The Hunger Hormone Ghrelin Dynamically Tunes Phasic Mesolimbic Signals Underlying Food-Directed Behaviors

BY

JACKSON J. CONE

B.A. University of California, Berkeley, 2008

THESIS

Submitted as partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Neuroscience
in the Graduate College of the University of Illinois at Chicago, 2014

Chicago, Illinois

Defense Committee:

Michael Ragozzino, Chair
Mitchell F. Roitman, Advisor
Jamie D. Roitman
Amy Lasek
Patricia H. Janak, Johns Hopkins University

ACKNOWLEDGEMENTS

High praise goes to my advisor, Dr. Mitchell Roitman, for countless contributions to my scientific development. Perhaps most importantly, Dr. Roitman knew when to keep me focused and when to let me explore my own ideas. I would also like to thank Dr. Jamie Roitman, who in many ways acted as a second advisor. I will carry the many valuable lessons they instilled throughout my career. Dr. James E. McCutcheon and Samantha Fortin deserve special recognition for their valued friendship, as well as the countless discussions that have helped shape my experiments. I am also immensely grateful to Dr. Patricia Janak for giving me my start in neuroscience. This thesis represents a critical step along a path that started in her laboratory.

My deepest appreciation and love goes to my mother, who always put my well being ahead of her own. I thank my stepfather for showing me the value of hard work and always going the extra mile for his family. I thank my grandmother, for her love and support and my grandfather for letting me disassemble everything he had laying around his workbench, as these experiences fostered my desire to discover how things work. I am eternally grateful to Dr. Kayla A. Chase for her endless intellectual and emotional support throughout graduate school. Her kind heart and playful personality enrich my life every day.

This research was primarily supported by a grant from the National Institute of Health to Mitchell F. Roitman (DA025634). My training was also supported by an Institutional Training Grant to Dr. Mark Rasenick (T32 MH067631-06A1), the UIC Center for Clinical and Translational Science Pre-doctoral Fellowship (TL1 TR000049), and the UIC Dean's Scholar Award. I received additional support from the Chicago Biomedical Consortium Scholars Award and the UIC Supplemental Graduate Research Fellowship.

AUTHORSHIP STATEMENT

Chapter II has been previously published as "Ghrelin acts as an interface between physiological state and phasic dopamine signaling" in the Journal of Neuroscience (34(14): 4905-4913; PMID# 24695709). I (J.J.C) was the primary author and Drs. James E. McCutcheon (J.E.M) and Mitchell F. Roitman (M.F.R.) were co-authors. Author contributions were as follows: J.J.C., J.E.M., and M.F.R. designed research; J.J.C. and J.E.M. performed research; J.J.C., J.E.M., and M.F.R. analyzed data; J.J.C., J.E.M., and M.F.R. wrote the paper.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER II

CHAPTER III

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AP	Anterior-posterior
AMP	Adenosine monophosphate
AgRP	Agouti related peptide
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
α-MSH	Alpha-melanocyte stimulating hormone
BMI	Body mass index
CCK	Cholecystokinin
CS+	Rewarded cue
CS-	Non-rewarded cue
DAG	Diacylglycerol
D1R	Dopamine 1 receptor family
D2R	Dopamine 2 receptor family
DAT	Dopamine transporter
DV	Dorsal ventral
FSCV	Fast-scan cyclic voltammetry
Gβγ	G beta-gamma
GABA	Gamma-aminobutyric acid
GOAT	Ghrelin o-acetyltransferase
GLP-1	Glucagon-like peptide-1
GHS-R	Growth hormone secretagogue receptor
GMP	Guanosine monophosphate

Hz	Hertz
HCl	Hydrochloric acid
IP_3	Inositol triphosphate
ip	intraperitoneal
kg	kilograms
LH	Lateral hypothalamus
LV	Lateral ventricle
LDTg	Laterodorsal tegmental nucleus
ML	medial-lateral
MSN	Medium spiny neuron
MCH	Melanin-concentrating hormone
μA	microamps
mg	milligrams
mm	millimeter
mV	millivolts
NMDA	N-Methyl D-aspartic acid
NPY	Neuropeptide Y
NAc	Nucleus accumbens
PVH	Paraventricular nucleus of the hypothalamus
PPTg	Pedunculopontine nucleus
PI3-K	Phosphoinositide 3-kinase
PLC	Phospholipase C
PCA	Principal component analysis

POMC	Pro-opiomelanocortin
PKA	Protein kinase A
PKC	Protein kinase C
s	second
Ag/AgCl	Silver/silver chloride
TH	Tyrosine hydroxylase
VTA	Ventral tegmental area

SUMMMARY

Food intake and body weight regulation has long been studied in the context of homeostasis. However, a strictly homeostatic view of feeding behavior cannot account for the dramatic rise in the incidence of obesity. Overconsumption is driven, at least in part, by the prevalence of calorically dense, highly palatable foods and the preponderance of environmental cues that signal their availability. Palatable food and their associated cues engage neural circuits underlying reinforcement and motivation, including the mesolimbic system, which is comprised of the midbrain dopamine neurons and one of their major targets, the nucleus accumbens. Food and food-predictive stimuli augment food-directed behavior by evoking brief (phasic) changes in nucleus accumbens dopamine and in the activity of nucleus accumbens neurons. However, the ability of food-related stimuli to drive food seeking depends on physiological state (hunger vs. satiety). Information about physiological state is relayed to the central nervous system through a variety of peptide hormones that are released by the digestive tract. Recent work indicates that peripheral feeding hormones act in a number of brain regions to augment food-directed behavior, including the mesolimbic system. Thus, peripheral feeding hormones may modulate mesolimbic encoding of food and food cues, and this could be a novel mechanism underlying their ability to augment food-directed behavior. However, this remains unresolved.

My experiments focus on ghrelin, a hormone released by the stomach that is strongly associated with hunger, and the only known peripheral peptide that promotes food intake. In the first study, I sampled sub-second fluctuations in dopamine concentration in the NAc using fast-scan cyclic voltammetry while rats retrieved sugar pellets (food reward). During these recordings, I manipulated central ghrelin signaling with intracranial injections of ghrelin or a ghrelin receptor antagonist to determine whether ghrelin regulated phasic dopamine release

evoked by food. In a second study, I sampled sub-second fluctuations in NAc dopamine using fast-scan cyclic voltammetry and recorded the activity of NAc neurons using in vivo electrophysiology in separate groups of rats that were trained to associate a cue with the delivery of food reward.

My results highlight novel mechanisms for ghrelin and physiological state to influence how the brain responds to food reward and food-related stimuli, particularly in areas of the brain linked to reinforcement and goal-directed behavior. I found that central administration of ghrelin increased, while central ghrelin receptor blockade decreased, the magnitude of dopamine release evoked by food. I went on to show that intra-LH, but not intra-VTA, administration of ghrelin recapitulated the results of central ghrelin infusions, thus pinpointing the LH as a potential site of action. Next, I demonstrated that intra-VTA orexin could be a plausible mechanism underlying the ability of central and LH ghrelin to regulate food-evoked dopamine release. In the second study, I found that central ghrelin administration augmented both phasic dopamine release and phasic increases in NAc activity evoked by a food-predictive cue. The data suggest that ghrelin may influence food seeking by modulating mesolimbic signals that contribute to reinforcement and goal-directed behavior.

Chapter I

Introduction

A. Preface

The neurobiology of food intake and body weight regulation has long been studied in the context of homeostasis. However, in today's obesogenic society, this framework is insufficient to explain the dramatic rise in the incidence of obesity. This is likely due to the prevalence of calorically dense, highly palatable foods and the preponderance of environmental cues that signal their availability. Palatable food and associated cues promote consumption by engaging the neural circuits underlying reinforcement and motivated behavior. Peptide hormones that signal energy status modulate behavior directed at food. However, these hormones have mainly been studied to identify their physiological effects on 'homeostatic' circuitry. Recently, it has come to light that these same hormones also act in motivational circuits to alter food intake. Thus, feeding hormones may modulate how motivational circuits encode food and food cues, which could be a novel mechanism underlying the ability of feeding hormones to influence food-directed behavior. However, this question remains unresolved and its resolution is the primary goal of this thesis.

