Klarman Family Foundation Grants Program in Eating Disorders Research

<u>**2009 Award Recipients, Two-Year Grants**</u>

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-corticostriatal 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 pharmaceutics, 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
Head, Laboratory of Molecular Genetics
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
Oregon Health and Science University School of Medicine

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 (BDNF2L/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 BDNF2L/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 puparation, 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.