

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.