Here, I start by highlighting the current state of the obesity epidemic. Next, I introduce the neural circuits involved in the homeostatic regulation of food intake and highlight the array of peripheral hormones that participate in the control of feeding behavior, with strong emphasis on the stomach hormone ghrelin. However, I recognize that a strict homeostatic view of feeding behavior fails to explain the prevalence of obesity. Thus, I introduce the mesolimbic system, a key contributor to behaviors motivated by food and associated cues. I will then discuss how the mesolimbic system encodes food and related cues, and how these signals have been directly

linked to food-directed behaviors. Next, I will posit that the mesolimbic system broadly integrates an array of signals associated with physiological state to produce a corresponding change in food-directed behavior. I conclude by introducing the key questions that this thesis aims to address – specifically regarding the modulation of mesolimbic signaling by the stomach hormone ghrelin.

B. Obesity has reached epidemic status

Obesity and excess body weight have rapidly become one of the greatest threats to public health. As of 2012, more than one third of the U.S. adult population fit the criteria for obesity, which is defined as a body mass index ≥30 (BMI; Ogden et al., 2014). This translates into roughly 78.6 million obese adults. Remarkably, another one-third of adult Americans can be classified as overweight (BMI≥25). This problem is not unique to the United States. A recent World Health Organization report indicates that worldwide obesity has more than doubled since 1980 (WHO, 2012). Obesity increases the risk of developing numerous chronic and debilitating health conditions, including type-2 diabetes, hypertension, and heart disease (Kopelman, 2007) and data suggests that the health consequences of obesity may outweigh the risk associated with smoking (Sturm and Wells, 2001). In addition to physical health, obesity is associated with mental health issues like anxiety and depression (Strine et al., 2008) and lower quality of life ratings (Kolotkin et al., 2001). Given these statistics, it follows that the obese spend up to 36% more on health care than normal weight individuals (Sturm, 2002) and this is likely contributing to rising health care costs across the entire U.S. medical system (Thorpe, 2005, 2006). The economic impact of obesity extends far beyond health care. Multiple studies have reported increased work absenteeism and fewer productive work hours in obese relative to normal weight

persons (for review see Hammond and Levine, 2010). Taken together, these data paint a dire picture, not only for public health, but also for the American economy.

According to Swinburn and colleagues, the 'obesogenicity' of an environment is defined as "the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations" (Swinburn et al., 1999). Perhaps it is not surprising then that the Centers for Disease Control have declared the U.S. an obesogenic society. Living in the U.S. enables an increasingly sedentary lifestyle while simultaneously providing easy access to inexpensive, highly palatable, and energy dense foods (Drewnowski and Specter, 2004; Owen et al., 2010). The difference between weight stability and weight gain comes down to simple mathematics: if energy intake exceeds energy expenditure, an individual gains weight. Thus, one way to understand obesity is to examine the factors that contribute to overconsumption.

In many parts of the country, it is impractical to walk, bicycle, or ride public transit to work, so over 90% of Americans drive to their place of employment (Winston, 2013). According to U.S. census data, the average commute time for U.S. workers is about 25.5 minutes one-way, but as many as 600,000 workers spend an hour and a half or more to get to their place of employment. Not surprisingly, there is a strong positive relationship between time spent driving and the likelihood of being obese (McCann and Frank, 2005). Moreover, the incidence of obesity is roughly half for people who live in walkable environments compared to areas that require cars for transportation (Frank et al., 2007). At work, the majority of Americans sit for long hours, which is known to increase the risk of obesity (Choi et al., 2010), however deficits in energy expenditure extend beyond our work lives. According to the U.S Department of Labor, Americans spend 50% of their leisure time watching television, yet another risk factor associated

with obesity (Hu et al., 2003). Many of these lifestyle and environmental insults could be offset with increased physical activity during leisure hours. However, only 20% of Americans meet the guidelines for weekly physical activity (U.S. Department of Health and Human Services, 2012). Together, the data suggest that sedentary behavior and the lack of energy expenditure in the hours before, during, and after work make considerable contributions to energy imbalance and the prevalence of obesity.

Deficits in energy expenditure only represent one part of the obesity problem. Between 1970 and the early 2000s, meal portion sizes more than doubled (Young and Nestle, 2002) and larger portions promote increased intake in laboratory settings (Rolls et al., 2006, 2007). Not surprisingly then, Americans consume more total calories per day than they did in 1970 (Prevention, 2004). Notably, this effect was driven by increases in sugar consumption, in spite of decreased fat intake. By some estimates, sugar consumption has increased by over 80 kilocalories per day than consumption patterns from the 1970s (Popkin and Nielsen, 2003), which may be due to the popularity of sweetened beverages (e.g.,soda, pop). Accessibility of inexpensive and highly palatable foods is another major contributor to the soaring rate of obesity (Drewnowski and Specter, 2004). For example, it is estimated that there are over 200,000 fast food restaurants currently in the U.S., and the proximity of one's home to a fast-food restaurant is associated with increased risk for obesity (Currie et al., 2010). This is likely because fast-food consumers eat more fat, saturated fat, sodium, and consume less vitamins, fruits and vegetables than those who do not eat fast food (Paeratakul et al., 2003). Increased caloric intake goes beyond formal meal times. Both children and adults snack more often, and the energy density of snacks has increased steadily over the last 20 years with most of the calories coming from candy and sweetened beverages (Piernas and Popkin, 2010a, 2010b). Thus, the current energy

imbalance encompasses both reduced energy expenditure and concurrent increases in the intake of energy rich but nutrient poor food.

Although the accessibility of calorie-dense food most certainly contributes to energy imbalance, explaining overconsumption comes down to more than availability. Highly palatable and positively reinforcing foods exert powerful effects on motivated behaviors aimed at food consumption and can promote intake, even in the absence of caloric need. In fact, sweetened foods can be so powerfully reinforcing, rats will forego the opportunity to obtain intravenous cocaine in favor of consuming sweet solutions (Lenoir et al., 2007). In addition to the foods themselves, environmental stimuli (sights, smells, advertisements) that signal the availability of food also influence feeding behavior. Humans are bombarded with promotions for food (Mink et al., 2010). This is especially problematic as cues associated with food powerfully enhance both ratings of appetite and food consumption (Ferriday and Brunstrom, 2008; Harris et al., 2009; Koordeman et al., 2010). Similar to humans, food-paired cues promote food intake and invigorate food-seeking behaviors, even in animals with no caloric need (Lovibond, 1983; Weingarten, 1983; Corbit et al., 2007). Thus, palatable foods that are heavily marketed through advertisements on TV, radio, and billboards are likely key contributors to overconsumption. Indeed, neighborhoods that have more advertisements for sugary beverages have higher incidence of obesity (Lesser et al., 2013). In summary, the prevalence of rewarding food and cues that signal food availability promote increased intake, even in the absence of caloric need, and their influence on food-seeking behavior is likely a key contributor to the obesity epidemic.

C. Hypothalamic control of energy balance

The hypothalamus is arguably one of the most heterogeneous brain structures in terms of the diversity of cell types and biological functions. Rather than elaborate on an exhaustive list, here I will focus on 3 major hypothalamic nuclei that have been strongly implicated in food intake and body weight regulation: the arcuate nucleus, paraventricular nucleus (PVH), and the lateral hypothalamus (LH). The arcuate nucleus is positioned at the base of the hypothalamus along the walls of the third ventricle. The vasculature near the arcuate is highly permeable, which permits greater diffusion of peripheral metabolic signals into the region (Ciofi, 2011). Thus, the arcuate exhibits vascular features characteristic of the circumventricular organs that are known to sense factors in the blood. This ideally positions neurons in the arcuate to integrate numerous signals related to physiological state (e.g., hunger, satiety, body adiposity). Indeed, arcuate neurons are sensitive to blood glucose, as well as multiple hormones related to physiological state (Hewson and Dickson, 2000; Morton and Schwartz, 2001; Wang et al., 2002; van den Top et al., 2004).

