professor, Department of Psychiatry and Neuroscience
Albert Einstein College of Medicine

“Neural Mechanisms Promoting Food Seeking in the Absence of Hunger”

Key Words: High-calorie food intake, Food-seeking behavior, Nucleus accumbens, Opioid receptors, Dopamine, Electrophysiology in behaving rats, Fast-scan cyclic voltammetry

Before food can be eaten, it must first be obtained. This is the case even under conditions of abundant food availability: one cannot eat the ice cream in the freezer without first approaching the freezer and removing the container. Food-associated stimuli -- smells, pictures, logos, etc. -- promote seeking of calorie-dense food even in the absence of caloric need, and this process may be dysregulated in individuals with eating disorders and obesity. Therefore, gaining a greater understanding of disordered intake regulation requires elucidation of the brain mechanisms underlying food seeking in subjects with no homeostatic need for food.

Pilot experiments suggest that activation of mu opioid receptors (MORs) in the nucleus accumbens (NAc) by endogenous opioid ligands is an important component of these mechanisms. Rats responded to auditory cues predicting heavy cream reward by approaching the reward location. Injection of a MOR antagonist into the NAc reduced the likelihood of approach when subjects were sated, but not when they were hungry. The proposed experiments combine behavioral, pharmacological, electrophysiological and electrochemical techniques to explore the mechanism underlying this effect. Many NAc neurons are excited by food-predictive cues, and these excitations are dependent on dopamine receptor activation. Therefore, we hypothesize that MOR activation increases cue-evoked dopamine release within the NAc, resulting in increased cue-evoked excitation of NAc neurons and, consequently, greater likelihood of food-seeking. To test this hypothesis, we will use fast-scan cyclic voltammetry to determine whether MOR blockade reduces dopamine release in sated but not hungry rats, as well as electrophysiological recording in awake, cue-responding rats to determine whether injection of a MOR antagonist into the NAc reduces the cue-evoked excitation of NAc neurons in sated but not hungry rats. These experiments will reveal a causal neuronal mechanism underlying seeking of high-calorie food in subjects with no homeostatic need for calories.

- **Lori Zeltser, Ph.D.**

Assistant Professor

Naomi Berrie Diabetes Center/Pathology and Cell Biology

Columbia University

“Maternal Influences on Susceptibility to Anorexia-like Behavior”

Key Words: Anorexia Nervosa, Mouse Model, BDNF, Maternal Programming

As anorexia nervosa (AN) is a complex disease involving interactions between genetic, environmental and psychological factors, developing a relevant 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 half of the mice subjected to this protocol will exhibit at least one "aphagic episode", defined as the refusal to consume any food for at least 24 hours. Aphagic episodes are often associated with >15% body weight loss, satisfying a major diagnostic criterion for AN.

An unanticipated feature of this model is that a genetic predisposition to anxiety in the dams is a critical risk factor for AN-like behaviors. We find that these anxiety-prone dams exhibit reduced maternal behavior during lactation, similar to models used to simulate "early life stress". The central hypothesis of the proposed studies is that epigenetic changes in neuronal circuits programmed by the anxiety-prone maternal environment are exacerbated by subsequent exposure to social stress during adolescence, promoting the initiation of anorexia-like behaviors. The proposed studies will take advantage of the extensive characterization of the consequences of early life stress and social isolation stress during adolescence on neuroendocrine and neural stress circuits to examine how these exposures interact to increase the risk of AN-like behavior. This issue is an important area for study, because there is evidence that many of the effects of exposure to early life stress can be mitigated by interventions in adolescence. In the long term, these studies could lead to the development of new strategies to identify at-risk children that might benefit from early intervention.

- **Yi Zhang, Ph.D.**

Fred Rosen Professor of Pediatrics
Boston Children's Hospital

“Insights into Epigenetic Mechanisms of Binge-eating Disorder”

Key Words: Binge-like eating behavior, Epigenetics, DNA methylation/hydroxymethylation, Tet, Transcription

Eating disorders represent a major economic and social problem, with up to 4.5% of the US population affected at least during one stage of their lives. Specifically, binge eating disorder (BED), characterized by excessive caloric consumption over a limited time period, represents one type of eating disorders. Indeed, chronic food overconsumption has been linked to perturbation of brain reward systems, and as such, the mesolimbic dopamine reward system has been a major subject of investigation within the context of BED. Despite insights into the neuroanatomical and neurochemical characterization of BED, the molecular mechanism underlying BED remains poorly understood. Given the recent discovery that epigenetic changes play an important role in brain reward system dysfunction, we hypothesize that epigenetic modifications may also have an important role in BED. To test this hypothesis, we established a mouse model of binge eating in which mice engage in excessive caloric intake of a highly palatable diet over a daily limited (1-hour) access. Our results reveal transcriptional and epigenetic differences in the ventral tegmental area (VTA) of mice with limited (binge) and extended access to a palatable diet. Interestingly, in addition to the genes already implicated in BED, our study also revealed novel genes with transcriptional changes concomitant with changes in DNA methylation. To extend this study, we propose the following two specific Aims:

Aim 1: Isolation of VTA dopamine neurons in a mouse model of binge-like eating behavior

Aim 2: Transcriptome and DNA methylation analysis of isolated VTA dopamine neurons in a mouse model of binge-like eating behavior

Completion of the proposed studies will not only enrich our molecular understanding of BED, but will also provide novel targets for therapeutic intervention of BED and other eating disorders.

- **Edward Ziff, Ph.D.**

Professor of Biochemistry and Molecular Pharmacology
New York University School of Medicine

“Synaptic Adaptation of the Brain Reward Circuits Associated with Binge Eating Disorder -- Why Diets Fail”

Key Words: Sugar, Binge eating, Depression, Craving, Synaptic Adaptation

Binge eating disorder (BED) is characterized by insatiable food craving and is strongly associated with depression. Recently, BED has been approved for inclusion in the diagnostic and statistical manual of mental disorders (DSM-5) as its own category of eating disorder, suggesting that BED is associated with significant physical and psychological problems. The goal of this pilot proposal is to understand the mechanisms of BED at the neurobiological, molecular and electrophysiological levels in order to provide biological basis for therapeutic intervention. We propose that contribution of BED to obesity involves modified activation of medium spiny neurons (MSNs) of the nucleus accumbens in the mesolimbic dopamine (DA) system of the brain. Changes in MSN circuitry are known to cause drug addiction, suggesting that palatable foods and drugs share common DA pathway. Key features of BED are food craving and depression, especially after attempts to stop overeating. We will use sucrose withdrawal as a model for food withdrawal and will study the MSN synaptic adaptations caused by the decrease in DA system activity that takes place after food withdrawal and dieting. Our preliminary studies show that withdrawal from sucrose after lengthy sucrose consumption induces the expression of K⁺ channels in MSNs that reduce excitability, an activity change associated with behavioral depression. Decreased excitability in turn leads to a decrease in intracellular Ca²⁺ that induces a homeostatic mechanism that trafficks calcium permeable AMPA receptors to MSN synapses, a step that with drugs of abuse causes craving. We will validate this pathway in vivo and in vitro and suggest that these synaptic modifications and the resulting abnormal behaviors prevent people from stopping overeating and result in BED. Therefore, in the long-term, this proposal will identify synaptic mechanisms of two changes that are critical for understanding how the disorder arises and for development of therapeutic drugs.

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

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

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

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

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

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

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

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

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

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

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.

Klarman Family Foundation Grants Program in Eating Disorders Research 2012 Award Recipients

Two-Year Awards

- **Cynthia Bulik, Ph.D.**

Director, UNC Eating Disorders Program
University of North Carolina at Chapel Hill

“Determining the Role of Uncommon Exon Variation in Anorexia Nervosa”

Key Words: Anorexia Nervosa, Genetic, Exon Variation

As part of the Genetic Consortium for Anorexia Nervosa (GCAN) and Wellcome Trust Case Control Consortium 3 (WTCCC3), we have amassed the largest anorexia nervosa (AN) sample in the world. We propose a well-powered evaluation of the hypothesis that uncommon coding variation plays a role in AN. We propose to evaluate the role of uncommon exon variation in AN in 3,000 female cases and 3,000 archived matched controls in a cost-effective manner by using the Illumina HumanExome chip, which captures missense and nonsense coding variation with $MAF = 0.005$, and a substantial fraction with $MAF 0.0005-0.005$. This chip contains ~230K missense and nonsense coding variants identified via exome sequencing of 12,028 European individuals. The chip contains SNPs from the Psychiatric GWAS Consortium mega-analyses for autism, major depression, schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder. We will genotype AN cases and controls using the Illumina HumanExome chip and conduct rigorous QC. We will evaluate the role of uncommon coding variants in AN conducting single SNP tests along with tests that aggregate variation within exons and genes, and will incorporate appropriately selected covariates and control for potential biases (e.g., population stratification). We will aim to identify biological pathways for AN with high confidence by rigorous assessment of the extent to which genomic results are enriched for smaller p-values in genes from both classical and novel empirical pathways. Finally, we will compare our results with exome chip genotyping conducted for other psychiatric disorders (autism spectrum disorders, bipolar disorder, schizophrenia, and attention deficit hyperactivity disorder) in order to look for shared genetic risk factors. The proposed study will provide a rigorous and well-powered evaluation of the role of uncommon exon variation in AN. If variants are identified, these discoveries could provide rapid and actionable insights into the genetic causes of AN.

