

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.