Davis Foundation
Postdoctoral Fellowship in Eating Disorders Research

2011 Fellows

- **Matthew Carter, Ph.D.**
  Postdoctoral Fellow
  University of Washington
  Mentor: Richard Palmiter, Ph.D.

  Optogenetic Investigation of the Parabrachial Nucleus in Food Intake
  The identification and functional characterization of the neural populations and circuits that mediate healthy and abnormal food-seeking behavior is paramount for understanding the neural basis of eating disorders. This project aims to investigate the role of the parabrachial nucleus (PBN) in mediating anorexia. Recent evidence demonstrates that inhibition of PBN neurons increases food intake. Blockade of inhibitory GABA-A receptors in the PBN or ablation of inhibitory neural inputs from orexigenic arcuate nucleus neurons to the PBN causes starvation. Interestingly, preliminary data suggest that ablation of glutamatergic signaling from the PBN causes weight gain. Taken together, these results strongly suggest that increasing activity in the PBN inhibits food intake. However, a causal role for the PBN in mediating anorexia has not been established. We currently lack a method of genetically targeting the subset of PBN neurons hypothesized to directly modulate food intake. Furthermore, we lack direct evidence that excessive neural activity in these neurons directly causes starvation or that inhibition of neural activity in these cells promotes feeding.

  My overall hypothesis is that hyperactivity of PBN neurons suppresses food intake while inactivity of PBN neurons increases food intake. I propose to test this hypothesis in three Specific Aims: (1) To validate methods of genetically targeting PBN neurons. I will target PBN neurons using multiple approaches so that I can eventually express Cre recombinase in that unique population for future experimental access and delivery of genetically-encoded probes. (2) To determine the sufficiency of PBN neural activity in suppressing food intake. I will target channelrhodopsin-2, a blue-light activated cation channel, into relevant PBN neurons and test the effects of stimulating these neurons on food intake in awake, behaving animals. (3) To determine if inhibition of PBN neural activity increases food intake. I will target halorhodopsin, a yellow-light activated chloride pump, into relevant PBN neurons and test the effects of inhibiting these neurons on food intake. Taken together, these experiments will provide insight into the role of the PBN in mediating anorexia and potentially establish a new target for treating eating disorders.

- **Yiran Guo, Ph.D.**
  Postdoctoral Fellow Researcher
  Children's Hospital of Philadelphia
  Mentor: Hakon Hakonarson, M.D., Ph.D.

  <u>Genome-Wide Association Study of Anorexia Nervosa</u>
  Anorexia nervosa (AN) is an eating disorder characterized by an obsessive fear of gaining weight, often coupled with a distorted self image resulting in an inability to maintain a healthy body weight. It is often maintained by various cognitive biases that alter how the affected individual evaluates and thinks about her or his body, food and eating. AN has strong evidence of heritability, with the high mortality rate amongst psychiatric disorders, mostly effecting adolescent females.

  We at the Center for Applied Genomics (CAG) at Children's Hospital of Philadelphia (CHOP), at the University of Pennsylvania, and with collaborators, recently carried out the first genome-wide association (GWA) study on AN, using ~610,000 single-nucleotide polymorphisms (SNPs) probes across the human genome in 1033 AN cases and 3733 pediatric control subjects of European ancestry. We confirmed that common SNPs within OPRD1 confers risk for AN, and also obtained suggestive evidence that common SNPs near HTR1D confer risk for restricting-type AN specifically. We also found a number of potential new genetic associations in compelling gene regions and we also investigated copy number variations (CNVs) in the same datasets and identified several regions with rare structural variants that were only observed in AN cases. These results point to intriguing new genes that await further validation in independent cohorts for confirmatory roles in AN. The fellowship applicant in this proposal, his mentors in CAG, CHOP and UPenn, and our additional collaborators are well positioned to expand the existing AN GWA study dataset and to increase the statistical power to detect and confirm additional AN genetic signals which will give the field greater insight into the biology of AN and potentially identify new and existing drug targets.

- **Sylvie Lardeux, D.V.M., Ph.D.**
  Postdoctoral Fellow
  Albert Einstein College of Medicine
  Mentor: Saleem Nicola, Ph.D.

  Control of Food Choice by Opioid and Dopamine Modulation of Nucleus Accumbens Neuronal Firing in a Rat Model of Binge Eating
  The neural mechanisms underlying binge eating in diseases such as binge eating disorder and bulimia nervosa are not yet well understood. However, recent results lead to the provocative hypothesis that binge eating disorders and addiction share a similar neurobiological mechanism. The opioid and dopaminergic systems in the nucleus accumbens (NAc) have been observed to undergo similar changes in animal models of binge eating and addiction. Few studies, however, have explored the precise mechanisms underlying binge eating. It is well known that mu opioid receptors in the NAc are involved in palatability-driven consumption, and that the dopaminergic system in the NAc is involved in stimulus-driven appetitive behavior. Therefore, modification of one or both of these systems in the NAc is likely to be part of the neural mechanism underlying binge eating disorders.