- **Kathryn Cunningham, Ph.D.**

Chauncey Leake Distinguished Professor of Pharmacology

University of Texas Medical Branch

“Fine-Tuning the 5-HT2AR:5-HT2CR Rheostat for Therapeutics in Binge Eating Disorder”

Key Words: Binge Eating Disorder, Serotonin, 5-HT2AR, 5-HT2CR, Heterodimer, Drug Discovery

Binge eating disorder (BED) is the most prevalent eating disorder in the U.S., and is linked to severe obesity and psychological and medical morbidity. Impulsivity and behavioral disinhibition factor into the multifaceted determinants that underlie the etiology of BED and its evolving pathogenesis. Our central hypothesis is that impulsive action and binge eating are mechanistically-linked to disrupted serotonin (5-HT) signaling through the 5-HT2A receptor (5-HT2AR) and 5-HT2CR in brain circuits that drive the incentive-motivational salience of food and cues that predict food. We postulate that 5-HT2AR:5-HT2CR homeostasis in the corticostriatal circuit sustains behavioral inhibition. Our discovery that 5-HT2AR:5-HT2CR heteromeric complexes exist in the brain, and that HI rats exhibit a deficit in heteromer expression relative to LI rats, supports the innovative concept that 5-HT2AR:5-HT2CR heteromeric signaling in single neurons may subserve the role of "rheostat" to fine tune neuronal function and behavioral control. Furthermore, we propose the provocative, high risk idea that a bivalent 5-HT2AR antagonist:5-HT2CR agonist may most effectively suppress both impulsivity and binge eating through promotion or stabilization of heteromeric formation. To this end, we will 1) establish the reciprocal association between impulsivity and binge eating, 2) demonstrate that impulsivity and binge eating impact the reinforcing efficacy of sweet-fat food and associated cues, and 3) explore the 5-HT2AR:5-HT2CR heterodimer as a neuronal rheostat to control impulsivity and binge eating. This innovative project addresses a fundamental gap in our knowledge of how the neural and behavioral aspects of impulsivity are related to binge eating and represents the first step in a continuum of research that may lead to the targeted development of pharmacological strategies to restore 5-HT2AR:5-HT2CR homeostasis and minimize deleterious behaviors that promote aggregate impulsivity/BED.

- **Bingshan Li, Ph.D.**

Assistant Professor of Human Genetics

Vanderbilt University Medical Center

“Mapping and Functional Characterization of Genetic Variants Associated with Anorexia Nervosa”

Key Words: Anorexia, Gene Mapping, Complex Diseases, Rare Variants, Linkage and Association Analysis, Genome Wide Association Studies, Whole Exome Sequencing, Transgenic Mouse

Anorexia Nervosa (AN) is a serious psychiatric disease characterized by inability to maintain a minimal normal weight, persistent fear of gaining weight and preoccupation about body shape. It affects 0.5–1% of the population and females are 10 times more than males to be affected. AN has the highest mortality rate among all psychiatric disorders with an estimated standard mortality rate over 10. Traditionally AN has been viewed as a disease influenced by sociocultural and environmental factors. However this view was recently challenged and family and twin studies showed a great genetic basis of AN. It is estimated that the heritability of AN is 56–75% from different studies and share environments account for negligible relative resemblance. All these lines of evidence suggest a strong genetic component of AN. However little is known about the biological mechanisms of AN and previous studies are often inconsistent due to the lack of understanding of the genetic basis of AN. Therefore it is crucial to identify genetic factors and to investigate their pathophysiological mechanisms for AN to have effective prevention, diagnosis and treatment in the long run. In the current AN GWAS we are involved we have successfully identified a single nucleotide polymorphism with unequivocal genome wide significance, providing a strong candidate gene for elucidating the genetic etiology of AN. To fine map the variants responsible for this GWAS signal and to identify additional genes associated with AN, in this study we aim to have a comprehensive and unbiased survey of the entire coding regions to identify genetic variants associated with AN via whole exome sequencing of our complex pedigrees, to replicate our initial findings in additional samples and to investigate the functional roles of these genetic variants in model organisms.

- **Marina Picciotto, Ph.D.**

Professor of Psychiatry

Yale University

“Acetylcholine: A Novel Regulator of Circuits Involved in Food Intake and Eating Disorders”

Key Words: Food Intake, Nicotinic Acetylcholine Receptors, Nicotine, Hypothalamus, Arcuate Nucleus

The propensity for development of eating disorders is complex and likely involves many brain systems, in addition to environmental factors such as stress. We hypothesize that abnormalities in acetylcholine signaling in the brain could increase susceptibility to eating disorders. It is known that smokers are leaner than non-smokers and use cigarettes to control appetite. We have found that nicotine, the primary psychoactive component in tobacco, highjacks nicotinic acetylcholine receptors (nAChRs) in the arcuate nucleus of the hypothalamus (ARC) to decrease food intake. The endogenous neurotransmitter that excites nAChRs is acetylcholine; thus, release of acetylcholine in ARC is likely to be important in matching nutrient need to food intake. Acetylcholine is released in several brain areas in response to stress, but the source of acetylcholine input to the ARC is not known. Anatomical studies in Aim 1 will therefore identify cholinergic inputs to ARC. In addition, nAChR subtypes in the ARC have not been fully identified and molecular studies in Aim 2 will identify the subunits expressed in POMC and NPY neurons. The effect of cholinergic input to the ARC on food intake has not been studied and optogenetic studies in Aim 3 will provide mechanistic data on the effects of acetylcholine release on food intake. We propose that stress-induced acetylcholine release in the ARC induces satiety even when calorie needs are high. In the behavioral studies in Aim 4 we will determine whether stress-induced anorexia can be blocked by genetic knockdown of specific nAChRs in ARC. Future studies can investigate the hypothesis that nAChR occupancy is altered in individuals with eating disorders, addressing the hypothesis that acetylcholine contributes to their etiology. Targeting the acetylcholine system may therefore be a novel strategy for therapeutic development to treat those suffering from eating disorders.

- **Leslie B. Vosshall, Ph.D.**

Professor

The Rockefeller University

“G Protein–Coupled Receptors Regulating the Sensation of Hunger and Satiety”

Key Words: *Drosophila melanogaster*, G Protein–Coupled Receptors, TALENs, Post–Fasting Feeding Response, EXPRESSO Automated Feeding Assay

The perception of hunger is a central control mechanism that allows humans and other animals to regulate food intake. An integrated system of external sensory input works together with peripheral body organs and the central nervous system to produce the sensations of hunger or satiety. The mechanisms that regulate the perception of hunger and satiety are poorly understood in any animal.

A number of peptide hormones that suppress or enhance feeding behavior in animals have been identified. These activate receptors that are members of the G protein–coupled receptor (GPCR) superfamily. The goal of this project is to perform a comprehensive analysis of GPCRs in the genome of the fly, *Drosophila melanogaster*, and identify and characterize those that regulate feeding behavior.

In preliminary studies, we identified mutations in 5 fly GPCRs that disrupt the sensation of hunger and developed an automated instrument called EXPRESSO that measures real–time food consumption, which will greatly accelerate the pace of this project. Two specific aims are proposed:

Specific Aim 1: Identification of GPCRs regulating feeding behavior

The EXPRESSO instrument will be used to perform a high–throughput screen of GPCRs and neuropeptides to identify those with a role in fly feeding behavior. The goal is to identify novel pathways regulating feeding behavior, which may be applicable to understanding eating behavior and its disorders in humans.

Specific Aim 2: Mechanistic analysis of GPCRs regulating feeding behavior

We will analyze the gene expression and visualize the neural circuits that express candidate feeding–related GPCRs and neuropeptides. Transgenic reagents will allow us to manipulate the function of neurons expressing these GPCRs as well as permitting in vivo calcium imaging of the circuit in different feeding states. The goal is to understand how these GPCRs participate in the sensation of hunger or satiety and the promotion or suppression of feeding.

- **Yong Xu, M.D., Ph.D.**
Assistant Professor
Baylor College of Medicine

“A Central Estrogen–5–HT Circuit Regulates Binge Eating”

Key Words: Estrogens, 5–HT, Brain, Receptor, Binge Eating

Binge eating, defined as the ingestion of a large amount of food in a brief period of time, afflicts approximately 5% of US adults and constitutes a significant public health concern. The pathophysiology of binge eating is poorly understood. Impaired central 5–hydroxytryptamine (5–HT) signals are associated with binge behavior and increased brain 5–HT content can inhibit binge eating in patients or animals. Interestingly, estrogens, which can act on central 5–HT neurons, also exhibit anti–binge properties. These findings led to a general hypothesis that the brain estrogen–5–HT circuit plays a physiologically relevant role in binge behavior. We will test this hypothesis with the following two Aims:

Aim 1: To determine whether activation/inhibition of brain 5–HT neurons inhibits/potentiates binge eating.

We will use newly developed DREADD viruses which allow activation or inhibition of selective subsets of neurons that express Cre–recombinase. The viruses will be stereotaxically delivered into the dorsal raphe nucleus (where brain 5–HT neurons are located) of SERT–Cre mice. This will selectively activate or inhibit 5–HT neurons. We will test if changes in 5–HT neural activities regulate binge eating.

Aim 2: To determine whether estrogen receptor–alpha (ERalpha) expressed by brain 5–HT neurons is required to mediate anti–binge effects of estrogens. We will use the Cre–loxP strategy to generate mice lacking ERalpha only in 5–HT neurons. We will use these mice to determine if loss of ERalpha in 5–HT neurons attenuates anti–binge effects of estrogens.

The proposed experiments will use the powerful genetic mouse models to delineate the complex brain circuits in the context of binge behavior. Results from these studies may not only reveal the fundamental mechanisms underlying the development of binge behavior, but also we may provide rational targets for the development of novel drugs that can treat binge eating and other related eating disorders.

One-Year Awards

- **Kenneth Carr, Ph.D.**

Associate Professor

NYU Medical Center / NYU School of Medicine

“Effects of Food Restriction on Sucrose-Induced AMPA Receptor Trafficking and Behavior”

Key Words: Food Restriction, Nucleus Accumbens, AMPA Receptors, Synaptic Plasticity, Binge Eating

Food restriction is a risk factor for the development of binge pathology. In the laboratory, food restriction induces neuroadaptations in brain reward circuitry that are likely to be among those that facilitate survival during periods of food scarcity in the wild. However, upregulation of mechanisms that promote foraging, reward-related learning and ingestive behavior may pose a hazard when food restriction is self-imposed in an ecology of abundant, palatable, energy-dense food. Past research of our laboratory indicates that food restriction upregulates D-1 dopamine receptor signaling in nucleus accumbens (NAc). Downstream consequences include increased phosphorylation of the AMPA receptor GluA1 subunit on Ser845, which enhances AMPA currents and facilitates trafficking to the postsynapse. Both cocaine and consumption of 10% sucrose increased GluA1 phosphorylation in food-restricted but not ad libitum fed rats. Further, episodic intake of 10% sucrose increased synaptic delivery of AMPA receptors, with marked increases in GluA1 and GluA2 in NAc postsynaptic density (PSD). In behavioral studies, NAc microinjection of an antagonist of GluA2-lacking AMPA receptors decreased the rewarding effect of D-1 receptor stimulation in food-restricted but not ad libitum fed rats, and slowed consumption of a small high sucrose meal. Microinjection of a nonspecific AMPA receptor antagonist blocked the conditioned hyperactivity of food-restricted rats expecting the meal. Given that AMPA receptor trafficking mediates experience-dependent behavioral plasticity, and food restriction is an important factor clinically as well as in animal models of binge eating, these findings suggest that AMPA receptor trafficking could play a role in the genesis of binge pathology. This pilot project will (i) evaluate the subunit composition of AMPA receptors in the NAc PSD following sucrose intake and (ii) determine whether a history of sucrose intake during food restriction alters AMPA receptor trafficking and sucrose-directed behavior in rats that have returned to ad libitum feeding.