Three neuropeptides synthesized by arcuate neurons are strongly associated with regulation of food intake and body weight. One population of arcuate neurons synthesizes both Neuropeptide Y (NPY) and Agouti-related peptide (AgRP). Both NPY and AgRP increase food intake, though through different mechanisms. NPY acts directly on NPY receptors to influence feeding (Gehlert, 1999), while AgRP acts as an inverse agonist at melanocortin receptors (Ollmann et al., 1997). Another separate population of neurons expresses pro-opiomelanocortin (POMC) peptide. One of the cleavage products of POMC yields α-melanocortin stimulating hormone (α-MSH). α-MSH is a potent agonist of melanocortin receptors, which normally suppress feeding (Fan et al., 1997). Thus, by blocking α-MSH binding to melanocortin receptors, AgRP attenuates a signal that would otherwise inhibit food intake. Ablating NPY/AgRP neurons

in adulthood results in rapid weight loss (Gropp et al., 2005; Luquet et al., 2005), thus illuminating a critical role for these neurons in food intake and body weight regulation. Conversely, ablating POMC neurons results in hyperphagia and obesity (Gropp et al., 2005). In one study, ablation of POMC neurons resulted in an obese phenotype, in spite of reduced food intake (Greenman et al., 2013). Augmenting NPY/AgRP or POMC neuron activity also induces changes in locomotion (Mesaros et al., 2008; Huo et al., 2009). Thus, NPY/AgRP and POMC neurons contribute to energy intake as well as expenditure. Interestingly, knockout mice born with either NPY, AgRP, or both peptides deleted from their genome exhibit no differences in body weight compared to controls (Qian et al., 2002). This finding speaks to the diffuse network of structures that regulate essential processes like feeding behavior.

All of the above data highlighting roles for NPY/AgRP and POMC neurons in feeding behavior were collected in response to long-term and global insults. However, recent work with optogenetics, has revealed that selectively activating NPY/AgRP cell bodies causes mice to rapidly initiate feeding, while food intake is attenuated by selective activation of POMC expressing neurons (Aponte et al., 2011). Moreover, optogenetic stimulation of a variety of NPY/AgRP projections to a diverse array of brain regions is sufficient to promote food intake, including the PVH and LH (Betley et al., 2013). Conversely, selectively ablating GABAergic signals from NPY/AgRP neurons to hindbrain nuclei results in starvation (Wu et al., 2009). These data suggest that NPY/AgRP neurons utilize diverse projections to strongly influence multiple nodes that control feeding.

Given PVH's strong connections with the arcuate nucleus (Légrádi and Lechan, 1998; Broberger et al., 1999) and the sympathetic nervous system (Swanson and Kuypers, 1980), it is positioned to regulate both energy intake and expenditure. Lesions of the PVH result in

hyperphagia and obesity (Leibowitz et al., 1981; Weingarten et al., 1985) and pharmacological manipulations of the PVH interfere with thermogenesis – which impacts energy expenditure and thus body weight (Madden and Morrison, 2009). Selectively exciting or inhibiting distinct populations of PVH neurons promotes feeding (Atasoy et al., 2012; Krashes et al., 2014), while selectively deleting subtypes of PVH neurons promotes hyperphagia and decreases energy expenditure (Xi et al., 2012; Pei et al., 2014). PVH neurons are modulated by both NPY and melanocortins to regulate food intake and body weight (Cowley et al., 1999). Optogenetic stimulation of NPY/AgRP terminals in the PVH is sufficient to rapidly evoke feeding behavior (Betley et al., 2013). Thus, the PVH represents one site where arcuate NPY/AgRP neurons act to control food intake.

The LH has long been known to link energy status and food-motivated behavior (Berthoud and Münzberg, 2011). This link was initially established upon the discovery that electrical stimulation of the LH increased food intake (Margules and Olds, 1962) and lesions of the LH produced aphagia (Teitelbaum and Epstein, 1962). More recently, it was found that selective stimulation of NPY/AgRP input into the LH is sufficient to promote food intake (Betley et al., 2013). Thus, in addition to the PVH, the LH is another key site responsible for the control of feeding by arcuate neurons. The LH is also home to two unique non-overlapping populations of neurons that express: 1) the peptides Orexin-A and Orexin-B (also called hypocretins; de Lecea et al., 1998; Sakurai et al., 1998); and 2) melanin-concentrating hormone (MCH; Adamantidis and de Lecea, 2010). Central administration of either orexin or MCH peptides promotes food intake (Qu et al., 1996; Sakurai et al., 1998). While there are only roughly 5000 orexin neurons per hemisphere, these cells integrate the numerous signals related to metabolism and physiological state (Adamantidis and de Lecea, 2009), and project throughout the brain and

regulate aspects of feeding, arousal, and motivation (Peyron et al., 1998). Orexin A and B can both activate Orexin-1 and Orexin-2 g-protein coupled receptors, and thereby enhance excitatory neurotransmission. For example, orexin-A and B both increase excitatory NMDA currents and regulate aspects of long-term potentiation (Borgland et al., 2006, 2008), with orexin-B also potentiating presynaptic glutamate release (Borgland et al., 2008). In addition to food intake, the LH and the orexin system also control aspects of arousal and motivation. Orexin-A excites locus coeruleus neurons, a key brain region involved in arousal (Hagan et al., 1999). Central orexin-A administration promotes locomotor activity in rats (Hagan et al., 1999; Nakamura et al., 2000). Lastly, in regards to motivation, central orexin increases the amount of effort exerted to obtain food under a progressive ratio schedule (Thorpe et al., 2005). Thus, the LH potently influences both energy intake as well as energy expenditure.

D. Peripheral hormones act centrally to control energy balance

Information about an organism's physiological state is relayed to the central nervous system through a wide variety of pre and post-ingestive signals that are released in the periphery. These signals include - but are not limited to - circulating blood glucose levels and the release of peptide hormones from the digestive tract. While these peripheral hormones influence many processes related to digestion and blood glucose management, they have also received considerable attention for their ability to regulate food intake and body weight through actions in the central nervous system.

Consumption of food elevates blood glucose levels. Glucose is actively transported into the brain and can be sensed by a variety of different nuclei involved in feeding behavior (Levin et al., 1999; Burdakov et al., 2005; Parton et al., 2007). Importantly, blocking glucose utilization

in the brain promotes food intake (Ritter et al., 2011). This demonstrates that resting glucose levels can act as a satiety signal to actively suppress feeding. Elevation in blood glucose stimulates the release of both insulin and amylin from the β-cells of the pancreas (Kahn et al., 1990; Rorsman and Braun, 2013). While insulin and amylin act in concert to regulate blood glucose (Scherbaum, 1998), they also act in the brain to control energy balance and suppress feeding (Begg and Woods, 2012; Lutz, 2012). In addition to the postprandial effects on insulin and amylin secretion, the arrival of nutrients in the stomach triggers the release of bombesin, thereby stimulating the release of gastrin, which causes HCl levels in the stomach to increase to aid in digestion. However, bombesin can also act centrally to suppress food intake (Gibbs et al., 1979; Gibbs and Smith, 1986).

The passage of nutrients from the stomach to the duodenum of the small intestine promotes the secretion of cholecystokinin (CCK), which acts peripherally through the vagus to suppress feeding behavior (Smith et al., 1981; West et al., 1984). The presence of nutrients in the small intestine also triggers the release of glucagon-like peptide-1 (GLP-1; Baggio and Drucker, 2007), which acts through the vagus to suppress gastric emptying (Imeryüz et al., 1997) and food intake (Abbott et al., 2005). Although the half-life of GLP-1 in the periphery is estimated to be only two minutes, GLP-1 receptors are expressed in the CNS (Shimizu et al., 1987) and GLP-1 can act centrally to regulate feeding (Turton et al., 1996). In contrast to the signals that are upregulated immediately following the consumption of calories, the hormone leptin is released by adipocytes in proportion to body adiposity (Maffei et al., 1995; Schwartz et al., 1996). Plasma leptin levels fluctuate slightly throughout the day, with central levels remaining relatively constant (Wong et al., 2004). Thus, brain leptin concentration signals long-term energy reserves and acts through many brain nuclei to regulate feeding and energy expenditure (Myers et al.,

2008). Notably, leptin resistance is thought to contribute to the development of obesity (Münzberg et al., 2005). This occurs in part because elevated triglyceride levels can interfere with leptin transport across the blood-brain barrier, thus preventing leptin from acting on central targets (Banks et al., 2004). To summarize, positive caloric balance is signaled by a diverse array of peripheral hormones that are secreted by the gut as well as adipocytes, and these peptides act both peripherally and centrally to suppress food intake and body weight.