  To better understand these mechanisms, we have developed a rat model of binge eating using intermittent access to a sweetened oil/cream solution. We will test the hypothesis that mu opioid and dopamine receptor activation in the NAc differentially influences the choice of food to consume in binge eaters and controls. Furthermore, we will determine whether these differences are due to different degrees of influence of these receptors on neural encoding of decision variables by NAc neurons in binge eaters and controls. To accomplish these aims, we will use a task in which rats will choose, in discrete operant trials, between two rewards that differ in fat or sugar content. We will determine how mu opioid dopamine receptor antagonists, injected into the NAc, affect food choice in naïve and binge eating rats. Next, we will determine how NAc neurons encode decision variables in these tasks, and determine how receptor antagonists injected directly into the NAc influence the encoding of decision variables by NAc neurons. These experiments will provide an extensive, detailed exploration of the physiological mechanisms underlying binge eating disorders, thereby filling a critical gap in our understanding of this disorder and providing new avenues of research towards novel treatments.

- **Byungkook Lim, Ph.D.**
  Postdoctoral Fellow
  Stanford University
  Mentor: Robert Malenka, M.D., Ph.D.

  The Role of Melanocortin Signaling in Nucleus Accumbens on Stress-induced Anorexia
  Elucidating the neural circuitry responsible for feeding behavior has important implications for understanding the development of obesity and anorexia. The anorexic hypothalamic neuropeptide, melanocortin (alpha-MSH), is elevated in the brain by high fat diets and chronic stress. I propose to use molecular manipulations combined with electrophysiological, anatomical and behavioral assays to address the hypothesis that alpha-MSH signaling via the melanocortin 4 receptor (MC4R) in the nucleus accumbens (NAc), a key component of the brain¡¯s reward circuitry, is critical for mediating pathological adaptations in feeding behavior. Preliminary data showed that the incubation of NAc slices with alpha-MSH induced a significant change in the stoichiometry of synaptic AMPA receptors (AMPARs) in one subtype of medium spiny neuron (MSN).

  Chronic restraint stress, which induced anorexia documented by weight loss, caused identical changes in AMPAR stoichiometry to those induced by alpha-MSH. Moreover, the downregulation of MC4Rs in NAc MSNs by in vivo expression of shRNA to MC4R blocked the stress-induced changes in both synaptic properties and body weight. Therefore, one aim of this proposal is to perform a more rigorous and comprehensive electrophysiological study of the synaptic modifications elicited by melanocortin signaling in NAc MSNs in response to chronic stress including the examination of long-term synaptic plasticity [i.e. long-term potentiation (LTP) and long-term depression (LTD)]. Another aim is to identity the alpha-MSH producing hypothalamic neurons that project to the NAc using a retrogradely transported rabies virus. Finally, the most ambitious aim is to examine the behavioral consequences on feeding behavior of modifying the activity of the ¥á-MSH-secreting hypothalamic neurons projecting to the NAc. This will be accomplished by expressing inwardly rectifying potassium channels or channelrhodopsin specifically in these neurons using injection of rabies virus expressing Cre recombinase into the NAc and injection of adeno-associated virus (AAV) expressing those proteins in a Cre-dependent manner into the hypothalamus. The results of this project will elucidate how a critical hypothalamic neuropeptide influences feeding behavior via regulation of the brain¡¯s reward circuitry.

- **Chen Liu, Ph.D.**
  Postdoctoral Fellow II
  University of Texas, Southwestern, Southwestern Medical School
  Mentor: Joel K. Elmquist, D.M.V, Ph.D.

  <u>Identification of Neural Pathways that Mediate Serotonin-Induced Anorexia</u>
  Perturbations of central serotonin (5-HT) function result in emotional and eating deficits that resemble core systems of Anorexia nervosa (AN), suggesting dysfunction of 5-HT signaling may contribute to the pathophysiology of AN. Indeed, serotonergic compounds that stimulate the central serotonin pathways such as d-fenfluoamine (d-Fen) and sibutramine elicit potent anorexic responses in both rodents and humans. However, the neural basis underlying the anorexigenic actions of these 5-HT agents remains largely unknown. Exploration of the neural pathways that mediate these processes may provide potential candidate brain sites for understanding the pathophysiology of AN.

  Among 5-HT receptors, 5-HT2CR has been implicated as the primary target that mediates the appetite-suppressing actions of 5-HT agonists. For example, the anorexigenic effect of 5-HT compounds is blunted in 5-HT2CR null mice, whereas treatment with m-chlorophenyl-piperazine (mCPP), a 5-HT2CR agonist, is sufficient to suppress food intake. 5-HT2CRs are widely expressed in the brain. However, the CNS sites where 5-HT2CR signaling mediates the anorexigenic effect of 5-HT agonists remain to be defined. To this end, we generated re-activatable 5-HT2CR null mice that are phenotypically identical to the conventional 5-HT2CR nulls. Unique to this model, endogenous 5-HT2CR expression can be selectively restored in the presence of Cre recombinase so that we can directly assess whether re-expression of 5-HT2CRs in distinct population of neurons is sufficient to restore the anorexic responses to 5-HT agonists in mice with global HT2CR deficiency. Using this strategy, we have recently identified hypothalamic pro-opiomelanocortin (POMC) neuron as one of the physiologically important targets that mediate the anorexigenic effect of 5-HT agonists.

