

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-HT_{2A}:5-HT_{2C} Rheostat for Therapeutics in Binge Eating Disorder”

Key Words: Binge Eating Disorder, Serotonin, 5-HT_{2A}, 5-HT_{2C}, 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-HT_{2A} receptor (5-HT_{2A}R) and 5-HT_{2C}R in brain circuits that drive the incentive-motivational salience of food and cues that predict food. We postulate that 5-HT_{2A}:5-HT_{2C} homeostasis in the corticostriatal circuit sustains behavioral inhibition. Our discovery that 5-HT_{2A}:5-HT_{2C} 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-HT_{2A}:5-HT_{2C} 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-HT_{2A} antagonist:5-HT_{2C} 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-HT_{2A}:5-HT_{2C} 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-HT_{2A}:5-HT_{2C} 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.