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

Professor of Surgery

University Health Network

“Deep Brain Stimulation for the Treatment of Refractory Anorexia Nervosa: Pilot Trial”

Key Words: Deep Brain Stimulation, Neurocircuitry, Anxiety, Neuroanatomy, Clinical Trial, Anterior cingulate

Although advances in understanding Anorexia Nervosa (AN) from neuroscientific and genetic perspectives have been made, these have thus far not translated into durable and effective treatments. Deep Brain Stimulation (DBS) is a non-lesional, reversible and targeted surgical therapy, that has been used for over 25 years in the management of movement disorders, such as Parkinson's Disease. Given overlapping neural circuits and co-morbidity between the neurologic and psychiatric disorders, DBS has begun to be explored in refractory psychiatric disease, with promising results in Major Depression. Several of the cardinal symptoms of AN, including depressed mood, anxiety, and preoccupation with body-image, can be mapped to distinct emotion- and action-regulating cortical-subcortical circuits comprised of key structural nodes. We propose a pilot trial of DBS in refractory AN, that targets two such structures, the subgenual cingulate gyrus (SCG), and nucleus accumbens (NAcc). Broadly, this study aims to establish the short and long-term safety of DBS in patients with treatment resistant AN and obtain evidence of initial efficacy. Specifically, we will use psychometric measures of AN severity, depression, anxiety, reward and quality-of-life, as well as neuroimaging with positron emission tomography (PET) and Magnetic Resonance Imaging (MRI) to track the short- and long-term influence of DBS on clinical and imaging (structural and functional) outcomes. Twelve patients will be enrolled in this single-arm, non-blinded study, with 6 receiving SCG and 6 NAcc DBS. Device activation will take place at 1-2 weeks post-surgery, and patients will be closely followed for the duration of the one-year study. We hypothesize that DBS is a safe and effective procedure in patients with refractory AN, as evidenced by minimal adverse events and improvements in baseline clinical and imaging measures at 6 months and 1 year. This would represent a significant step forward in the care of the most severely afflicted anorexic patients.

- **Jacqueline McGinty, Ph.D.**

Professor of Neurosciences

Medical University of South Carolina

“Prefrontal Cortical BDNF and High Fat Food Seeking”

Key Words: BDNF, Prefrontal Cortex, Epigenetics, Obesity, High Fat Food Seeking, Self-Administration

Repeated bouts of excessive food consumption in the absence of energy deprivation are characteristic of binge eating that is associated with dysregulated energy balance and eating disorders, such as bulimia and obesity. To control overconsumption of palatable sugary or high fat foods (HFF), individuals attempt to restrict their access to energy dense foods by dieting but relapse to palatable food-seeking and binge eating commonly overcome self-control. In contrast to the considerable attention paid to the relationship between homeostatic regulation of energy balance and food intake, the neurobiological mechanisms that contribute to seeking and overeating palatable foods (“hedonic” eating) are not well understood. In a manner that may be similar to addictive drug-seeking, dopamine and glutamate in the prefrontal cortex (PFC) and nucleus accumbens (NAc) are implicated in stress and cue-induced food-seeking. Intriguingly, brain-derived neurotrophic factor (BDNF), a critical mediator of food intake and energy balance, is not only expressed in the hypothalamus but in two critical reward-related pathways, PFC-NAc neurons, where it is a key regulator of glutamate transmission, and ventral tegmental area (VTA)-NAc neurons, where it is a key regulator of dopamine transmission. Although *Bdnf* knockdown in the ventromedial hypothalamus augments HFF and standard chow consumption and *Bdnf* knockdown in the VTA augments only HFF consumption, the role of corticolimbic BDNF in hedonic food-seeking is completely unknown. In contrast, BDNF has emerged as a key regulator of addictive drug-seeking. For example, BDNF infusion into the dorsomedial PFC at the end of restricted access cocaine self-administration suppresses subsequent cocaine-seeking in a phospho-ERK-dependent manner. Thus, in this pilot study, we propose to investigate (1) the effects of HFF self-administration and HFF-seeking on BDNF-related chromatin remodeling, and gene and protein expression in the PFC and NAc and (2) whether intra-PFC BDNF infusion decreases HFF-seeking in rats with a HFF self-administration history.

Klarman Family Foundation Grants Program in Eating Disorders Research 2011 Award Recipients

Two-Year Awards

- **Nicole Barbarich-Marsteller, Ph.D.**

Assistant Professor of Clinical Neurobiology
Columbia University

“Hippocampal Neurogenesis in a Translational Model of Anorexia Nervosa”

Key Words: Anorexia Nervosa, Activity-Based Anorexia, Animal Model, Rat, Neurogenesis

The lack of effective treatments and high mortality rate for anorexia nervosa provides strong justification for utilizing animal models to identify neurobiological mechanisms that may play a role in perpetuating weight loss and hyperactivity. In the activity-based anorexia model, limited food access is combined with unlimited access to a running wheel resulting in significant weight loss, hyperactivity, and failure to adapt food intake to increasing energy demands. The biological basis for this maladaptive cycle is unknown. Previous work on activity-based anorexia in our lab has found 1) a significant suppression of hippocampal cell proliferation after 3 days, 2) two distinct behavioral phenotypes characterized as vulnerable and resistant, and 3) a sensitization to wheel running following relapse, suggesting that an initial exposure produces lasting changes in neurobiology. While it is unlikely that impaired neurogenesis is responsible for the development of activity-based anorexia, it is plausible that food restriction and hyperactivity trigger neurobiological adaptations that perpetuate the maintenance of these behaviors.

Our long-term goal is to identify the neurobiological mechanisms maintaining these maladaptive behaviors, and to translate this information into a better understanding of the neurobiological mediators underlying anorexia nervosa. Specific aims: Quantify cell proliferation (with BrdU) and the number of young neurons (with doublecortin) in rats with an onset of activity-based anorexia during adolescence vs. adulthood (Aim 1) and with phenotypes characterized as vulnerable or resistant (Aim 2). Compare cell proliferation and the number of young neurons in rats after initial onset and recovery from activity-based anorexia (Aim 3). Utilize irradiation to determine whether complete ablation of hippocampal cell proliferation induces an immediate and severe state of activity-based anorexia (Aim 4). Overall, the biological basis for perpetuating food restriction and hyperactivity is not well understood, however these translational studies will enhance our understanding of the mechanisms maintaining these maladaptive behaviors.

- **Deborah Clegg, Ph.D.**

Assistant Professor, Department of Internal Medicine
and Department of Nutrition
University of Texas Southwestern Medical Center

“Estrogens Enhance the Therapeutic Effects of Leptin through Estrogen Receptor–
Dependent Mechanisms”

Key Words: Leptin, Estrogens, Anorexia Nervosa, Estrogen Receptor Alpha (ERalpha),
Estrogen Receptor Beta (ERbeta), Blood Brain Barrier (BBB), Activity–Based Anorexia
Paradigm (ABA)

Our goal is to enhance our understanding of the molecular manifestations of Anorexia Nervosa (AN) with the hope of developing new treatment methodologies. AN is an eating disorder characterized by profound weight loss, amenorrhea, semi–starvation–induced hyperactivity, and modulation of bone formation. Leptin is a circulating factor secreted from adipocytes in direct proportion to body adiposity; however due to weight loss, hypoleptinemia becomes a key endocrinological feature of AN. No medications have been approved by the Food and Drug Administration for the treatment of AN; however, research has focused on the potential therapeutic actions of leptin, as leptin may promote restoration of menstrual cycles, reduce motor restlessness in severely hyperactive patients, and prevent osteoporosis.

AN is more prevalent in women, although AN is manifested by severely reduced estradiol levels. Estrogens modulate leptin synthesis and secretion directly via sex steroid receptor–dependent transcriptional mechanisms. Estrogen response elements (EREs) are found in the genes for leptin and its receptor. Therefore, we are testing the hypothesis that estrogens may enhance the pleiotropic beneficial effects of leptin through ER–dependent mechanisms.

Mechanisms involved in adaptation to starvation are similar in rodents and humans; therefore, we will mimic the hypoleptinemia – induced endocrinological features of AN using the activity–based anorexia paradigm (ABA) in transgenic mice. We will test the hypotheses: 1) estrogen receptor ER α (not ER β) transcriptionally increases the expression of the critical leptin receptor isoform, OB–Rb in the central nervous system; 2) ER α and not ER β enhances the expression of leptin mRNA from adipocytes; and 3) ER α potentiates the transport of leptin across the blood brain barrier. We believe that activation of these ER–dependent mechanisms will combine to enhance leptin's ability to resolve amenorrhea, reduce hyperactivity, and improve bone mineralization. To our knowledge, estrogenic enhancement of leptin treatment has not previously been tested with respect to AN.

- **Luis de Lecea, Ph.D.**

Associate Professor, Department of Psychiatry and Behavioral Sciences
Stanford University School of Medicine

“The Role of Hypocretins and the Monoaminergic Nuclei in Anorexia: How to Control Fatal Motivation for Negative Energy Balance”

Key Words: Anorexia Nervosa, Hyperactivity, Self-Starvation, Anhedonia, Hypocretins, Serotonin, Dopamine, Lateral Hypothalamus, Dorsal Raphe Nucleus, Ventral Tegmental Area, Optogenetics, Activity-Based-Anorexia Model, Forced-Swim-Test, Tail-Suspension-Test

Anorexia nervosa (AN) is an eating disorder of which neuronal cause is poorly understood. The multifaceted pathology evolves around the imbalance between food intake and energy expenditure.

Anorexics actively engage in strenuous physical activity while substantially restricting their diet. Food-restriction is followed by loss of pleasure and motivation to eat while the energy deficit escalates over time turning into a fatal condition. The hypothalamus has long been a key structure in the integration of metabolic variables and motivation to seek food. In particular, neurons producing the neurotransmitter Hypocretin (Hcrt) also known as orexin, have been associated with triggering hyperactivity in AN. Our laboratory discovered the Hcrt system and has pioneered the implementation of optogenetic methods in vivo to manipulate the activity of genetically defined neuronal circuits with unprecedented temporal resolution.