E. The stomach hormone ghrelin acts centrally and peripherally to promote food intake

All of the hormones discussed above are released in response to positive energy balance and act to suppress feeding behavior. However, ghrelin, a 28 amino acid peptide secreted primarily by endocrine cells in the fundus of the stomach, is the only hormone known to promote food intake (Kojima et al., 1999; Nakazato et al., 2001). Upon its' discovery, ghrelin was so named because it was found to bind to the growth hormone secretagogue receptor 1a (GHS-R) and promote the release of growth hormone from the pituitary gland (the root word *ghre* is taken from Proto-Indo-European languages meaning 'to grow'). However, very soon after the findings that linked ghrelin with growth hormone secretion, ghrelin levels were found to be increased by food restriction (Tschöp et al., 2000) and central injections of ghrelin were shown to potently stimulate appetite in rats (Wren et al., 2000; Nakazato et al., 2001). Only a few months later, ghrelin was found to increase ratings of appetite and promote food intake in humans (Wren et al., 2001). Plasma ghrelin levels rise preceding meals and fall shortly after eating, which led to the hypothesis that ghrelin was involved in meal initiation (Cummings et al., 2001). Moreover, plasma ghrelin levels correlate strongly with subjective ratings of hunger (Cummings et al., 2004), suggesting that ghrelin plays a role in the perception of hunger. These results, along with

many subsequent studies led to ghrelin becoming known as a 'hunger hormone.' In the years that followed, ghrelin has been shown to be involved in a wide array of phenomenon, including neuroprotection, anxiety, motivation, and memory (for a brief review see Andrews, 2011). Nonetheless, ghrelin is primarily studied for the regulation of food intake. Given that this thesis investigates ghrelin as it relates to food intake, I will focus on ghrelin's feeding-related functions exclusively.

Ghrelin undergoes post-translational modification by the enzyme ghrelin o-acetyltransferase (GOAT) in the stomach, which adds an octanoyl fatty acid group to the Serine 3 position (Gutierrez et al., 2008; Yang et al., 2008). The octanoyl modification is critical for GHS-R binding. Given that a ghrelin transporter has yet to identified, some believe the fatty acid chain assists in the ability of peripheral ghrelin to cross the blood brain barrier and enter the brain (Banks et al., 2002). The GHS-R is a seven transmembrane g-protein receptor that is coupled to Gαq. GHS-R mRNA is expressed throughout the brain, including the hypothalamus, hippocampus, amygdala, midbrain, and many hindbrain nuclei (Zigman et al., 2006). The gene encoding the GHS-R is highly conserved across species (Howard et al., 1996), and the ligand-binding domain has remained relatively unchanged across the last 400 million years of evolution (Palyha et al., 2000). Interestingly, the GHS-R rapidly desensitizes and the rate of receptor recycling is quite slow compared to other GPCRs. It can take up to 6 hours for surface receptor expression to return to baseline following ghrelin exposure (Camiña et al., 2004). Notably, the long delay between receptor internalization and surface re-expression closely mirror time between meals, and could perhaps contribute to ghrelin's physiological effects on food intake. The GHS-R is constitutively active, and can signal with roughly 50% efficacy in the absence of a ligand (Holst et al., 2004). However, mutations that alter constitutive activity have been linked to

short stature syndrome and do not affect ghrelin binding or ligand dependent signaling (Pantel et al., 2006). Thus, the GHS-R may serve multiple functions that differentially depend on the constitutive signal versus ligand-dependent activation.

Ghrelin binding to the GHS-R activates Phospholipase C (PLC), which generates diacylglycerol (DAG) and inositol triphosphate (IP_3). In turn, DAG triggers the release of calcium from internal stores, while IP_3 activates protein kinase C (PKC), which closes potassium channels (Cruz and Smith, 2008; Shi et al., 2013), although one report suggests that GHS-R activation closes potassium channels through cyclic GMP (Han et al., 2005). Nonetheless, activation of the GHS-R can depolarize the cells on which it resides through both calcium and potassium dependent mechanisms. Depolarization resulting from DAG and IP_3 stimulation also likely activates voltage-dependent calcium channels, further increasing intracellular calcium independent from the release of internal stores (Delmas et al., 2005; Balla, 2006). Indeed, GHS-R stimulation has been shown to increase intracellular calcium through both mechanisms resulting in a long lasting depolarization (Herrington and Hille, 1994). While some of these experiments were conducted before ghrelin was identified, thus requiring the use of exogenous GHS-R ligands, ghrelin induced stimulation of the GHS-R exhibits identical effects on intracellular calcium concentration (Yamazaki et al., 2004). Remarkably, there is also evidence that GHS-R activation can act through protein kinase A (PKA) and cyclic AMP, which also results in elevated intracellular calcium (Kohno et al., 2003). Together, these actions likely contribute to the ability of ghrelin to potentiate LTP (Diano et al., 2006), independent from NMDA receptor activation (Chen et al., 2011). In addition to post-synaptic effects, ghrelin has also been shown to modulate presynaptic neurotransmitter release through reduction in paired pulse suppression (Chen et al., 2011). Lastly, aside from effects on ion channels, central ghrelin

activates phosphoinositide 3-kinase (PI3K) and its' downstream effector Akt, which contribute to ghrelin's effects on both feeding and memory related processes (Chen et al., 2011; Kanoski et al., 2012). Taken together, GHS-R activation increases neuronal excitability through multiple independent pre and postsynaptic mechanisms.

Plasma ghrelin levels rise before meals and fall shortly after feeding (Cummings et al., 2001, 2004). The preprandial rise in ghrelin appears to be due to the activity of the vagus nerve, as destruction of the vagus disrupts ghrelin secretion that is normally observed in response to fasting (Williams et al., 2003). Thus, in addition to relaying the peripheral effects of ghrelin to the central nervous system, the vagus nerve is also involved in ghrelin secretion. Interestingly, postprandial suppression of ghrelin is unaffected by vagotomy, suggesting that elevation in ghrelin in response to fasting is controlled separately from postprandial suppression (Williams et al., 2003). Following food consumption, ghrelin levels are suppressed in proportion to the caloric density of the meal (Callahan et al., 2004; Le Roux et al., 2005a). Notably, different macronutrients are differently effective at suppressing ghrelin secretion. Carbohydrates and protein suppress ghrelin levels longer than fat, however carbohydrate ingestion results in a robust rebound in ghrelin secretion approximately three hours after ingestion (Sánchez et al., 2004; Foster-Schubert et al., 2008). Thus, ghrelin levels are modulated not only by food intake but also by the type of nutrients consumed. In addition to signaling the need to eat, ghrelin may also convey information about body adiposity. Steady-state circulating ghrelin levels are reduced in obese individuals compared to normal weight controls (Tschöp et al., 2001), and are negatively correlated with visceral and subcutaneous fat mass (Katsuki et al., 2004). Ghrelin levels increase following gastric bypass surgery or weight loss (Cummings et al., 2002). Lastly, ghrelin levels increase in exercising women who lose weight compared to controls that exercised but remained

weight stable (Leidy et al., 2004). Thus, surges in plasma ghrelin likely signal the need for calories, while resting ghrelin levels may reflect energy reserves. However, more work must be done to more fully understand this possibility.

There is also evidence that ghrelin may contribute to anticipatory responses associated with feeding. Mice trained to eat at a specific time of day exhibit increased locomotor activity leading up to the receipt of food, a behavior thought to represent food anticipation. Injection of ghrelin increases, while ghrelin receptor blockade decreases the pre-meal increase in locomotion (Merkestein et al., 2012), suggesting ghrelin levels contribute to the anticipatory response. In support of this, daily rhythms in ghrelin secretion can be entrained to meal times. Normally, rats exhibit a peak in ghrelin secretion in the hours leading up to the start of the dark cycle, which is typically their largest meal. However, rats that were trained to eat their largest meal in mid-day exhibited a peak prior to the meal and no elevation in plasma ghrelin at the onset of the dark cycle (Drazen et al., 2006). In humans, viewing images of food increases plasma ghrelin compared to viewing non-food images (Schüssler et al., 2012). Thus, ghrelin may play a role in preparing physiological processes for the receipt of calories. The normal suppression of ghrelin after meals is also sensitive to whether or not the meal was expected to occur (Ott et al., 2012). In sum, these data imply that while ghrelin secretion and suppression may be regulated by physiological state, cognitive and learned factors such as the anticipation of calories can also influence ghrelin secretion and suppression.