  To further explore the neural networks that underlie 5-HT-indcued anorexia, we propose to selectively reactivate 5-HT2CRs in other key hypothalamic or brain stem neurons that have been suggested to contribute to 5-HT's regulation of food intake. The long-time goals of this research plan are set to map functional brain targets underlying 5-HT-induced anorexia and identify critical neural circuits for complex feeding behaviors whose malfunction may contribute to the pathophysiology of AN.

- **Simal Ozen, Ph.D.**
  Postdoctoral Fellow
  Stanford University
  Mentor: Luis de Lecea, Ph.D.

  The Role of Lateral Hypothalamus in Anorexia: The Trigger of Hyperactivity
  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 the energy expenditure. Two cell groups in lateral hypothalamus (LH), hypocretinergic cells (Hcrt) and leptin-receptor containing (LepRb) cells, are key players in regulation of homeostatic balance. Leptin is a circulating hormone that reflects the condition of existing fat stores in relation to the current activity levels. Low levels of leptin, such as during food-restriction, promote arousal and high energy-expenditure for the individual to seek out food and to be active and available when the food becomes accessible. This survival strategy is disrupted in AN; self-starvation induced hyperactivity is intentionally allocated for facilitation of the energy-deficit. This hyperactivity in presence of voluntary-starvation might be explained by the imbalanced activation of Hcrt and LepRb cells. Low leptin levels would, likely, decrease the activity of LepRb cells which would diminish their inhibitory control over Hcrt cells. Such a disinhibition-induced up-regulation of Hcrt cells could explain the self-destructive hyperactivity, especially when considering the strong projections from Hcrt cells to the dopaminergic reward circuitry. However, the current knowledge on the role of LH in AN pathology is limited since studying the functional significance of these inputs requires the use of cell-type specific manipulation techniques that were not possible until recently. Here, we propose to perform an integrative study combining optogenetics with behavioral and physiological recordings in order to identify the role of LepRb and Hcrt cells in an activity based anorexia mouse model. First, we will explore the effects of optogenetic silencing of Hcrt cells on wheel-running and food-intake. We will also assess the hedonic aspects of hyperactivity by recording the unit activity in dopaminergic reward circuitry. Second, we will focus on LepRb cells as the potential cause of Hcrt activation and will investigate the effects of optogenetic activation of LepRb cells on anorexia. Overall, our study will provide a novel approach to understand the neurobiological causes of hyperactivity associated with anorexia and will, hopefully, open new areas for therapeutic interventions.

2010 Fellows

- **Haijiang Cai, Ph.D.**
  Postdoctoral Fellow
  California Institute of Technology
  Mentor: David J Anderson, Ph.D.

  Role of Central Amygdala Neural Circuits in Emotion and Eating Disorders
  Emotional disorders such as depression and anxiety are co-morbid with eating disorders, however, their causality is still unknown. The long term goal of this project is to investigate the neural basis of emotion and eating disorders, understanding of which we believe will help us know better about the etiology of eating disorders, and develop novel drugs or cognitive therapies for them.

  Central amygdala is a brain region that has long been known for emotion control and has recently been suggested to be involved in eating behavior. However, the underlying neural circuits are difficult to determine due to its cellular complexity. In this proposal, we will use transgenic mice to test the hypothesis that central amygdala neural circuitry controls both conditioned fear responses (emotional disorder) and aversive cue-induced cessation of feeding (eating disorder), focusing on two populations of neurons in the lateral part of central amygdala (CEl), PKC-delta and CRF neurons.

  First, we will test the hypothesis that fear and eating behaviors are affected by optogenetic manipulation of these CEl neurons with channelrhodopsin (ChR2) or halorhodopsin (NpHR). Then, we will map their downstream target neurons in brain regions that are responsible for emotion or eating. We will also map their presynaptic input neurons from regions involved in emotion or eating control. We will investigate whether the neural circuits that underlie emotion and eating behavior are overlapping or separate in central amygdala. We have already found that these CEl neurons are innervated by insular cortex (IC), a brain region involved in eating disorders. Therefore, we will also test the hypothesis that fear and eating behaviors are affected after optogenetic manipulation of IC-CEl neural pathway. These experiments might help to identify the specific neural circuits that are abnormal in emotion and eating disorders, possibly enabling the development of more restricted and specific drugs.