Our core hypothesis is that the Hcrt system has a central role in the pathophysiology associated with AN by affecting the activity of monoaminergic nuclei, mainly the dopaminergic (DA) ventral tegmental area and the serotonergic (5-HT) dorsal raphe nuclei, and thus affecting brain reward function and the hedonic value of food consumption. Here, we propose to perform an integrative study combining optogenetics with behavioral measurements in a mouse model of anorexia. In an attempt to recover negative energy balance, we will selectively manipulate (activate and silence) the activity of Hcrt, DA and 5-HT neurons during food intake and physical activity while monitoring the homeostatic balance and the behavioral state of the animals. Overall, our study will provide a novel approach to understand the neurobiology of the vicious circle of self-starvation, hyperactivity, anhedonia, and depression, and will open new areas for therapeutic interventions in AN.

- **Richard Palmiter, Ph.D.**
Professor of Biochemistry
University of Washington

“Optogenetic Analysis of an Anorexia Neural Circuit”

Key Words: Anorexia, Mouse Model, Parabrachial Nucleus, Light-Induced Neuronal Activation (or Inhibition)

Our previous research established that ablation of hypothalamic neurons that express the neuropeptide, agouti-related protein (AgRP) along with neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA) in adult mice leads to severe anorexia. Loss of these AgRP-expressing neurons results in hyperactivity (Fos induction) of post-synaptic target cells. We showed that suppression of Fos induction by chronic administration of a GABA receptor agonist (bretazenil) within the parabrachial nucleus (PBN), but not other brain regions, prevented Fos induction and ameliorated the anorexia caused by ablation of AgRP neurons. Thus, we hypothesized that hyperactivity of PBN neurons is responsible for anorexia. We then established that excitatory input to the PBN arises from the nucleus tractus solitarius. Genetic suppression of either the excitatory glutamatergic input to the PBN or its excitatory output protects against severe anorexia caused by AgRP neuron ablation.

These and other observations led us to hypothesize that hyperactivity of a select population of neurons within the PBN mediates anorexia, while hypoactivity of those neurons stimulates feeding. We propose to use optogenetic techniques to selectively activate or inactivate PBN neurons while monitoring food intake. Initial experiments will target all glutamatergic neurons in the PBN; follow-up experiments will target a more select population of glutamatergic PBN neurons. We anticipate that increasing or decreasing the activity of PBN neurons will have significant effects on food intake (in opposite directions), which would substantiate our hypothesis and establish the PBN as an important integrator of hypothalamic and sensory information that modulates food intake. We also propose to use optogenetic and fluorescent tracer techniques to map the critical outputs of the PBN. These experiments will help establish the neuronal circuit that mediates anorexia in this mouse model, a neuronal circuit that likely mediates anorexia in humans as well.

- **Maribel Rios, Ph.D.**

Associate Professor, Department of Neuroscience
Tufts University School of Medicine

“Investigations of Cellular and Molecular Mechanisms Underlying the Effects of BDNF and Restricted Food Intake on Binge Eating”

Key Words: BDNF, Ventral Tegmental Area, Dopamine, Synaptic Plasticity, Mesolimbic, Reward, Bulimia Nervosa, Binge Eating Disorder, Restricted Palatable Food Intake

Binge eating is a cardinal symptom of binge eating disorder (BED) and bulimia nervosa (BN), which afflict a significant number of individuals with considerable medical consequences. Dieting or restricted intake of palatable, energy-rich foods is considered a high risk factor but the underlying pathological mechanisms are poorly understood. Involvement of the mesolimbic dopamine pathway was suggested because intermittent access to palatable food elicits both bingeing and alterations in this reward circuit in rodents. Evidence suggests that diminished mesolimbic activity produces reward deficiency syndrome and compensatory overeating. Similar to drugs of abuse, palatable food withdrawal might induce synaptic modifications in the mesolimbic reward circuitry that impair dopamine secretion and elicit binge eating as a maladaptive behavior. As not all individuals that diet binge eat, genetic factors might also contribute. Indeed, humans afflicted with BED and BN that also carried the Val66Met polymorphism in the brain-derived neurotrophic factor (Bdnf) gene exhibited more severe binge eating compared to wild type carriers. This mutation impedes regulated secretion and signaling of BDNF, a prominent regulator of neuronal synaptic plasticity.

We found that central BDNF depletion impairs mesolimbic dopamine secretion and interacts with limited palatable food access to induce severe binge-like behavior in mice. We seek to test the hypothesis that deficient BDNF cooperates with intermittent palatable food access to induce synaptic modifications that decrease mesolimbic dopamine tone and drive binge eating. In Aim 1, we combine genetic, electrophysiological and biochemical approaches to quantify changes in excitatory and inhibitory transmission in VTA dopamine neurons induced by palatable food restriction and how loss or gain of BDNF function influences these responses. Aim 2 will identify molecular mechanisms activated by deficient BDNF signal and restricted palatable food access, leading to reduced mesolimbic activity. These investigations will inform cellular and molecular mechanisms underlying binge eating and novel therapeutic avenues.

One-Year Awards

- **Traci Czyzyk, Ph.D.**

Associate Consultant in Research

Mayo Clinic in Arizona (beginning on September 1st)

“Kappa Opioid Receptor Control of Binge-Eating in Mice”

Key Words: Kappa Opioid Receptor, Binge-Eating, Mice, siRNA, Norbinaltorphimine

Binge-eating disorder (BED) in humans has comorbid conditions including depression and substance abuse, suggesting that BED and psychiatric disorders might share common neurocircuitry. The repeated use of commonly abused drugs leads to molecular neuroadaptations in the kappa opioid receptor (KOR) pathway in brain regions associated with reward. We have recently developed a novel mouse model to examine binge-like eating behavior in mice that utilizes a schedule of intermittent access to a palatable, energy-dense diet. We will use this model to determine whether increased activity of KORs increases binge-like eating, and the corollary that blockade of KORs will reduce binge-like eating in mice. Our preliminary results demonstrated that KOR knockout mice exhibit enhanced binge-like eating suggesting a mechanistic link between KOR signaling and binge-eating. We will induce binge-like eating in mice and will address the following questions. These *in vivo* experiments will allow us to obtain the first preclinical data that pharmacological KOR antagonism might be therapeutically beneficial for the treatment of BED.

1. Does KOR signaling increase after repeated binge-like eating? We will use both gene expression and protein analysis to determine if dynorphin A1-17 levels increase after repeated bingeing. The effect of inducing binge eating on KOR activation will also be assessed using an antibody that recognizes Ser369 phosphorylated KOR.

2. Does pharmacological antagonism of KORs reduce binge-like eating behavior? We will determine if systemic administration of the KOR antagonist norbinaltorphimine reduces binge-like eating.

3. Does genetic knockdown of KORs within the nucleus accumbens (NAcc) reduce binge-like eating behavior? We will determine if genetic knockdown of KORs within the NAcc reduces binge-like eating using adeno-associated viral delivery of KOR siRNA.

- **Lori Zeltser, Ph.D.**

Assistant Professor of Clinical Pathology
Columbia University

“Mouse Knock-in of Human Bdnf Val66Met Polymorphism to Study Susceptibility to Anorexia Nervosa”

Key Words: Anorexia Nervosa, BDNF, Critical Period

As the maintenance of energy balance is essential for survival, animals evolved biological systems that defend against caloric restriction. We hypothesize that impairments in the establishment of metabolic set-points during the development of neuronal circuits regulating energy homeostasis can increase susceptibility to restrictive anorexia nervosa (ANR). Genetic studies in families with eating disorders identified an association between a polymorphism in the gene encoding brain-derived neurotrophic factor (Bdnf) and ANR. The Bdnf Val66Met polymorphism is reported to impair activity-dependent BDNF release. This mode of BDNF release is best-characterized in the context of promoting the maturation of GABAergic synapses during critical periods for the development of sensory circuits, and we predict that it would perform a similar function in developing circuits regulating feeding and body composition.

The central hypothesis of the proposed study is that deficits in the maturation of GABAergic synapses in circuits regulating energy homeostasis underlie the increased susceptibility to ANR observed in humans homozygous for the BdnfMet allele. To test this theory, we will calorie-restrict a mouse model segregating for the human BdnfMet variant and examine whether defense of metabolic baselines and/or return to initial levels of food intake after restoration of ad libitum feeding is impaired. If hBdnfMet/Met mice recapitulate increased susceptibility to ANR reported in humans, it would provide a novel model to study physiological and neuroanatomical correlates of ANR. We will examine whether the initial pattern of GABAergic projections in hypothalamic circuits regulating energy balance is altered in hBdnfMet/Met mice. Analyses at later timepoints will provide insight into whether these circuits respond differently to puberty and/or changes in feeding status. The novel implication of developmental processes in the brain in mediating susceptibility to anorexia would open up new avenues of research and ultimately, could lead to more effective treatment strategies for Eating Disorders.

Klarman Family Foundation Grants Program in Eating Disorders Research 2010 Award Recipients

Two-Year Awards

- **Chiye Aoki, Ph.D.**

Professor of Neural Science and Biology
New York University

"The Role of GABA in Regulating Synapses Altered by Puberty and Anorexic Behavior"

Key Words: Puberty, GABAA Receptors, Hippocampus, Hormone, Synapse, Neurosteroid, Activity-Based Anorexia (ABA), Stress

Our goal is to understand the neurobiological basis for anorexia nervosa (AN) vulnerability, with the hope of providing the rationale for better pharmacologic treatments. We will test a novel hypothesis -- namely, that AN vulnerability increases among females at the onset of puberty, due to developmental changes in the GABAergic receptor (GABAR) subtypes expressed within the cortico-limbic pathway, leading to elevation of stress-induced anxiety. The hippocampus is a key structure regulating anxiety as well as behavioral plasticity. We have shown that, for female rodents, entry into puberty is associated with a dramatic change in the expression of GABAR in the hippocampus, from those containing alpha1 and gamma subunits to those with alpha4 and delta subunits, thereby rendering the hippocampus less sensitive to benzodiazepines and more sensitive to THP (allopregnanolone), a neurosteroid released during stress. Without THP, this subunit change causes a reduction in the excitability of pyramidal neurons and impairment of hippocampus-dependent learning. With THP, hippocampal excitability and learning are restored. Importantly, the GABAR change converts THP from being anxiolytic to anxiogenic. If AN vulnerability is linked to this GABAR subunit switch, then we should be able to reduce AN vulnerability by blocking the action of THP upon GABARs. This idea will be tested by using an animal model of AN - activity-based anorexia (ABA). We will also test the hypothesis that AN intractability is due to GABAR subunit switch, by using (1) Western blots to identify pubertal female brain regions with elevated expression of alpha4 and delta subunits; (2) electron microscopy to determine whether these subunits are increased at synapses to modulate phasic inhibition and/or at extrasynaptic sites to modulate dendritic excitability through shunting inhibition; and (3) whether pharmacologic intervention of THP action that is successful for reducing AN vulnerability also reduces GABAR subunit switch in ABA brains.

