

Davis Foundation
Postdoctoral Fellowship in Eating Disorders Research

2009 Fellows

• **Andrew Adams, Ph.D.**

Postdoctoral Fellow

Beth Israel Deaconess Medical Center

The Role of Melanin Concentrating Hormone (MCH) in the Pathology of Anorexia Nervosa (AN)

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.

- **Patricia Bonnavion, Ph.D.**

Postdoctoral Fellow

Stanford University School of Medicine

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 distortions, 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 activates 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 San Francisco

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

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

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

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.

• **Leah Kelly, Ph.D.**

Postdoctoral Associate

The Rockefeller University

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 be investigated in mice displaying a phenotype of reduced feeding and bodyweight. Recordings will be made from both the MCH/ataxin-3 mouse 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.

• **Timo Mueller, Ph.D.**

Postdoctoral Fellow

University of Cincinnati

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

- **Karli Watson, Ph.D.**

Postdoctoral Fellow

Duke University

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.

- **Gabor Wittmann, Ph.D.**

Postdoctoral Fellow

Tufts Medical Center

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.