- **Toni-Kim Clarke, M.Sc., Ph.D.**
  Postdoctoral Fellow
  University of Pennsylvania
  Mentor: Wade Berrettini, M.D, Ph.D

  Genetics of Anorexia Nervosa
  This proposal addresses genetic susceptibility to anorexia nervosa (AN). It will advance the field by identifying alleles which increase AN risk, thus enhancing our understanding of AN pathophysiology. Twin studies have estimated AN heritability to range from ~ 50% to 75%. Previous genetic studies of AN include a whole genome association study (GWAS) on ~1000 AN DNA samples and ~ 4600 control DNA samples using women of European origin with no AN history. The results of these analyses reveal intriguing findings associating the AKAP6 gene locus and several dopaminergic genes with AN. The purpose of this proposal is to test these initial associations in a second sample of ~ 500 unrelated women of European origin with AN and 1500 unrelated control women with no history of AN using Taqman genotyping assays. Single SNP and haplotype analysis will be conducted to determine whether the same alleles identified in the GWAS are associated with AN in this new sample. Although the AN GWAS has potentially important findings, this approach is useful only for relatively common SNPs and copy number variants. It is hypothesized that both frequent and rare SNPs predispose to common, complex traits, thus, re-sequencing genes where common alleles may be implicated in risk is a method to discover uncommon variants. Any confirmatory association signals detected in the second sample will be extended with exon sequencing of 500 unrelated AN DNA samples to detect uncommon alleles of large effect, which may substantially increase AN risk. Exon sequencing will be carried out using 454 sequencing technology on 50 DNA pools, each containing the DNA of 10 individuals. After conducting extensive quality control of exon sequence data, any valid rare variants will be prioritized for further analysis dependent on their proposed biological function. These biologically relevant rare variants will be confirmed for association in the remaining sample of ~1000 AN individuals and ~1500 controls. Such analyses will be carried out in order to estimate the frequency of rare variants in cases and controls and determine their potential role in susceptibility to AN.

- **Penny Dacks, Ph.D.**
  Postdoctoral Fellow
  Mount Sinai School of Medicine
  Mentor: Charles Mobbs, Ph.D.

  Molecular Mechanisms of Estrogen-induced Anorexia

  Estrogen is a likely biological contributor to anorexia nervosa. The mechanisms underlying estrogen-induced anorexia are unknown but may involve a shift in the metabolic profile in the hypothalamic ventromedial hypothalamus (VMH, including ventromedial nucleus (VMN) and arcuate nucleus), with decreased beta-oxidation and increased glucose metabolism. We propose that this shift is mediated by estrogen receptor alpha (ERalpha) inhibition of the transcriptional activity of CBP. For example, silencing of ERalpha in the VMN increases food intake and reduces glucose sensitivity; ERalpha can inhibit CBP activity; and inhibition of CBP can prevent metabolic shifts from glycolysis to beta-oxidation. We propose to use the robust model of estrogen-induced anorexia in male mice on a high-fat diet. First, we will examine whether estrogen induces anorexia in ERalpha knockout mice and whether rescue of VMH ERalpha expression (via AAV-mediated in vivo gene delivery) restores the anorexic effect. Glucose sensitivity will be measured to test whether estrogen effects on food intake correlate with glucose metabolism. Glucose-regulated gene expression in the VMH will be examined with qRT-PCR to test whether estrogen effects on food intake and glucose sensitivity correlate with a shift toward greater sensitivity to glucose. Glucose-regulated genes that exhibit estrogen-dependent changes in expression will be tested for physical association with ERa and CBP (CHiP studies) to examine whether decreased sensitivity to glucose is associated with ERalpha displacement of CBP from these genes. In the second Specific Aim, we will identify ERa domains necessary for estrogen-induced anorexia to narrow down potentially involved cellular pathways. Two potentially necessary domains are the ligand-binding and helix 12 (coactivator) domains, which have been shown to be necessary for ERalpha inhibition of CBP transcriptional activity. Estrogen effects on food intake will be tested after expressing various ERalpha deletion mutants in ERalpha knockout mice (either selectively in the VMH via AAV-mediated gene delivery or systemically via transgenic expression under control of the ERalpha promoter). All of these studies are designed to elucidate cellular and metabolic pathways by which estrogen and ERalpha can reduce food intake and potentially contribute to anorexia nervosa.

- **Jaime Maldonado-Avilés, Ph.D.**
  Postdoctoral Fellow
  Yale University
  Mentor: Ralph J. DiLeone, Ph.D.

  Role of the Prefrontal Cortex and Related Molecular Mechanisms in Altered Feeding Behaviors