- **Jeffrey Friedman, M.D., Ph.D.**

Professor; HHMI Investigator
The Rockefeller University

“Molecular and Neuronal Basis of Reward Driven Ingestive Behavior”

Key Words: Binge Eating, Reward Driven Behavior, BAC-TRAP Technology

Mouse models of behavioral disorders have proven to be of immense value for the identification and validation of druggable molecular targets. For instance, the early stage development of SSRIs relied on mouse behavioral assays such as the Forced Test Swim. When SSRIs are administered, mice are able to swim or float for longer periods of time than controls. This behavioral readout had great predictive value for the efficacy of antidepressant drugs in later stages of clinical testing. Numerous mouse behavioral assays have also been developed for other neuropsychiatric diseases such as autism or OCD.

Current mouse behavioral models of binge eating disorder fail to recreate key traits of a binge episode in humans where patients compulsively overeat highly palatable food in discrete hedonic episodes but can have normal homeostatic eating (meal cycles). Patients also report a lack of self-control similar to reward seeking behavior.

We have created a new murine behavioral assay in which a binge episode is reward driven and can be induced with optogenetic tools. We will characterize our newly developed 'optogenetic binge', and use Halorhodopsin to inactivate specific downstream targets of DA projections. Halorhodopsin is a chloride channel that hyperpolarizes neurons with exposure to yellow light. This approach will enable us to dissect the behavioral role of specific sites to which VTA-DA neurons project.

A rational pharmacological approach to treat binge eating disorders would be to use drugs that regulate the excitability of reward neurons directly involved in bingeing, avoiding other dopaminergic neurons belonging to circuits mediating other reward driven behaviors. This is pertinent for minimization of side effects, in case validated targets lead to future drug development.

Dissociating the neuronal basis of reward driven behaviors will advance our understanding of binge eating behavior and other reward related dysfunctions. Importantly, it will lead to new generation of drug targets and potentially guide future drug design strategies to address the treatment of binge eating disorder. All together, this project opens new avenues of research addressing binge eating disorder.

- **Joanna Steinglass, M.D.**

Assistant Professor of Clinical Psychiatry, Eating Disorders Clinic
Columbia University

“Delayed Gratification: A Biobehavioral Characteristic of Anorexia Nervosa”

Key Words: Anorexia Nervosa, Delay Discounting, fMRI, Adolescence,
Prefrontal Cortex

Anorexia nervosa (AN) is a severe mental illness affecting primarily young women. The substantial morbidity and mortality of AN are, in part, related to its high relapse rate following successful initial treatment and its often chronic illness course. The mechanisms that underlie the development and impressive persistence of this illness are poorly understood. The proposed research will begin to examine a novel neurobiological model of AN that focuses on the capacity to delay gratification.

Individuals with AN are unusually able to override innate, normal impulses, such as the drive to eat. Illness commonly emerges during adolescence, a time when most individuals exhibit reduced capacity to control impulses, reflecting the slower maturation of the prefrontal cortex relative to limbic subcortical regions. Using a non-food temporal delay discounting task known to reflect activation of the prefrontal cortex, we have obtained preliminary data suggesting that individuals with AN are more prone than controls to favor delay of reward.

We now propose to evaluate 30 individuals with AN, ages 16 to 25, before and after weight restoration on our inpatient unit and 30 age matched healthy controls using the delay discounting task to measure tendency to delay receipt of monetary reward. We will use simultaneous fMRI to examine associated neural functioning. Both before and after weight restoration, we hypothesize that patients with AN will manifest hyperactivity of the lateral prefrontal cortex, compared with controls, during performance of this task.

If established, these hypotheses would provide a foundation for the development of a coherent neurobiological model that may account for enduring characteristics of individuals with AN that are adaptive in many circumstances but may also increase the risk of developing chronic AN.

- **Andrew Steele, Ph.D.**

California Institute of Technology

“Elucidating the Neural Circuitry of Over-Exercise in a Mouse Model of Anorexia Nervosa”

Key Words: Anorexia Nervosa, Hunger-Sensing, Mouse, Home Cage Behavior, In Vivo Electrophysiology, Neural Circuitry

Our goal is to determine the neuronal circuitry of how decreased nutrient levels leads to hyperactivity in mice. This work proposal is relevant to the understanding of the underlying cause of anorexia nervosa because almost all cases of anorexia nervosa are accompanied by hyper-exercising. The methodology employed will be a combination of direct measurement of electrical activity in hunger sensing and reward associated brain regions using in vivo electrophysiology and mouse genetics to delete a key nutrient responsive enzyme, SIRT1, which likely mediates hyperactivity in response to low nutrient conditions. Elucidating the neuronal circuit mediating increased activity on a low calorie diet will facilitate the understanding and treatment of anorexia nervosa by implicating both a brain region and a neurotransmitter/neuropeptide system for therapeutic targeting.

Specifically, we aim to:

1. Characterize neuronal activation and firing associated with low calorie intake, or calorie restriction (CR). We will determine where in the brain neuronal activation markers are increased in CR mice compared to that of mice with ad libitum (AL, free access to food) diets by immunohistochemistry for c-fos induction, a "gold standard" marker for neuronal activity. We will record from neurons in the hypothalamus and ventral tegmental area of awake, behaving mice on chronic low calorie diets where they show temporally regulated hyperactivity to uncover correlations between neuronal activity in hunger sensing, reward, and locomotor areas.
2. Determine the neural circuit mediating the behavioral response to CR using deletion of the nutrient sensing enzyme, SIRT1. Test whether neuronal-specific deletion of SIRT1 abolishes the up-regulation of activity. Selectively restore expression of SIRT1 in brain regions to determine the region(s) sufficient for restoration of CR-induced hyperactivity. Record electrical activity in the putative SIRT1-dependent circuit using in vivo electrophysiology to determine neuronal firing correlates of behavior.

- **Baoji Xu, Ph.D.**

Associate Professor
Georgetown University

“Roles of BDNF in the Development of Arcuate Neurons”

Key Words: Brain-Derived Neurotrophic Factor, TrkB, Eating Behavior, Obesity, Axonal Growth, Neuronal Maturation, Neuronal Survival, Arcuate Nucleus, Paraventricular Hypothalamus

The long-term goal of this research project is to understand molecular and neural mechanisms governing eating behavior. Brain-derived neurotrophic factor (BDNF) plays crucial roles in the control of eating behavior, as mutations in the genes for BDNF and its receptor TrkB lead to hyperphagia and obesity in both mice and humans. Furthermore, *Bdnf* gene variants have been linked to human obesity and eating disorders in several association studies. The organization and functional activity of hypothalamic neural circuits plays critical roles in the control of eating behavior and BDNF is a potent regulator of neuronal development and synaptic function; however, the precise role of BDNF in the regulation of eating behavior remains unknown. Our preliminary results show that many neurons in the arcuate nucleus (ARC) express TrkB and their projection to the paraventricular hypothalamus (PVH) is greatly diminished in a *Bdnf* mouse mutant that develops severe hyperphagia and obesity. These results lead us to posit that BDNF controls eating behavior in part by regulating the assembly of the neural circuit between the ARC and the PVH. BDNF is expressed in the PVH and ventromedial hypothalamus (VMH), but not in the ARC. To test this hypothesis, we will examine the expression of BDNF in the PVH and the VMH during development and under different feeding states and investigate the role of BDNF in the development of ARC neurons by deleting the *TrkB* gene in a subset of ARC neurons and the *Bdnf* gene in the PVH, the main target of ARC neurons. Furthermore, energy homeostasis of these mutant mice and their responses to physiological factors controlling appetite will be examined. If successful, this research project will identify a source of BDNF (the PVH) and a mechanism (the development of ARC neurons) governing the action of BDNF in the regulation of eating behavior.

One-Year Awards

- **Saleem Nicola, Ph.D.**

Assistant Professor

Albert Einstein College of Medicine

“Nucleus Accumbens Opioid- and Dopamine-Dependent Neural Mechanisms of Binge Eating”

Key Words: Opioids, Nucleus Accumbens, Dopamine, Appetitive Behavior, Multi-Unit Electrophysiology in Behaving Rats, Appetitive Behavior, Addiction, Rodent Models of Binge Eating

Studies of recently-developed animal models of binge eating have led to the proposal that binge eating results from long-term changes in the brain similar to those that cause drug addiction. Addiction is characterized by decreased hedonia during drug-taking, coupled with enhanced motivation to seek drug. Here, we propose experiments to test the hypothesis that a similar shift occurs in binge eating: specifically, that consumption of highly palatable food in rats binging on high fat/high sugar food is driven by increased motivation to seek out such food, whereas in non-binging rats, it is driven by the high palatability of the food. We focus our studies on the nucleus accumbens (NAc), which may control both motivation (in concert with its dopamine projection from the midbrain) and palatability (through activation of mu opioid receptors). First, we use a combined electrophysiological and pharmacological approach in behaving animals to determine the mechanisms by which opioid receptor activation in the NAc regulates palatability. Next, we implement a rat models of binge eating (using limited access to a high fat/high sugar food) to determine how the NAc dopamine- and opioid-dependent mechanisms controlling motivation and palatability change across the development of binge eating, again using both electrophysiological and pharmacological techniques in behaving animals . Our goal is to elucidate the neural mechanisms that underlie binge eating, so that pharmaceutical treatments for binge eating disorders can be developed that specifically target these mechanisms.

