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 and 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 mechanis 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

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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

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Disappointing responses of Eating-Disorder (ED) patients to "best-practice" treatments call for innovative therapy approaches. Repeated transcranial magnetic simulation (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.