Once released, ghrelin acts both peripherally and centrally to affect feeding behavior. Peripheral ghrelin likely augments feeding through actions on the vagus nerve. Ghrelin alters vagal activity, and lesioning vagal afferents attenuates feeding in response to peripheral, but not central ghrelin administration (Date et al., 2002). In addition, peripheral ghrelin fails to promote

intake in human subjects who have undergone a vagotomy procedure (Le Roux et al., 2005a). In concert with peripheral actions, ghrelin crosses the blood brain barrier and accesses a large number of regions in the central nervous system (Banks et al., 2002; Cabral et al., 2013). Indeed, ghrelin has been shown to promote food intake via action in many of the regions that express the GHS-R, including the arcuate nucleus of the hypothalamus (Nakazato et al., 2001), hippocampus (Kanoski et al., 2012), ventral tegmental area (VTA; Abizaid et al., 2006), and nucleus accumbens (NAc; Naleid et al., 2005). Thus, the widespread nature of GHS-R expression and the ability of ghrelin to access this extended network to regulate feeding behavior suggests that ghrelin acts in a diverse array of brain circuits, potentially influencing a wide array of neurological processes.

As mentioned earlier, the arcuate nucleus of the hypothalamus is known to control food intake and body weight. Importantly, the arcuate was one the first identified central targets for ghrelin's effects on feeding behavior (Nakazato et al., 2001). Subsequent studies revealed that ghrelin's influence on feeding was due to actions on NPY/AgRP as well as POMC neurons. Peripheral ghrelin injection increases fos expression in arcuate neurons (Hewson and Dickson, 2000; Wang et al., 2002), once again providing evidence that peripheral ghrelin enters the brain and interacts with neural centers involved in feeding behavior. Bath application of ghrelin increases spontaneous activity of NPY/AgRP neurons and decreases the activity of POMC neurons (Cowley et al., 2003). Not only are NPY/AgRP cells activated by ghrelin, their activation is critical for ghrelin to promote food intake. Ghrelin fails to stimulate feeding in rats with arcuate lesions (Tamura et al., 2002) and central antagonists of either NPY or AgRP block feeding induced by central ghrelin infusion (Nakazato et al., 2001). Additionally, central ghrelin administration increases both NPY and AgRP mRNA expression in the arcuate (Hidekitamura et

al., 2000; Shintani et al., 2001), suggesting that ghrelin could exert both immediate and long term influence on feeding behavior. Interestingly, many NPY/AgRP neurons that respond to ghrelin are also sensitive to glucose, and the effect of ghrelin on NPY/AgRP neurons is tempered by co-administration of leptin (Kohno and Yada, 2012). Thus, not only does ghrelin directly influence arcuate NPY/AgRP neurons, their output appears to integrate multiple signals linked to physiological state.

There is a small body of evidence indicating that ghrelin also acts both directly and indirectly in the PVH to influence energy balance. Ghrelin attenuates inhibitory transmission in the PVH (Cowley et al., 2003) and infusion of ghrelin into the PVH induces fos expression in PVH neurons (Olszewski et al., 2003a). Interestingly, viral knockdown of GHS-R in the PVH has no effect on food intake, but does decrease body weight (Shrestha et al., 2009), suggesting that PVH GHS-R signaling preferentially contributes to energy expenditure rather than energy intake. In contrast, two separate studies reported that ghrelin induces feeding when infused in the PVH (Melis et al., 2002; Olszewski et al., 2003a). Consequently, more experiments are required to establish whether ghrelin acts in the PVH to control food intake, energy expenditure, or both. Importantly, peripheral injection of ghrelin induces fos expression in PVH neurons, implying that peripheral ghrelin recruits the PVH (Rüter et al., 2003). In summary, ghrelin clearly acts in the PVH to influence energy balance, but more data will be required to fully understand the role of ghrelin in this brain region.

In addition to the arcuate and PVH, ghrelin also has robust effects on behavior through actions in the LH. Ghrelin induces feeding when infused directly into the LH (Olszewski et al., 2003b; Szentirmai et al., 2007; Cone et al., 2014). Intra-LH ghrelin augments feeding behavior for several hours after administration, suggesting LH ghrelin receptor activation can exerts both

immediate and delayed effects on feeding (Olszewski et al., 2003b). Within the LH, ghrelin likely regulates feeding behavior through orexin neurons. Ghrelin increases the frequency of action potentials in LH orexin neurons (Yamanaka et al., 2003) and increases fos expression in LH orexin, but not MCH neurons (Olszewski et al., 2003b). Consistent with this result, blocking orexin peptide signaling with antibodies disrupts food intake elicited by LH ghrelin, while antibodies against MCH have no effect (Toshinai et al., 2003). Together, these data strongly support the possibility that LH ghrelin augments feeding primarily through LH orexin neurons. Critically, central ghrelin promotes fos in orexin neurons even when NPY/AgRP expression is compromised (Toshinai et al., 2003). Thus, central ghrelin has direct effects within the LH that are independent of indirect actions on arcuate NPY/AgRP neurons. Given that central orexin promotes feeding (Sakurai et al., 1998), activation of LH orexin neurons by ghrelin could be a potential mechanism underlying LH ghrelin's effects on food intake. LH ghrelin may also contribute to energy expenditure, as LH ghrelin infusion increases the amount of time mice spend awake (Szentirmai et al., 2007).

Interestingly, there are ghrelin positive neurons in the central nervous system (for review see Ferrini et al., 2009). Ghrelin has been localized in axon terminals in the arcuate nucleus, PVH, and LH and these neurons make synapses with arcuate NPY/AgRP and POMC neurons (Cowley et al., 2003) and LH orexin neurons (Toshinai et al., 2003). However, virtually nothing is known about how ghrelin expressing neurons contribute to feeding behavior, as they have not been studied *in vivo*. Nonetheless, the arcuate, PVH, and LH are positioned to augment energy balance in response to both central and peripheral sources of ghrelin.

F. The mesolimbic system is a likely contributor to the obesity epidemic

The hypothalamus has traditionally been associated with the homeostatic balance between energy intake and energy expenditure (Morton and Schwartz, 2001; Morton et al., 2006). However, modern society is highly obesogenic, in that many aspects of the environment promote consumption in excess of homeostatic requirements. Two prominent factors include the prevalence of highly palatable food and the preponderance of cues associated with their availability, which can promote food intake in the absence of caloric need. Therefore, studying food intake strictly in terms of homeostasis is insufficient to explain the current obesity epidemic. Palatable foods strongly reinforce future behaviors aimed at their consumption because of their highly rewarding hedonic properties (taste, caloric density, etc.). Here, the term "reward" describes the positive value an animal attributes to an object (Schultz et al., 1997). Moreover, stimuli that signal the availability of food reward, and therefore predict the positive hedonic experience associated with consumption, exert powerful influence on behavior. Thus, in order to understand how food reward and food-related cues contribute to overconsumption, one must examine neural circuits that control reward-directed behaviors.

The neurobiology underlying the effects of food and associated cues on food-directed behavior encompasses a diverse array of brain circuits (Kelley, 2004; Morton et al., 2006; Baldo and Kelley, 2007; Volkow et al., 2011; Petrovich, 2013; Richard et al., 2013). However, one critical candidate is the mesolimbic system, which is comprised of the dopamine neurons in the ventral tegmental area (VTA) and one of their major target nuclei, the nucleus accumbens (NAc). Both the VTA and NAc are rapidly gaining appreciation for their role in non-homeostatic or hedonic feeding, which is food consumption in excess of caloric need (often of highly palatable foods). Dopamine and the NAc critically participate in aspects of motivation, reinforcement, and learning associated with food reward and food cues (Dayan and Balleine, 2002; Baldo and

Kelley, 2007; Lex and Hauber, 2008; Salamone and Correa, 2012). Thus, the ability of food and associated cues to activate mesolimbic circuitry likely contributes to overconsumption. In addition, obesity is known to compromise multiple aspects mesolimbic signaling. Insults to mesolimbic circuitry could potentially drive further intake as well as reduce energy expenditure. Consequently, the mesolimbic system may contribute to imbalance in both energy expenditure as well as energy intake.