- **Howard Steiger, Ph.D.**

Professor

McGill University

“Repeated Transcranial Magnetic Stimulation (rTMS), Clinical Features and Brain-Activation Patterns in Adults with Anorexia and Bulimia Nervosa: A Pilot Study”

Key Words: Eating Disorders, Anorexia Nervosa, Bulimia Nervosa, Repeated Transcranial Magnetic Stimulation (rTMS), Brain Activity, Functional Magnetic Resonance Imaging (fMRI), Neurobiology, Treatment

Disappointing responses of Eating-Disorder (ED) patients to "best-practice" treatments call for innovative therapy approaches. Repeated transcranial magnetic stimulation (rTMS) is a novel treatment, yielding alterations in cerebral activation and improvements in various psychiatric symptoms. We will examine rTMS-induced changes in clinical symptoms, brain-activation patterns, and neurotransmitter/neuroendocrine levels in adults with Anorexia Nervosa (AN: n= 30) or Bulimia Nervosa (BN: n= 30). We will: a) Use rTMS as a probe to establish relationships between anomalous frontostriatal brain activation and eating-disorder (ED) syndromes. b) Evaluate potentials of rTMS as an ED-treatment adjunct. AN is frequently associated with behavioural/ affective "over-regulation" and excessive frontostriatal activation, whereas BN is typically associated with behavioural/ affective "dysregulation" and frontostriatal under-activation. rTMS can selectively increase or decrease activity in underlying cortical regions, yielding corresponding behavioural changes. We will study effects of three 1-week (5-day) rTMS protocols: a) low frequency (1 Hz) stimulation presented bilaterally over the sensory motor area (SMA)--believed to decrease cortical activation, b) high-frequency (10 Hz) stimulation presented over the left dorsolateral prefrontal cortex (DLPFC)--believed to stimulate cortical activation, or c) "sham" (no stimulation) rTMS. We will obtain pre- and post-intervention measures of clinical symptoms, brain activation (using functional magnetic resonance imaging, or fMRI), and indices of neurobiological activity. Event-related brain activation will be measured during the performance of Wisconsin Card Sorting and Simon Spatial Incompatibility Tasks, respectively requiring cognitive flexibility and response inhibition. We expect low-frequency rTMS to decrease frontostriatal activity and to improve bodily (and generalized) preoccupations in AN, high-frequency stimulation to increase frontostriatal activity and improve binge/purge urges, mood and impulsivity in BN, and sham rTMS to have no effects. We expect symptom-matched rTMS to normalize neurobiological indices. In full form, this will be a comprehensive study of brain activation and rTMS treatments in the EDs.

- **Greg Suh, Ph.D.**

Assistant Professor

NYU Medical Center / NYU School of Medicine

“Identification of the Internal Caloric Sensor”

Key Words: Internal Caloric Sensor, Eating, Appetite regulation, Palatability, Nutritional Value

Both the nutritional value and palatability of food play a fundamental role in the control of eating behavior. Recent studies showed that sugar blind mice are attracted to sugar-rich food upon starvation. This suggests a hypothesis that there exists a taste-independent, internal sugar sensor that allows animals to develop sugar preference solely based on caloric content. Such an internal sensor continuously monitors the internal energy state of organisms and regulates food intake accordingly. Here, we propose to identify the internal caloric sensor using the fruit fly, *Drosophila melanogaster*. Like mice, flies in which sugar receptors (*GR5a* and *GR64a*) functioning in taste system are mutated are sugar blind, but could discriminate sugar solution over plain water upon starvation. These starved flies are attracted to sucrose or D-glucose, but not to non-metabolizable sugars, sucralose or L-glucose. This result indicates that the internal sugar sensor of the fly is responsive to the nutritional value of food, but not to its palatability. To identify such sensors, the cells and the molecule machinery, we will conduct forward genetic screens, highly amenable in *Drosophila*, for neural circuits and genes required for activation of the internal sugar sensing and subsequent feeding behavior. *Drosophila* has been used to dissect fundamental behaviors such as circadian rhythm, learning and memory, courtship behavior. Many behavioral genes and their signaling pathways are conserved from flies to humans. Likewise our studies will reveal the identity of the internal calorie sensors and provide a foundation for understanding the mechanisms by which appetite is regulated by the internal energy state in normal and eating disorder patients.

- **Lori Zeltser, Ph.D.**

Assistant Professor
Columbia University

"Mouse Knock-in of Human Bdnf Val66Met Polymorphism to Study Susceptibility to Anorexia Nervosa"

Key Words: Anorexia Nervosa, BDNF, Critical Period

As the maintenance of energy balance is essential for survival, animals have evolved biological systems that defend against caloric restriction. We hypothesize that impairments in the establishment of metabolic "set-points" during the development of neuronal circuits regulating energy homeostasis can increase susceptibility to restrictive anorexia nervosa (ANR). Genetic studies in families with eating disorders identified an association between a polymorphism in the gene encoding brain-derived neurotrophic factor (Bdnf) and ANR. The Bdnf Val66Met polymorphism is reported to impair activity-dependent BDNF release. This mode of BDNF release is best-characterized in the context of promoting the maturation and pruning of GABAergic synapses during critical periods for the development of sensory circuits, and we predict that it would perform a similar function in developing feeding circuits.

The central hypothesis of the proposed study is that deficits in the maturation of GABAergic synapses in feeding circuits during the critical period underlie the increased susceptibility to ANR observed in humans homozygous for the BdnfMet allele. To test this theory, we will calorie-restrict a mouse model segregating for the human BdnfMet variant and examine whether defense of metabolic baselines and/or return to initial levels of food intake after restoration of ad libitum feeding is impaired (Aim 1). Pruning of extraneous GABAergic synapses is central to the acquisition of sensory acuity and is disrupted in mice homozygous for the hBdnfMet/Met allele. We will define the timing of the analogous processes in the hypothalamic feeding circuits, initially focusing on the GABAergic projections from the arcuate nucleus to the paraventricular nucleus of the hypothalamus (Aim 2). We will then examine whether these events are perturbed in hBdnfMet/Met mice. The novel implication of developmental processes in mediating susceptibility to anorexia would open up new avenues of research and ultimately, could lead to more effective treatment strategies for Eating Disorders.

Klarman Family Foundation Grants Program in Eating Disorders Research

2009 Award Recipients, Two-Year Grants

Catherine Dulac, Ph.D.

Chair and Higgins Professor of Molecular and Cellular Biology
Howard Hughes Medical Institute Investigator
Harvard University

Genetic and Epigenetic Pathways Underlying the Neural Circuits of Feeding Behavior

Scientific Abstract

Anorexia nervosa, bulimia nervosa, and binge eating are complex neurological disorders, which arise primarily in women and, in addition to environmental factors, involve heritable genetic factors and abnormalities in social and motivated behaviors. We hypothesize that social, motivational, and homeostatic circuits regulating feeding behavior are governed by a conflict between maternally and paternally expressed imprinted genes in the adult brain. In a pilot study funded by the Klarman Foundation, we discovered hotspots for imprinting in brain regions known to regulate feeding behavior, including two major serotonergic inputs, the dorsal raphe and raphe pallidus. Aberrant functioning of the serotonergic system has been strongly implicated in both anorexia and bulimia nervosa. We next undertook a large-scale investigation of imprinted genes expressed in the embryonic and adult CNS using a novel approach that involves Solexa sequencing and single nucleotide polymorphisms that distinguish paternal and maternal allele-specific expression. We have uncovered numerous brain circuits involved in feeding behavior that express imprinted genes and the majority of imprinted genes expressed in the embryonic and adult CNS. We now seek to build upon the results of our pilot study to carry out a rigorous investigation into the role of genomic imprinting in the regulation of feeding behavior. Specifically, we now seek to: (1) Characterize the repertoire of imprinted genes, sexually dimorphic imprinted genes and the cell types expressing imprinted genes within feeding-related circuits of the brain; (2) Investigate the potential for heritable and nonheritable changes in the imprintome in response to major alterations in caloric intake; and (3) Investigate the function of specific imprinted genes in the regulation of feeding using a combination of mouse genetics and virus-based gene expression strategies. This study should reveal genetic and epigenetic pathways that are causally-linked to the onset of eating disorders or that can be used as targets for drug development.

Charles V. Mobbs, Ph.D.

Professor of Neuroscience, Department of Neuroscience
Mount Sinai School of Medicine

Role of Hypothalamic Metabolism in Estrogen-induced Anorexia

Scientific Abstract

The long-term objective of the proposed studies is to develop a treatment for anorexia. Anorexia is much more common in young women than men or older women or in children. Therefore a plausible hypothesis is that post-pubertal estrogen triggers anorexia in susceptible individuals. The proposed studies would assess the hypothesis

that estrogen triggers anorexia by inhibiting CPT-1 or inducing glucokinase leading to increased glycolysis and thus increasing hypothalamic sensitivity to the satiety effects of glucose. In particular, the proposed studies would test the effectiveness of a dietary intervention to prevent estradiol-induced anorexia, and the molecular mechanisms by which that diet might produce these protective effects.

Richard Palmiter, Ph.D.

Professor of Biochemistry
Howard Hughes Medical Institute Investigator
University of Washington

Elucidation of Mechanisms by which Dopamine and AgRP Neurons Affect Feeding Behavior

Scientific Abstract

We have developed two genetic mouse models in which loss of specific neurotransmitters from small populations of neurons results in severe anorexia. One model involves loss of dopamine from mid-brain dopaminergic neurons that project to the caudate putamen and the other involves loss of GABA from hypothalamic AgRP neurons that project to the parabrachial nucleus. In both cases, we have devised viral or pharmacological replacement therapies to restore feeding behavior. We propose to delve further into these two models to elucidate the neural circuits involved and to determine whether the loss of GABA from AgRP neurons and loss of dopamine affect the same circuits. We hypothesize that dysregulation of certain brainstem nuclei -- perhaps the same ones that are activated in response to gastrointestinal malaise -- are responsible for the anorexia that is observed when these specific neurotransmitters are eliminated. Elucidating the neural circuits involved in these mouse models should help in understanding and treating human eating disorders.

One-Year Awards

Kathryn Cunningham, Ph.D.