  Much deserved attention has been given to the role of the hypothalamic in food intake, food motivated behaviors, and their relationship to eating disorders. However, convergent lines of evidence suggest that cortical circuits known to regulate executive control, including the Prefrontal Cortex (PFC) potentially contribute to the clinical features of eating disorder, including Anorexia Nervosa (AN). Yet, less in known about how the PFC control integrated feeding behaviors. The overall hypothesis of this proposal is that deficits in the circuitry of the PFC can results in altered self-regulation of feeding behaviors. The experiments proposed in this application will combine molecular and behavioral tools to understand the relationship between the PFC and feeding behaviors. Feeding states can induce gene expression and lead to changes in feeding behaviors. We recently found that a short exposure to a high fat diet resulted in a significant reduction in the expression of the microRNA miR-155 in the mouse mPFC. Given that microRNAs suppress the translation of many targeted genes, Specific Aim 1 we will determine: 1) the expression pattern of miR-155 across neuronal mPFC neuronal populations; 2) which genes are targeted by miR-155 and show increased expression after high fat; 3) the effect of viral-mediated miR-155 overexpression or inhibition on feeding behaviors. The mPFC circuitry mediates executive control, including inhibitory control, over numerous behaviors. To determine how the mPFC regulates integrated feeding behaviors, in Specific Aim 2 we will: 1) determine the activity of mPFC neurons, as reflected by immediate early gene expression, after performance of two behavioral tasks that involve inhibitory control over food intake: anticipatory negative contrast (ANC) and differential reinforcement of low rate of response (DRL). We will also determine 2) the effect of mPFC temporary inactivation on inhibitory control in ANC and DRL and 3) the molecular mechanisms underlying inhibitory control in ANC and DRL. Our goal is to determine which molecules/circuits within the mPFC can results in excessive or diminished self-regulation of integrated feeding behaviors.

2009 Fellows

- **Andrew Adams, Ph.D.**
  Postdoctoral Fellow
  Beth Israel Deaconess Medical Center
  Mentor: Eleftheria Maratos-Flier, M.D.

  <u>The Role of Melanin Concentrating Hormone (MCH) in the Pathology of Anorexia Nervosa (AN)</u>
  Anorexia Nervosa (AN) is a serious illness in which severe self-imposed food restriction is commonly associated with hyperactivity. Without treatment AN leads to a severe reduction in body mass to the point of starvation and has a high degree of morbidity and mortality. Understanding of the mechanisms underlying the pathology of AN is limited and treatments are largely inadequate. Animal models for the study of AN are largely lacking. We propose that mice lacking the hypothalamic neuropeptide melanin concentrating hormone (MCHKO) may serve as a novel model in which to study the pathology of AN. Mice without MCH have a lean phenotype, increased metabolic rate and increased physical activity. In addition, when allowed access to running wheels MCHKO run significantly more than wild type mice. Furthermore, when these mice are subjected to calorie restriction (CR) they begin to exercise to extreme, losing almost all their body fat; this is in contrast to WT mice which markedly decrease their activity during CR in order to conserve body mass. MCHKO mice are known to have increased dopaminergic tone which leads to an increase sensitization to the addictive effects of amphetamine. We propose that MCHKO mice are also hypersensitive to the rewarding aspect of physical exercise, to the point of an addictive phenotype. We predict this phenotype is mediated by the transcription factor DeltaFosB in the nucleus accumbens (NAc) specifically via modulation of DeltaFosB target genes Cdk5 and dynorphin which have been previously reported to alter dopaminergic signaling in the striatum. We also predict that running will act further exaggerate amphetamine hypersensitivity in MCHKO mice.

  The MCHKO animal represents a unique model in which animals engage in counterproductive exercise behavior in the context of food restriction. This mimics the hyperactivity syndrome observed in patients with AN. Analysis of this model will allow definition of some of the pathways and molecular mechanisms involved in this complex and debilitating disease.

- **Gabor Wittmann, Ph.D.**
  Postdoctoral Fellow
  Tufts Medical Center
  Mentor: Ronald Lechan, M.D., Ph.D.

  Identification of Neural Pathways that Mediate Stress-induced Anorexia
  The long-term goals of the proposed research plan are to fully elucidate the neural pathways in the rodent brain responsible for stress-induced anorexia and identify their peptide/neurotransmitter mediators. It is proposed that this approach will generate new insights about the pathophysiology of anorexia nervosa and potentially identify new targets for treatment. To test the hypothesis that neural inputs arising from the limbic system mediate the effects of psychological stress on centers of food intake regulation, and that the malfunctioning of these pathways contribute to the development of anorexia nervosa and other eating disorders, five specific aims will be addressed. Neuronal populations that are activated by psychological stress and project to the hypothalamic arcuate and ventromedial nuclei and specifically feeding-related neurons that synthesize proopiomelanocortin (POMC) and neuropeptide Y, will be identified by retrograde and anterograde transport techniques and nuclear immunolabeling for immediate-early gene expression following restraint immobilization. On the basis of this analysis, selective lesions will be made in each identified region by stereotaxic injection of the excitotoxin, ibotenic acid, and evidence for alterations in stress-induced anorexia and activation of feeding-related neurons sought. In addition, the importance of a specific neuronal group in the perifornical area that co-expresses the anorexic peptides, urocortin 3 (Ucn3) and thyrotropin-releasing hormone (TRH), in mediating stress-induced anorexia will be assessed by measuring transcriptional regulation of their peptide mRNAs in response to a variety of stress paradigms and determining whether they innervate anorexigenic POMC neurons in the arcuate nucleus. As an extension of these studies, the expression of the type 2 corticotropin-releasing factor receptor (CRF-R2, that binds Ucn3) and TRH receptors in POMC neurons will be determined, and the importance of CRF-R2 signaling to POMC neurons in response to psychological stress studied in the CRF-R2 knockout mouse.