G. Anatomical organization of mesolimbic system

VTA dopamine neurons are located medially in the ventral midbrain and are histologically defined by the expression of tyrosine hydroxylase (TH), the enzyme that catalyzes the rate-limiting step in dopamine synthesis. In addition to dopamine, the VTA is also home to both GABAergic and glutamatergic projection neurons, but their biological functions are beyond the scope of this thesis. While much work has been done to trace inputs to VTA neurons (Sesack and Pickel, 1992; Futami et al., 1995; Carr and Sesack, 2000; Fadel and Deutch, 2002; Dommett et al., 2005; Geisler et al., 2007), recent studies demonstrate that VTA dopamine neurons receive a wide variety of input from all over the brain, including striatal, pallidal, midbrain, and hypothalamic efferents. Specifically, some of the densest projections to VTA dopamine neurons originate from pedunculopontine nucleus (PPTg), laterodorsal tegmental nucleus (LDTg), LH, PVH, central amygdala, NAc, orbitofrontal cortex, and lateral habenula (Watabe-Uchida et al., 2012). This positions VTA dopamine neurons to respond to a range of visual, gustatory, and reward-related input. VTA dopamine neurons project to a range of targets, including the NAc, amygdala, prefrontal cortex, and the motor cortex (Domesick, 1988; Hosp et al., 2011). In particular, VTA dopamine neurons project to the NAc in a topographical fashion. Medial

dopamine neurons project to the NAc shell, while more lateral dopamine cells innervate the NAc core (Ikemoto, 2007). This suggests a level of functional specialization organized along anatomical lines. A single dopamine neuron can extensively arborize in the striatum, suggesting that dopamine neuron activity modulates ensembles, rather than individual NAc neurons (Matsuda et al., 2009). Indeed, experimental and modeling data indicates that dopamine acts primarily by diffusing through large areas following release (i.e., volume transmission; Garris et al., 1994; Rice, 2000; Dreyer et al., 2010).

Dopamine activates two distinct families of dopamine receptors, D1-like and D2-like, which are both coupled to g-proteins. The D1 family consists of D1 and D5 receptors (D1Rs), while the D2 family consists of D2, D3, and D4 receptors (D2R). Dopamine receptors are primarily located in extrasynaptic areas on striatal neurons (Levey et al., 1993; Hersch et al., 1995; Yung et al., 1995). D1R binding activates $G\alpha_{olf}$, which generates adenylyl cyclase and activates PKA (Neve et al., 2004). PKA activation following D1R binding increases surface expression of both AMPA and NMDA receptors (Snyder et al., 2000; Dunah and Standaert, 2001; Chao et al., 2002; Hallett et al., 2006). In addition to NMDA receptor trafficking, D1R receptor activation potentiates NMDA currents (Blank et al., 1997). D1R activation also increases the excitability of striatal neurons through activation of L-type calcium channels (Hernández-López et al., 1997) and inhibition of potassium channels (Podda et al., 2010). In contrast, D2R largely acts to suppress activity in striatal neurons. D2R are coupled to $G\alpha_{i/o}$ proteins, which inhibit adenylyl cyclase activity (Neve et al., 2004). D2R activation influences multiple potassium channels, including inward rectifying and leak channels (Perez et al., 2006) as well as reduces calcium currents through voltage dependent calcium channels (Hernandez-Lopez et al., 2000). D2R binding also decreases NMDA receptor currents (Higley and Sabatini,

2010). Taken together, postsynaptic NAc dopamine receptors serve to regulate the efficacy of synaptic inputs and thus shape glutamatergic transmission with the NAc.

Approximately 90-95% of the neurons in the NAc are medium spiny neurons (MSNs), which are GABAergic projection neurons. In the dorsal striatum, D1Rs and D2Rs are expressed on different populations of MSNs, and largely segregate between striatonigral and striatopallidal projections (Gerfen et al., 1990). Interestingly, NAc MSNs that innervate the ventral pallidum do not segregate by dopamine receptor expression (Lu et al., 1997). Moreover, the D1R and D2R dichotomy is less reliable in the NAc where D1R and D2R are more often found on the same MSNs (Meredith et al., 2008). Thus, the anatomical characteristics that define the dorsal tier of the striatum are absent in ventral areas. Given their opposing functions, it is interesting that D1Rs and D2Rs can be found on the same NAc MSNs. However, coexpression of D1R and D2R on NAc MSNs likely function in a cooperative manner, as animals will work to self-administer a mixture of D1R and D2R agonists into the NAc, but neither drug alone are sufficient to reinforce responding (Ikemoto et al., 1997). In addition, coincident activation of both D1R and D2R increases spike firing in NAc neurons and this is depends on both PKA and Gβγ (Hopf et al., 2003). In addition to shaping postsynaptic activity, D1Rs can also be found on presynaptic terminals that innervate the NAc (Dumartin et al., 2007) where they can act to facilitate neurotransmitter release (Mizuno et al., 2007). D2Rs are also expressed presynaptically. D2R activation has been shown to suppress the activity of weakly active cortical terminals, perhaps acting to filter out weak inputs onto striatal MSNs (Bamford et al., 2004). Taken together, dopamine alters synaptic transmission in the NAc through distinct and cooperative effects on pre- and postsynaptic targets alike.

Extracellular dopamine concentration in the NAc is regulated by multiple factors, including D2Rs, the dopamine transporter (DAT), and cholinergic interneurons. On dopamine terminals, D2Rs act as autoreceptors to negatively regulate dopamine release by reducing quantal size (Pothos et al., 1998) and suppress dopamine release in response to repeated stimulation (Kennedy et al., 1992; Benoit-Marand et al., 2001). D2R activation has also been shown to reduce phosphorylation of TH, which may downregulate dopamine synthesis in the terminal (Lindgren et al., 2001). DATs are expressed only on the axons of dopamine neurons in the striatum (Ciliax et al., 1995; Hersch et al., 1997). By clearing dopamine from the extracellular milieu, the DAT is critical for terminating dopamine action on postsynaptic targets. Given that dopamine signals primarily through volume transmission (Garris et al., 1994), the DAT is thought to have very little influence on dopamine concentration near the release site, as it is not present in high enough numbers to prevent dopamine spillover due to diffusion. Instead, the DAT regulates the spread of dopamine over time and space (Cragg and Rice, 2004). Indeed, injections of DAT blockers increase both the magnitude and duration of electrically evoked dopamine signals (Sulzer, 2011) and increases the size of naturally occurring dopamine spikes (Owesson-White et al., 2012). Lastly, cholinergic interneurons make up only a small fraction of the cells present in the striatum, but they exert robust effects on extracellular dopamine levels. For example, recent optogenetic studies have shown that selective activation of cholinergic interneurons is sufficient to evoke dopamine release in the striatum (Cachope et al., 2012; Threlfell et al., 2012). Thus, cholinergic interneurons strongly influence dopamine release in the NAc through actions on dopamine terminals.

In addition to dopaminergic input, the NAc receives projections from cortical, limbic, and thalamic structures (for review see Meredith et al., 2008) and sends efferents to motor-related

structures like the ventral pallidum, VTA, SN pars compacta, SN pars reticulata, and retrorubral area (Groenewegen et al., 1999). Thus, the NAc connects cortical and limbic structures with motor circuitry. When the anatomical data was considered along with the available behavioral, pharmacological, and electrophysiological evidence, Mogenson and colleagues termed the NAc a 'limbic-motor' interface. That is, the NAc is the neural substrate by which motivation is converted into action (Mogenson et al., 1980). Mogenson's interpretation has been largely supported but is somewhat of an oversimplification. The sub-divisions of the NAc, the core and shell play different, but related roles in processing primary affective stimuli and goal-directed behavior. This specialization owes partially to their unique patterns of connectivity. The NAc core integrates numerous excitatory inputs from limbic regions, including the amygdala, hippocampus, thalamus, and medial prefrontal cortex (Kelley et al., 1982; Berendse and Groenewegen, 1990; Brog et al., 1993; Groenewegen et al., 1999; Voorn et al., 2004). The NAc shell receives input from infralimbic and piriform cortices, hippocampus, lateral hypothalamus, amygdala, and the thalamus (Kelley et al., 1982; Berendse and Groenewegen, 1990; Brog et al., 1993; Groenewegen et al., 1999; Voorn et al., 2004). Interestingly, NAc shell inputs are differentially weighted, as EPSCs from hippocampus to NAc projections are larger in magnitude than those from amygdala or prefrontal cortex projections (Britt et al., 2012). Whether a similar pattern of synaptic input is observed in the NAc core is unknown. At a surface level, the similarities between core and shell inputs could suggest similar functional roles in behavioral processes. However, a finer dissection of core and shell inputs reveals strong differences between the two subregions (Voorn et al., 2004). For example, many NAc projecting nuclei differentially innervate the NAc core and shell, including the amygdala (Ragsdale and Graybiel, 1988; Wright et al., 1996), cortex (Wright and Groenewegen, 1996), thalamus (Wright and