Professor and Interim Chair, Department of Pharmacology and Toxicology
University of Texas Medical Branch

Novel Chemical Therapeutics in Binge Eating Disorder

Scientific Abstract

Binge eating disorder (BED) is the most prevalent eating disorder in the U.S. and is linked to severe obesity as well as psychological and medical morbidity. Characterized by bursts of brief, compulsive eating binges in the absence of hunger, the neural mechanisms of BED are likely to involve serotonin (5-HT) circuits in hypothalamus which regulate feeding and satiety as well as circuit in limbic-cortico-striatal regions which affect reward and motivation. Therapeutic gains in BED may be achievable through the selective and sustained activation of 5-HT signaling through its cognate 5-HT_{2C} receptor (5-HT_{2C}R). The 5-HT_{2C}R is linked to the G $\alpha_{q/11}$ family of proteins and is known to activate phospholipase C (PLC), induce phosphoinositide metabolism and subsequently increase intracellular calcium (Ca_i⁺⁺). The 3rd intracellular loop of the 5-

HT_{2C}R makes a physical interaction with an intracellular protein known as *phosphatase and tensin homologue deleted on chromosome 10* (PTEN; Ji et al., *Nat. Med.* 12: 324, 2006), a tumor suppressor which is localized to limbic-corticostriatal pathways. A small peptide fragment of the 5-HT_{2C}R (3L4F; corresponds to Pro283-Arg297 of the 5-HT_{2C}R) competes with the 5-HT_{2C}R for binding to PTEN and evokes 5-HT_{2C}R agonist-like properties *in vivo* with a limited side effect profile (Ji et al., 2006). This discovery suggests that either a brain-penetrant peptide or small molecule that inhibits the 5-HT_{2C}R:PTEN interaction will be a novel method to enhance 5-HT_{2C}R function, possibly for sustained period of times. We will analyze the biology of this protein-protein interaction as a first step toward testing our overall hypothesis that pharmacological attempts to improve functionality of the 5-HT_{2C}R would be therapeutically efficacious in BED.

In Specific Aim 1, we will test the hypothesis that 5-HT_{2C}R-induced activation of downstream effector pathways is limited by PTEN assembly with the 5-HT_{2C}R and establish the selectivity of the partnership relative to PTEN interaction with the homologous 5-HT_{2A}R and 5-HT_{2B}R. A cell-based assay will be used to study the interaction between 5-HT_{2C}R:PTEN with endogenous Ca_i⁺⁺ release as a readout. In Specific Aim 2, we will test the hypothesis that modifications to the backbone of the 3L4F peptide (Pro283-Arg297 of the 5-HT_{2C}R) will generate molecules that functionally mimic the peptide in the cell-based assay. Peptides are subject to enzymatic degradation thereby limiting their utility as pharmaceuticals, and the knowledge of the smallest functional peptide will guide the design of non-peptide small molecules of functional interest in BED.

This project is innovative, potentially high impact research on a novel target, and the planned approaches have not been applied in either eating disorders or obesity. The outcomes will help us understand the neurobiology of the 5-HT_{2C}R system and how the formation of a protein-protein complex with PTEN controls activation of 5-HT_{2C}R signaling pathways. With this advanced understanding of regulatory mechanisms for the 5-HT_{2C}R and the availability of new cellular models, peptides and other reagents generated from these studies, the future presents the opportunity to explore these systems in preclinical models of binge eating with the goal to develop new therapeutic approaches to treat BED.

Jeffrey M. Friedman, M.D., Ph.D.

Professor; HHMI Investigator, Department of Molecular Genetics
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The Rockefeller University

Molecular Profiling of Feeding Neurons

Scientific Abstract

Feeding disorders such as binge eating, bulimia and anorexia nervosa are pressing public health concerns, yet, no medication exist that is effective at treating them. At present, serotonin reuptake inhibitors have been used to treat some feeding disorders, but there is a notable lack of efficacy of these agents for the long-term treatment of these conditions. This application proposes to identify novel therapeutic targets that modulate activity in neurons that control feeding so that future drug development strategies target molecules specific to neurons directly controlling feeding.

First, we test the functional capacity of neurons in the hypothalamus to induce or repress feeding when activated with channelrhodopsin. Second, we will generate a comprehensive list of druggable targets in neurons that can regulate feeding using the newly developed BAC-Trap technology which allows one to generate transcriptional profiles from specific classes of neurons. Drugs that target GPCRs, ion channels and kinases are used to treat a wide array of conditions, including hypertension, cancer, epilepsy, heart arrhythmias, hypertension and pain. In particular, Ion channels regulate membrane potential and neuronal excitability, and are attractive drug targets for diseases that originate from abnormal neuronal activity. Ion channels are especially well characterized as a class owing to advances such as voltage and patch-clamp methodology and crystal structures. Advances in high-throughput methods that screen for drugs that can modulate these targets open new opportunities for identifying new classes of drugs to treat nutritional disorders. A rational pharmacological approach to treat feeding disorders would be to use drugs to regulate the excitability of hypothalamic neurons directly controlling feeding. Profiling feeding neurons for gene expression will enable the identification of a new generation of drug targets and potentially guide future drug design strategies to address the treatment of eating disorders.

Jeri Janowsky, Ph.D.

Professor of Behavioral Neuroscience and Neurology
Director of Neurological Sciences Institute
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Modifying Body Image Using Prefrontal Cortical Control Mechanisms

Scientific Abstract

A central feature of anorexia, bulimia and other eating disorders is a pervasive misperception about the size and shape of one's body along with intense negative affect regarding body size and its relation to eating. In this pilot study, we will test the hypothesis that cognitive control imposed by the prefrontal cortex can significantly alter the negative affect associated with body image. This hypothesis stems from our prior work on prefrontal functional and hormonal changes of aging, and the control of emotion in women. We further hypothesize that this neural and cognitive mechanism is used by women who have recovered from an eating disorder. To test this, we will compare brain activity (fMRI) and behavioral assessments of body image and affect in adult women who have not had a diagnosis of an eating disorder as well as in women who have recovered from an eating disorder earlier in life. Women will rate their own bodies that have been computer morphed to be fatter or thinner than their actual size. They will rate perceived size and affective response under conditions that vary their ability to use cognitive control. The focus of the imaging study is the activity and interaction among the amygdala, prefrontal cortex and a region of the fusiform that responds specifically to bodies. We hypothesize that fatter images will increase negative affective responses in all women, but women who have recovered from eating disorders will show amplified prefrontal and lower amygdala activity even when their behavioral ratings match those of women who have not had eating disorders. Further, degradation of cognitive control will result in much greater negative affect, a perception that the body is larger than it is, and higher amygdala activity in women who have recovered from an eating disorder than those who never had an eating disorder. The

ultimate goal of studies that will build on this pilot study is an understanding of the neural and hormonal control of body image. Future studies will examine cognitive control in women with anorexia, and in collaboration with clinicians who treat anorexia, utilize the concept and features of cognitive control as a potential therapy tool.

2008 Award Recipients

Wade Berrettini, M.D., Ph.D.

University of Pennsylvania

Genome-wide Association Study of Anorexia Nervosa

Scientific Abstract:

The long-term goals of this project are to delineate the alleles which increase risk for anorexia nervosa (AN). Genetic epidemiologic studies suggest that ~ 50% of the risk for AN is inherited. Identifying the alleles which increase AN risk will lead to better diagnosis and treatment through a more complete understanding of the underlying pathophysiology.

Geneticists have hypothesized that both common and rare alleles predispose to relatively common diseases, such as AN. Common alleles are theorized to have limited odds ratios (< two), while rare alleles (at the same genes) have larger odds ratios.

As a first step in identifying AN risk alleles, aim one of this proposal is a genome-wide association (WGA) analysis of ~ 1500 comprehensively assessed, unrelated female AN probands, all of European origin. The AN results will be compared to results for ~ 4500 female control subjects of European origin, whose genotyping was completed at the same core facility where the genotyping will occur. This should allow for provisional identification of common AN risk alleles. As part of aim 1, supplemental genotyping will be done in the genes of highest statistical significance and biological plausibility, to maximize genetic information from those genes. This will identify the common variants in these plausible AN risk genes. In aim 2, these same plausible AN risk genes will be re-sequenced in ~ 200 AN unrelated probands, selected for having the risk alleles at the provisionally identified genes. This will identify the uncommon risk alleles at these genes. Thus, this approach will capture the common AN risk alleles of relatively small effect, through the WGA aim, and, it will detect the uncommon AN risk alleles (possibly of relatively larger effect) at these same loci through re-sequencing.

Catherine Dulac, Ph.D.

Harvard University

Genetic and Epigenetic Pathways Underlying the Neural Circuits of Feeding Behavior

Scientific Abstract:

Despite the wealth of knowledge regarding the circuitry and mechanisms that govern homeostatic aspects of feeding, very little is known about the biological basis of eating disorders. Anorexia nervosa (AN), bulimia nervosa (BN), and binge eating (BE) are complex neurological disorders, which, arise primarily in women and, in addition to environmental factors, involve heritable genetic factors and abnormalities in social and

motivated behaviors. We propose the hypothesis that the social, motivational, and homeostatic circuitry regulating feeding behavior is governed by a conflict between maternally and paternally expressed imprinted genes in the adult CNS. Imprinted genes only express either the maternal or the paternal allele as a result of inherited epigenetic modifications. They are thought to have evolved as a result of a parental conflict over the asymmetrical investment of resources by the mother versus the father in the growth and development of offspring. Importantly, feeding has been proposed as a primary point of parental conflict. We propose three specific research aims for a comprehensive investigation of this hypothesis: (1) Identify and map the expression of the entire repertoire of imprinted genes in brain areas involved in feeding and motivated behavior; (2) Identify sexually dimorphic imprinted genes expressed in feeding-related circuitry; and (3) Investigate the function of specific imprinted genes in the regulation of feeding using mouse genetics and established behavioral paradigms. To accomplish these aims we have developed a novel approach to discover and characterize imprinted genes in the adult male and female CNS and will investigate the functional role of imprinted genes of interest in feeding using knockout and BAC transgenic mice and viral gene-expression based approaches. This study is anticipated to reveal genetic and epigenetic pathways that are either causally-linked to the onset of eating disorders or that can be used as targets for drug development.

Guido Frank, M.D.