- **Patricia Bonnavion, Ph.D.**
  Postdoctoral Fellow
  Stanford University School of Medicine
  Mentor: Luis De Lecea, Ph.D.

  Optogenetic manipulation of serotonergic neurotransmission in feeding behavior
  Anorexia nervosa is a growing concern in mental health, often inducing death. Food rejection with loss of pleasure to eat, body image distorsions, resistance to treatment often associated with chronic anxiety and obsessive-compulsiveness traits make the pathogenesis of this disorder poorly understood. Imaging studies in anorexic patients showed central serotonin (5-HT) system dysfunction suggesting 5-HT overactivity. However, selective serotonin reuptake inhibitors that increase brain 5-HT extracellular levels may help in some anorexia cases. Hence, 5-HT contribution in anorexia remains largely unclear addressing the following question: is 5-HT system overactivated or depleted in eating disorders such as anorexia? It is well established that 5-HT system participates in food intake regulation and satiety via its action on hypothalamic nuclei. In addition, its impairment is associated with several psychiatric disorders including depression, anxiety and obsessive-compulsive disorders partially due to 5-HT altered function on the meso-limbic system. We believe that symptoms and traits that characterize anorexia suggest modification in food reward and hedonic aspects of feeding, and therefore may involve inherent dysregulation of emotional and reward pathways involving 5-HT. Our main hypothesis is that 5-HT may be a link between the control of feeding and reward brain mechanisms that generate hedonic impact. In this context, our present project aims at establishing a causal role for 5-HT neurons of the dorsal raphe nucleus (DRN) in driving food reward and motivation to eat palatable food. This will be addressed thanks to in vivo optogenetic stimulation that directly actives or inhibits serotonergic neurons with cell-type specificity and millisecond-scale temporal resolution in freely moving mice. Optogenetic approach will allow us to paired immediate modulation of 5-HT neurotransmission with specific-feeding motivated behaviors. This method should help to clearly characterize the necessity and sufficiency of 5-HT neurotransmission in food reward and appetitive behaviors. In future studies, we plan to dissect out the food reward circuitry under 5-HT influences and identify 5-HT preferential targets. Elucidating the neurobiology of motivated feeding behavior should help in understanding 5-HT system impairments associated with psychiatric eating disorders such as anorexia.

- **Shinjae Chung, Ph.D.**
  Postdoctoral Scholar
  University of California Irvine
  Mentor: Olivier Civelli, Ph.D.

  The MCH System as a New Target for the Management of Eating Disorders
  Eating disorders are characterized by compulsive eating or extreme reduction of food intake accompanied by metabolic dysfunction. Defining its basic processes has however not been easy. Recently, several neuropeptide and monoamine systems have been identified to play an important role in eating disorders. The Melanin Concentrating Hormone (MCH) system is such a new neuropeptide system which is known to regulate energy homeostasis and various brain functions. Therefore, we propose to study whether the MCH system is involved in the etiology of eating disorders by testing whether its activity has any impact on two physiological responses associated with eating disorders.

  First, we hypothesize that the MCH system is involved in hedonic food reward. The MCH receptor is highly expressed in the shell of the nucleus accumbens (NAcSh), an important brain region to regulate various reward behaviors. We have recently identified that the MCH system can modulate cocaine addiction behavior. These two evidences lead us to propose that the MCH system might regulate hedonic food reward. The reward that palatable food brings is an important component of obsessive eating. Thus our aim is to show whether blockade of the MCH system can decrease the compulsive nature of palatable food seeking behavior.

  Secondly, we will study the MCH system's role in modulating the HPT axis. Hormonal abnormalities are commonly associated with eating disorders. In particular, thyroid hormone imbalance is closely related to cold intolerance which is often seen in eating disorders patients. Indeed, MCH1RKO mice cannot control body temperature when they are exposed to cold. We therefore propose to study whether modulating the MCH system can be beneficial to treat abnormal HPT axis function which is seen in patients with eating disorders.

  Our long term goal is to identify new physiological functions of the MCH system that contribute to the treatment of various human disorders, most notably eating disorders. We know the sites of action of MCH, having defined its receptor expression pattern. We want to link the activity of the MCH system at these sites to physiological responses relevant to the etiology of eating disorders ultimately to improve their treatments.

- **Monica Dus, Ph.D.**
  Postdoctoral Fellow
  NYU Medical Center / NYU School of Medicine
  Mentor: Greg Suh, Ph.D.

  Dissection of Neural Circuits Underlying Internal Caloric Sensing in Drosophila
  Both the nutritional value and palatability of food play a fundamental role in controlling eating behaviors and reward circuits. Recent studies show that mice respond to calorie-rich food even in the absence of taste, suggesting the existence of a taste-independent internal caloric sensor that modulates feeding. However, very little is known about how the internal energy state of organisms affects feeding. In particular, how the metabolic value of food is encoded in the brain is still unclear. We propose to study the internal caloric sensing pathway in the fruit fly Drosophila melanogaster. The availability of genetic, behavioral, and imaging tools in this model organism will allow us to dissect both the neural and genetic basis of feeding behavior with specific focus on the internal energy state and nutritional value of food in the absence of taste. In particular, we will use behavioral assays in sugar receptor mutant flies to test for the presence of an internal caloric sugar sensor. We will then perform both genetic and functional screens to identify the neural circuits and genes necessary for the activity of the internal caloric sensor. Our experiments will provide a valuable strategy and a framework for future studies on the neurogenetic control of feeding behavior and eating disorders.