Groenewegen, 1996), and hippocampus (Groenewegen et al., 1991). Moreover, the dopaminergic projections to the NAc core and shell have different origins, with medial VTA dopamine neurons projecting preferentially to the shell, while more lateral dopamine neurons project to the core (Ikemoto, 2007). In addition to differences in the origins of inputs, NAc core and shell neurons are very different morphologically. For example, NAc core neurons have a greater number of dendritic spines, more dendritic arbors, and longer dendrites (reviewed in Meredith et al., 2008), and may have as much as 50% more surface area available for synaptic connections (Meredith et al., 1992). Together, these data indicate that NAc core and shell neurons process very different sets of information, and morphological differences in MSNs across the two subregions could further contribute to how incoming information is integrated to produce behavior.

H. The role of the mesolimbic system in food intake and energy expenditure

Consumption of food affects mesolimbic circuitry through many pre and post-ingestive mechanisms. Prior to the onset of a meal, the sight of food signals the impending consumption of calories. Importantly, passively viewing images of food can activate many areas of the brain, including the striatum (Malik et al., 2008; Dimitropoulos et al., 2012). As the meal begins, the taste of food predicts the arrival of calories. In humans, consumption of food activates both the dorsal and ventral striatum (Small et al., 2003; Stice et al., 2013a). In rodents, researchers have utilized sham feeding to isolate how taste regulates mesolimbic activity. These studies found that orosensory stimulation for both sugars and fat, in the absence of post-ingestive consequences, evokes dopamine release in the striatum (Hajnal et al., 2004; Liang et al., 2006). Experiments have also been conducted in genetically modified mice that cannot taste sweet compounds to investigate how post-ingestive signals influence the mesolimbic system. Following consumption

of a sugar solution, dopamine levels increase in the ventral striatum, even in animals who are 'sweet-blind' (de Araujo et al., 2008). Additionally, 'sweet-blind' animals learn to prefer solutions that contain calories to non-caloric sweeteners, suggesting that post-ingestive signals alone are sufficient to condition a preference for a caloric solution. Intra-gastric infusions of either sugar or fat following consumption of a calorie-free solution promotes a strong preference for that solution compared to a solution paired with intra-gastric water (Sclafani and Glendinning, 2005). In addition, intra-gastric infusions of nutrients alone are sufficient to elicit dopamine release, demonstrating that the mere arrival of nutrients in the gut recruits mesolimbic circuitry (Ren et al., 2010). Taken together, both pre and post-ingestive signals can regulate mesolimbic activity and alter food-directed behaviors.

While food consumption broadly increases dopamine release and turnover in the rodent NAc (Hernandez and Hoebel 1988; Taber and Fibiger 1997), multiple studies have found that augmenting NAc dopamine signaling does not affect food intake (Baldo et al., 2002; Beeler et al., 2010; Salamone and Correa, 2012). Thus, we are left with a critical question: If NAc dopamine does not regulate food intake per se, how does dopamine contribute to feeding behavior? Experimental evidence seems to converge on the idea that dopamine participates in motivational and learned aspects of feeding behavior and likely serves to reinforce behaviors that result in food consumption.

Genetically deleting the ability of dopamine neurons to produce dopamine results in rapid starvation (Szczypka et al., 1999). However, these animals can approach and consume food, but do not eat enough to survive unless dopamine production is rescued by injections of L-DOPA (Szczypka et al., 1999). This suggests that dopamine is more critical for the locomotor behavior (i.e., motivation) needed for ingestion. Moreover, rescuing dopamine synthesis selectively in the

ventral striatum has no effect on food intake, whereas feeding is restored following rescue of dopamine synthesis in the dorsal striatum (Szczypka et al., 2001). Thus, NAc dopamine is not required for animals to consume food, but appears to facilitate activity in motor circuits that promote food approach and consumption. Further support for dopamine's contribution to motivational aspects of feeding behavior stems from the work of Salamone and colleagues. In one study, they systematically increased the number of lever presses required to receive each subsequent food reward (progressive-ratio). The session was limited to 30 minutes and the animals were food-restricted. Together, these manipulations put a premium on maximizing the number of reinforcers obtained during the session. Dopamine depletions in the NAc or blockade of D1R or D2Rs reduced the effort animals would exert to obtain food, resulting in fewer food rewards (Aberman et al., 1998). This suggested that NAc dopamine was critical for the motivation to continue responding when costs were high. In another set of studies, Salamone and colleagues used a paradigm allowing rodents to choose between working (lever pressing) for a highly palatable food reward or consuming freely available (and less palatable) chow. As above, NAc dopamine depletions or blockade of NAc dopamine receptors shifted animals' preference away from effortful responding for palatable food (Salamone et al., 1991). However, these rats still consumed freely available chow, indicating that NAc dopamine manipulations did not affect total caloric intake. Another group genetically deleted the DAT in mice, which should chronically elevate NAc dopamine levels. The mice were then placed in cages where all of their food was earned from pressing two levers with different response requirements. They found that DAT knockout mice would press more on the high cost lever, yet consume the same amount of food as controls (Beeler et al., 2010). Together, these data support the idea that NAc dopamine does not alter intake, but instead affects the allocation of effort required to obtain food.

I. Food intake and food-related cues evoke dopamine release in the NAc

Although dopamine levels increase for up to an hour following a meal (Wilson et al., 1995), dopamine neurons also encode the receipt of food reward in a temporally precise manner. At rest, midbrain dopamine neurons typically fire between 3-8 Hz in slow, irregular patterns (Grace and Bunney, 1984). However, upon the receipt of food reward, or following the presentation of salient or reward-predictive stimuli, dopamine neurons emit a brief, high frequency (20 Hz; phasic) increase in firing rate that lasts only a few hundred ms (Mirenowicz and Schultz, 1994, 1996). Phasic dopamine responses to food reward have been characterized in a variety of species including mice, rats, and non-human primates (Mirenowicz and Schultz, 1994, 1996; Hyland et al., 2002; Roesch et al., 2007; Matsumoto and Hikosaka, 2009; Cohen et al., 2012). Phasic fluctuations in the activity of VTA dopamine neurons, like those evoked by food reward, are thought to generate 'spikes' in NAc dopamine concentration. Over the last two decades, tools have been developed that allow precise measurement of phasic dopamine fluctuations in the NAc. Fast-scan cyclic voltammetry (FSCV), a technique originally used in analytical chemistry, allows the identity and concentration of multiple electroactive species to be measured, even in complex chemical mixtures. Thus, FSCV is an ideal tool for monitoring concentration changes of neurotransmitter levels in brain tissue with high temporal resolution, including dopamine (Wickham et al., 2013). Indeed, FSCV recordings demonstrates that blocking VTA activity with lidocaine or impairing VTA burst firing with NMDA receptor antagonists disrupts phasic fluctuations in NAc dopamine concentration (Sombers et al., 2009b; Owesson-White et al., 2012). With respect to food reward, FSCV data is largely consistent with the electrophysiological measurements of dopamine cell body activity. Delivery or retrieval of

unpredicted food reward evokes phasic spikes in NAc dopamine concentration (Day et al., 2007; Stuber et al., 2008; Brown et al., 2011; Beeler et al., 2012). Interestingly, the magnitude of phasic dopamine release to food is modulated by the presence or absence or calories (Beeler et al., 2012) and reward magnitude (Gan et al., 2010). Thus, the receipt of food reward evokes brief spikes in NAc dopamine concentration and the magnitude of release is sensitive to the caloric content of the food.