University of Colorado Denver

The Brain Reward System Across the Major Eating Disorders and its Relationship to Genotype

(funded by the Davis Foundation)

Scientific Abstract:

The Eating Disorders (EDs) Anorexia Nervosa (AN), Bulimia Nervosa (BN), and Binge Eating Disorder share symptoms involving disturbed food intake behavior. Food is considered a "natural reward stimulus", and abnormal eating behavior in EDs suggests brain reward system disturbances. In this application we will study the brain reward system using food (sucrose solution) and non-food (monetary) reward learning paradigms together with functional brain imaging and neurocomputational methods. In addition, we will collect DNA in order to test whether the reward anticipation related dopamine D2 receptor A1 genotype predicts aspects of reward brain function differently in EDs compared to controls. We will recruit female ill AN, BN, and BED subjects and age and gender matched healthy controls, 16-45 years old, with 17 subjects per cell. Aim 1 is to investigate ED brain reward pathways. Aim 1.a. will test the hypothesis that AN will have reduced, while BN and BED will have increased reward brain activation in areas such as the ventral striatum in response to food stimuli. Aim 1.b. will test the hypothesis that in an immediate monetary reward task, AN will have reduced, while BN and BED will have increased brain activation in the striatum, insula, and orbitofrontal cortex. In contrast, a delayed monetary reward task will show, in AN, increased, but in BN and BED, reduced brain activation in the dorsolateral prefrontal and parietal cortex. Aim 2. is to test the hypothesis that the dopamine D2 receptor A1 genotype will predict an even greater reduced brain reward response in ill AN, while in BN and BED subjects, that genotype will predict a less reduced brain reward response compared to the healthy controls. This will indicate that, in all ED groups, the normal gene-behavior

relationship is disturbed compared to controls, but in opposite directions, and specifically related to reward anticipation.

Angela Guarda, M.D.

Johns Hopkins University School of Medicine
Role of the Cannabinoid (CBI) System in Bulimia Nervosa

Scientific Abstract:

This project will characterize alterations in the endocannabinoid system in women with bulimia nervosa (BN) and in a behavioral rodent model of the bulimic restrict-binge cycle. We hypothesize that cyclical calorie restriction followed by binge-eating on palatable food induces state-related cannabinoidergic changes that sustain bulimic behavior. Activation of endocannabinoid CB1 receptors is implicated in both addiction and in motivated eating behavior with CB1 receptors being highly expressed in hypothalamic feeding-related nuclei and in the mesolimbic reward circuitry. Cannabinoids impact aspects of feeding behavior relevant to BN including hyperphagia, preference for palatable foods and motivation for food reinforcement.

This project will include two studies. In the first study positron emission tomography (PET) will be used to compare CB1 receptor binding in women with BN and in healthy controls. We hypothesize differences in striatal and frontal cortex CB1 receptor availability between groups and predict that in the bulimic subjects, receptor availability will correlate with both chronicity and severity of bulimic behaviors. The second study will employ micro-PET, receptor autoradiography and regional gene expression to quantify alterations in brain cannabinoid systems in a state-related model of bulimia in female rats. Our preliminary data support altered CB1 receptor gene expression using real-time RT-PCR in the striatum of rats cycled on this restrict-binge protocol. This behavioral paradigm provides intermittent access to a sweet, high-fat "binge" food in calorie-restricted rats, resulting in escalating binge-like eating behavior over a 6-week period. We further hypothesize altered CB1 receptor availability by micro-PET imaging as well as alterations in CB1 receptor binding and mRNA expression by autoradiographic and in-situ hybridization techniques, respectively. Characterization of underlying neurobiological alterations in the endocannabinoid system in BN could clarify mechanisms involved in maintaining eating disordered behavior, lead to novel targets for therapeutic drug development and inform behavioral interventions for eating disorders.

Alvaro Pascual-Leone, M.D., Ph.D.

Beth Israel Deaconess Medical Center

The Role of the Right Prefrontal Cortex in Binge Eating Disorder: a Translational Research Study using Transcranial Magnetic Stimulation (TMS) and Functional Magnetic Resonance Imaging (fMRI)

(funded by the Davis Foundation)

Scientific Abstract:

Binge eating disorder (BED) is the most common eating disorder in the U. S. Despite it being increasingly recognized as a major cause of morbidity and a public health burden,

the pathophysiology of BED remains poorly understood. Based on several lines of evidence, we propose a multidisciplinary investigation under the hypothesis that a dysfunction in right prefrontal cortex (PFC) circuits critically contributes to the maladaptive eating patterns that characterize BED. The long-term goals of this project are to shed light on the neurocognitive mechanisms underlying BED, and to open a new therapeutic approach with neuromodulation-based interventions. Specifically, we will assess whether enhancing the activity of the right dorsolateral PFC (DLPFC) in BED patients using repetitive transcranial magnetic stimulation (rTMS) can lead to a decrease in the frequency of binge episodes, energy intake and the experience of loss of control, and whether these effects correlate with cognitive and neuroimaging changes. We will study 36 patients fulfilling DSM-IV criteria for BED in a sham-controlled, randomized, proof-of-principle study, involving three parallel groups, where participants will receive 10 days of rTMS targeting: (a) the right DLPFC (active site), (b) the left DLPFC (topographic, active control) or (c) sham rTMS (placebo). Evaluations will be performed at baseline, immediately after rTMS and at 6 weeks of follow-up. To assess outcomes, we will use a combination of behavioral, nutritional, cognitive, and neuroimaging measures. Patients will complete take-home diaries, perform a battery of cognitive tests assessing decision-making, self-awareness, response inhibition and body image, and take part in a laboratory buffet meal test. Before and after stimulation, structural and functional brain magnetic resonance imaging will also be performed to evaluate potential changes in brain responses to high- and low-calorie food both below and above the conscious level, as well as grey matter changes.

Maribel Rios, Ph.D.

Tufts University School of Medicine

Examination of the Role of Brain-Derived Neurotrophic Factor in Binge Eating Behavior

Scientific Abstract:

A recent national study revealed that within the US population, binge eating disorder (BED) afflicts 3.5 and 2.0% of women and men, respectively, and that bulimia nervosa (BN) affects 1.5% of women and 0.5% of men. Effective treatment strategies for affected individuals are greatly needed. However, the neuromolecular mechanisms contributing to the etiology of these disorders remain largely unknown. Defective brain-derived neurotrophic factor (BDNF) signaling through the tropomyosin related kinase B (TrkB) receptor emerged recently as a candidate mechanism. Human association studies suggest a link between BED and BN with the Val66Met polymorphism in the *Bdnf* gene, which impedes regulated secretion of BDNF. Furthermore, mice (BDNF^{2L/2LCK-cre}) in which we deleted *Bdnf* across the brain, exhibited dramatic hyperphagic behavior, reminiscent of bingeing behavior in humans. This proposal aims to elucidate the role of deficient BDNF signaling in binge eating behavior, a feature of both BED and BN. We propose behavioral studies to ascertain whether BDNF^{2L/2LCK-cre} mutants exhibit increases in food reward under baseline conditions and following food restriction and stress. Moreover, we will conduct a systematic analysis of the mesolimbic dopaminergic system in BDNF mutants as this pathway mediates reward and motivated behavior, including consumption of palatable food. Analysis will include measurements of dopamine signaling, synthesis and secretion in wild types and BDNF mutants. The effect of palatable food ingestion on TrkB signaling within the mesolimbic system will also be determined. Finally, to pinpoint regions of the brain that are essential suppliers of BDNF for the regulation of motivated eating, we will evaluate the

effect of selectively deleting Bdnf in the ventral tegmental area (VTA) and medial prefrontal cortex (mPFC), which are the chief sources of BDNF within the mesolimbic system. Collectively, these studies will determine whether the BDNF/TrkB pathway is a viable target for the treatment of BED and BN.

Leslie Vosshall, Ph.D.

The Rockefeller University

Identification of Novel Genes and Circuits in an Animal Model of Binge Eating Disorder

Scientific Abstract:

The etiology of compulsive feeding behaviors including bulimia nervosa and binge eating disorder in humans is poorly understood. We propose that studying these important clinical conditions in a simpler genetic model system, the larva of the fruit fly *Drosophila melanogaster*, may shed new light on this important health problem. Fruit flies go through four distinct life stages: embryo, larva, pupa, and adult. While adult flies regulate their feeding according to hunger status and the circadian clock just like normal humans, the larva resembles a binge eater because it feeds continuously for nearly 72 hrs, eating 3-5 times its own weight in food. About 24 hrs before pupation, the larva abruptly leaves the food medium and stops eating. This highly stereotyped behavior provides an attractive experimental model to explore the neuronal mechanisms that drive and sustain continuous (compulsive) feeding. The overall hypothesis to be evaluated is that continuous feeding in the *Drosophila* larva is a behavior accessible to genetic and pharmacological modulation. We will carry out microarray analysis to identify candidate genes subject to regulation during continuous feeding. Using a genome-wide RNA interference (RNAi) screen, we hope to identify genes that modulate food intake. We will complement the RNAi screen with a small molecule screen that will look for compounds that reduce food intake. Finally, we will study the neuronal circuits modulating continuous feeding. Our long-term goal is to identify genes and neuronal circuits mediating the continuous feeding behavior of larvae and to prove that this compulsive-like behavior can be decreased by specific pharmacological interventions. We hope to illuminate common principles underlying the regulation of feeding behavior that will be applicable to parallel processes occurring in human patients suffering from compulsive eating disorders.

Jeffrey Zigman, M.D., Ph.D.

U.T. Southwestern Medical Center

Mechanisms by which Ghrelin and Orexin Defend against Depression and Anxiety

Scientific Abstract:

Anorexia Nervosa and Bulimia Nervosa are both associated with high rates of depressive and anxiety disorders. It is likely that these co-morbid conditions contribute greatly to the progression of both AN and BN and the frequent relapses that occur during treatment. AN and BN also are both associated with high levels of the hormone ghrelin. Ghrelin effects change in several behaviors and physiologic processes as a response to negative energy balance, including potent stimulation of feeding. We have recently shown that ghrelin can induce both antidepressant and anxiolytic behaviors in

mice, that chronic stress increases ghrelin levels, and that mice unable to respond to ghrelin experience more depression and eat less upon exposure to chronic stress. We also have shown that caloric restriction induces antidepressant and anxiolytic behaviors and that these effects are blocked upon deletion of the ghrelin receptor or orexin, which is a putative downstream neuropeptide target of ghrelin. We hypothesize that elevated ghrelin levels arising from the disordered eating patterns characteristic of AN and BN may help individuals cope with their underlying depressive and anxiety symptoms and therefore reinforce the feeding behaviors. In the current application, we provide a series of studies designed to further explore the mechanism by which ghrelin and orexin interact to promote anxiolytic and antidepressant behaviors. We will investigate the requirement of orexin signaling for ghrelin's effects on mood and anxiety by characterizing the behavioral effects of ghrelin administration to mice deficient in orexin and of direct microinjection of ghrelin into the lateral hypothalamic area, which houses orexin neuronal cell bodies. Furthermore, we will determine if selective ghrelin receptor expression within orexin neurons is sufficient for ghrelin's anxiolytic and antidepressant actions. We believe that these proposed studies present a direct channel for translation into the development of new effective treatments for eating disorders.