- **Vikas Duvvuri, M.D., Ph.D.**
  Chief Resident
  University of California, San Diego
  Mentor: Walter Kaye M.D.

  Dopaminergic Signaling in Anorexia Nervosa
  Individuals with anorexia nervosa (AN) restrict food intake and become emaciated, and are overcontrolled, inhibited, anhedonic, and preoccupied with future consequences. Data suggests such symptoms in AN may be due to disturbances of dopamine (DA) function in the anterior ventral striatum (AVS) and frontal regions (impaired processing of immediate rewards), as well as the dorsal lateral prefrontal cortex (DLPFC) and striatal regions (overactive planning and concern about consequences). This 3 year study will compare 20 women recovered from AN (REC AN) and 20 healthy control women (CW). We will study REC AN to avoid the confounding effects of malnutrition and because they have behaviors and neural circuit alterations relevant for this study. In AIM 1, we expect that blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) and a delayed discounting task will show REC AN have diminished immediate but enhanced delayed gratification alongside a skew in neural correlates. AIM 2 interrogates delayed discounting by adding a double blind, randomly ordered single oral dose of amphetamine (AMPH) (a drug which stimulates DA release and reduces feeding), olanzapine (OLAN) (a drug with DA receptor antagonist activity which increases food intake and weight), or placebo one month apart (so each trial occurs at the same phase of menses). Data supports the prediction that AMPH will make subjects more tolerant of delays and increase inhibition (and OLAN will have opposite effects), thus affecting the rewarding or self-control aspects of palatable foods. These drugs will have asymmetric effects on REC AN due to inherent disturbances in neural function. In AIM 3, we will explore functional, genetic variations that predict behavioral and neuronal output by concurrently sequencing a set of DA signaling genes in the same subjects. The applicant is a 4th year psychiatry resident at UCSD, who completed a PhD in neurochemistry at Stanford and seeks to become an independent investigator in the neurobiology of eating disorders. Under the mentorship of Dr. Kaye, this research plan prepares the applicant in interdisciplinary approaches towards characterizing the molecular mechanisms of aberrant neural circuit function in AN and finding targets for effective treatments for this deadly illness.

- **Pouneh Fazeli, M.D.**
  Clinical Fellow in Medicine
  Massachusetts General Hospital
  Mentor: Anne Klibanski, M.D.

  The Neurobiology of Appetite Regulation in Anorexia Nervosa
  Anorexia nervosa (AN) is a disease predominantly affecting young Caucasian women characterized by self-induced starvation and complex genetic and environmental etiologic factors. Hormonal abnormalities identified involving appetite regulation and response to stress may increase susceptibility to AN. Gastrointestinal symptoms are prevalent. Altered hunger and satiety perception have emerged as candidate physiologic characteristics in AN and appear to have significance in maintaining low weight. Recent advances in our understanding of complex regulatory systems involving the brain and gastrointestinal tract, and the identification of novel gut-derived peptides that play a role in eating behavior and appetite have led to the finding that these pathways are dysregulated in AN.

  Our proposal uses functional Magnetic Resonance Imaging (fMRI) to measure differences in activation of affect-driven food motivation circuitry between women with restricting-type AN, women who have recovered (AN-R) from restricting-type AN, and healthy control (HC) subjects in response to a paradigm that has been shown to activate this circuitry in a pre- and post-meal state. Inclusion of an AN-R group will contribute to unconfounding the disease state from traits associated with AN. We will combine the imaging paradigm with pre- and post-meal blood sampling to investigate differences in the response of appetite-regulating hormones to food between AN, AN-R and HC and to examine correlations between neuroendocrine factors and activation of food motivation circuitry.

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  Eating Disorders: Connectivity and plasticity of neuronal circuits underlying abnormal feeding behaviour
  Eating disorders such as anorexia and bulimia nervosa are life-threatening conditions, notoriously difficult to treat owing to the complex psychological factors thought to be involved. Recent advances have indicated that feeding behaviour is strongly regulated by the hypothalamus. Alterations in hypothalamic function can perturb feeding behavior raising the possibility that its dysregulation could contribute to eating disorders.

  Little is known about how neurons within the hypothalamus communicate to control feeding behavior. It is not clear how the balance between known orexigenic and anorexigenic pathways is altered under conditions where food intake is reduced. This study proposes to identify novel targets for the treatment of eating disorders by answering the following questions:

  Broadly: What is the neural circuitry that regulates feeding? How do neurons regulating feeding respond to signals in the periphery and how do they process this information leading to long-term changes in connectivity? How is this circuitry altered in pathological states?

  The study will be carried out in 3 stages:

  Firstly, the connectivity of specific hypothalamic neurons (POMC, NPY and MCH) will be mapped by making in vitro patch clamp recordings from murine brain slices in which both the pre- and postsynaptic neurons are specifically labeled.

  Transgenic mice expressing ChR2m-Cherry in the presynaptic neuron of interest and GFP in the postsynaptic neuron will be generated via injection of lox-stop-lox ChR2-mCherry adenovirus or using BAC recombineering. Connections will be identified by stimulating the presynaptic neuron electrically or optically7 via ChannelRhodopsin, whilst simultaneously recording from the postsynaptic neuron.

  Secondly, once connections have been identified, long and short-term synaptic plasticity will be investigated, both in response to neuromodulators, e.g. leptin, and changes in behaviour, e.g. starvation.

  Thirdly, any changes in connectivity and plasticity of these neurons will investigated in mice displaying a phenotype of reduced feeding and bodyweight. Recordings will be made from both the MCH/ataxin-3mouse and the temporally inducible NPY knockout mouse (Agrp DTR/DTR).

  By comparing feeding circuitry in both normal conditions, and where one element of the circuit had been specifically ablated, it may be possible to provide insight into biochemical and neurological basis of eating disorders.

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  Pathogenetic role and therapeutic potential of the novel enzyme Ghrelin octanoyl-acyl transferase (GOAT) for Anorexia Nervosa and Bulimia nervosa
  Anorexia nervosa (AN) and bulimia nervosa (BN) are devastating eating disorders affecting approximately 1-5% of young adolescents. Neuroendocrine circuitries implicated in energy homeostasis are believed to be involved in both AN and BN. For this project, we propose to test a series of hypotheses which focus on an important novel component of the gut-brain axis as a potential neuroendocrine mechanism involved in anorexia and cachexia. Using a translational approach, we will a) dissect the possible mechanistic role and b) determine the potential value as a therapeutic target of the novel lipid sensor ghrelin octanoyl acyl transferase (GOAT) and its link to the neuropeptidergic control of food intake and body weight via acyl-ghrelin signaling in the CNS. For this purpose we will use animal models as well as analysis of an existing store of plasma samples from human patients with AN. We have recently made substantial progress in understanding the biological function of the novel lipid sensor GOAT and its ability to regulate food intake and body weight via activation of the afferent hormone ghrelin and its hypothalamic target circuits. We therefore propose to study the GOAT/ghrelin system as a functional link between nutrient sensing and CNS control of energy balance in the pathogenesis of AN and as a therapeutic target for AN. Our encouraging preliminary data show that GOAT regulates body weight and body fat. We have all necessary tools, models and assays in place to test if 1) mutations in the human GOAT gene are relevant for the etiology of AN or BN, 2) GOAT enzyme activity is decreased in AN or BN, 3) diet enriched with GOAT-activating substrate (medium chain triglycerides, MCT diet) modulates hypothalamic neuropeptidergic circuitry to promote food intake and weight gain and 4) chronic treatment of MCT diet induces or genetic GOAT activation rescues body weight in two established rodent models of AN. These proposed experiments will determine if the only known afferent endocrine pathway to promote a positive energy balance is involved in the pathogenesis of anorexia nervosa or represents a novel treatment target for this disease

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  Neural Encoding of Aversive Stimuli in Anorexia Nervosa
  The long term objective of this project is to create an animal model of anorexia nervosa in order to study the neural components underlying the disorder on a single cell level. Rhesus macaques exhibit spontaneous aversion to bitter tastants and to images of familiar subordinate conspecifics. This behavior mirrors that exhibited by individuals with anorexia nervosa, who have heightened aversion for high calorie foods and images of overweight bodies. We hypothesize that the symptoms of anorexia nervosa are caused by a heightened experience of disgust for a variety of stimuli resulting from the disinhibition of anterior insula by the perigenual anterior cingulate. We will study how neurons in the anterior insula of the rhesus macaque encode aversive stimuli, and how these neurons respond differently to various types of disgusting stimuli. In order to parallel our work in the animal model with a human clinical population, we will also determine whether the social processing of disgust results in altered communication between the anterior cingulate and the anterior insula in individuals with anorexia nervosa. Finally, we will attempt to recreate the disorder of anorexia nervosa in the macaque monkey model by inhibiting the anterior cingulate cortex, which we expect will strengthen the disgust responses in the anterior insula and cause greater sensitivity to aversive stimuli in the monkey.
  Specific Aim 1: To compare how neurons in the primate anterior insula encode aversive gustatory and social stimuli.
  Specific Aim 2: To measure disgusted facial expression in anorexic individuals, control individuals, and macaque monkeys in response to social and non-social stimuli.
  Specific Aim 3: To determine whether functional connectivity between insula and anterior cingulate is altered in individuals with anorexia nervosa.
  Specific Aim 4: To determine whether the inactivation of anterior cingulate cortex sensitizes rhesus macaques to aversive social and gustatory stimuli.