Phasic dopamine signals, including those evoked by food, were long hypothesized to play a pivotal role in aspects of reinforcement. Importantly, the recent advent of optogenetics, has revealed a causal role for phasic dopamine signaling in behavioral reinforcement. Phasic, but not tonic, stimulation of VTA dopamine neurons leads to a preference for the chamber paired with phasic stimulation (Tsai et al., 2009), while animals allowed to nose-poke for phasic stimulation of VTA dopamine neurons will work for thousands of stimulations in a single hour (Witten et al., 2011). These studies indicate that phasic activation of dopamine neurons is sufficient to reinforce goal-directed behavior. Importantly, NAc dopamine action is critical for the ability of phasic dopamine signaling to reinforce behavior. Blocking D1R and D2Rs in the NAc attenuates responding for phasic activation of dopamine neurons (Steinberg et al., 2014). Moreover, phasic activation of ventral striatal MSNs also reinforces operant behaviors (Britt et al., 2012). These data suggest that the ability of food to evoke phasic dopamine release could reinforce food-directed actions. In support of this, pairing consumption of sucralose (a sweetener which lacks calories) with phasic stimulation of VTA dopamine neurons induces a preference for sucralose over sucrose, thus establishing that phasic dopamine signaling is sufficient to bias food choices (Domingos et al., 2011).

In addition to the foods themselves, cues that predict the availability of food reward evoke phasic increases in dopamine neuron activity (Schultz et al., 1997; Waelti et al., 2001; Roesch et al., 2007; Cohen et al., 2012) and elicit phasic dopamine release in the NAc (Roitman et al., 2004; Day et al., 2007; Stuber et al., 2008; Gan et al., 2010; Brown et al., 2011; Wassum et al., 2013). The temporal shift in dopamine signaling from the reward to its earliest predictor is thought to underlie associative learning (Schultz et al., 1997; Waelti et al., 2001). Indeed, the emergence of cue-evoked phasic dopamine signaling is correlated with behavioral features of learning, including approach behavior and discrimination of reward predictive from non-predictive stimuli (Roesch et al. 2007; Stuber et al. 2008). Moreover, blocking NAc dopamine receptors impairs cue-reward learning (Smith-Roe and Kelley, 2000; Di Ciano et al., 2001), while attenuating phasic dopamine release disrupts the ability to learn about spatial cues that signal food availability (Zweifel et al., 2009). Overall, these data suggest that cue-evoked dopamine release reinforces the association between rewards their predictors. Indeed, it was recently shown that phasic dopamine signaling is sufficient to promote cue-reward learning (Steinberg et al., 2013). Conversely, pauses in phasic dopamine signaling occur for expected, but omitted, rewards (Tobler et al., 2003; Bromberg-Martin et al., 2010). In contrast to phasic increases in dopamine, the brief decrease in extracellular dopamine should create a momentary lapse in D2R activation. This would result in increased activity in D2R expressing MSNs, which alone is negatively reinforcing (Kravitz et al., 2012). The momentary cessation of D2R signaling is hypothesized to facilitate learning from negative outcomes (i.e., when no reward is delivered). Indeed, pharmacological blockade of NAc D2Rs disrupts optimization of choice behavior following reward omission (Porter-Stransky et al., 2013). Thus, phasic changes dopamine neuron activity connects environmental stimuli (sights, smells of food) with the outcomes they predict

(receipt of palatable food). In environments with scarce resources, this function would be highly adaptive. However, in the current environment where food reward and cues are ample, it may facilitate overconsumption.

Once cue-reward associations are acquired, food-predictive cues exert powerful effects on motivated behavior, and can promote food seeking in the absence of caloric need (Lovibond, 1983; Weingarten, 1983; Corbit et al., 2007). Importantly, NAc dopamine likely mediates the effects of food cues on food-motivated behavior. Interfering with NAc dopamine receptors impairs conditioned approach behaviors elicited by food cues (Di Ciano et al., 2001; Lex and Hauber, 2008; Saunders and Robinson, 2012; Gore and Zweifel, 2013). Moreover, the magnitude of cue-evoked dopamine spikes are correlated with measures of approach behavior (Day et al. 2007; Stuber et al. 2008; Flagel et al., 2011) and operant responses for food reward (Wassum et al., 2013). Animals engaged in a self-timed task that involves pressing a series of levers in sequence finish the series more quickly when the magnitude of phasic dopamine release at the start of the sequence is larger (Wassum et al., 2012). Given that the animals chose when to initiate responding, these data strongly suggest that phasic dopamine release can invigorate food-seeking behavior. Furthermore, optogenetic stimulation of VTA dopamine neurons in phasic bursts can promote food-seeking behaviors (Adamantidis et al., 2011; Ilango et al., 2014). These data establish a causal link between phasic dopamine signaling and the initiation of goal-directed actions. This suggests that cue-evoked phasic dopamine release may underlie the influence of food cues on food-seeking behaviors.

Phasic dopamine release may also contribute to aspects of food-related decision-making (i.e., foraging behavior). Phasic dopamine release is differentially evoked by cues that signal the opportunity to choose between an uncertain large reward and a certain but smaller reward

(Sugam et al., 2012). Interestingly, the dopamine response to an uncertain option relative to the certain option is correlated with individual risk preference (Sugam et al., 2012). Moreover, the magnitude of cue-evoked dopamine signaling scales with anticipated reward, with greater dopamine release in response to cues that signaled larger payoffs (Day et al., 2010; Gan et al., 2010). While phasic dopamine signaling encodes variables associated with reward-based decision-making, how these signals factor into the actual decision process is unknown. Pharmacological manipulation of the NAc during risky decision-making provides some insight. Blockade of NAc D1Rs decreases preference for larger uncertain rewards (Stopper et al., 2013). Stimulating D1Rs with a D1R agonist improved behavior by increasing risky responding when the probability of payoff for the risky option was high and reduced responding for the risky option when the payoff probability was low. Interestingly, altering D2R tone had no effect on behavior (Stopper et al., 2013). Given that phasic dopamine release is thought to preferentially activate D1Rs (Dreyer et al., 2010), the pharmacological data suggest that phasic dopamine signaling may play an active role in decision-making processes through D1R activation. Moreover, individual differences in decision-related dopamine signals may help determine to food choices.

In addition to food seeking, some evidence suggests that subsecond changes in mesolimbic activity also contribute to aspects of food consumption. Phasically activating VTA GABA neurons, which disrupts dopamine release in the NAc, interferes with licking for food reward (e.g., sucrose; Van Zessen et al., 2012). Optogenetic stimulation of dopamine neurons with tonic patterns of activity (thereby preventing phasic changes) interferes with ethanol consumption in rats trained to drink alcohol (Bass et al., 2013). In summary, globally blocking NAc dopamine has no effect on food intake. However, the ability of food and food cues to evoke

phasic dopamine signaling has broad implications for food-related reinforcement, food-related learning, motivation, and may contribute to food choices. In addition, there is some evidence that impairing phasic signaling during food consumption disrupts intake, but more work is needed on this topic.

J. Food and food-related cues activate NAc neurons

Phasic fluctuations in NAc dopamine influence reward-directed behavior by modulating NAc output. NAc neurons typically exhibit a hyperpolarized resting membrane potential but given enough convergent excitatory input can transition into an elevated resting potential that facilitates spiking in response to synaptic input (Goto and O'Donnell, 2001). D2R activation can impede, while D1R activation can facilitate the transition into the activated state (Surmeier et al., 2007). Phasic dopamine release is thought to preferentially activate D1R (Dreyer et al., 2010). Thus, phasic dopamine release and subsequent activation of D1Rs should act to facilitate striatal output. In support of this, coincident fMRI and PET measurements in the ventral striatum reveal that striatal activation correlates with striatal dopamine release (Schott et al., 2008), suggesting that striatal dopamine release influences ongoing striatal activity *in vivo*. Additionally, coincident changes in NAc cell firing and phasic dopamine release have been observed at the same recording site (Cheer et al., 2007; Cacciapaglia et al., 2011). Moreover, pharmacological manipulations of the VTA that disrupt phasic dopamine release impair cue-evoked activity in the NAc and attenuate reward-seeking behavior (Yun et al., 2004; Cacciapaglia et al., 2011). Taken together this suggests that phasic dopamine release influences reward seeking behavior by gating the response of NAc neurons to reward-related stimuli.

While the NAc core and shell each make unique contributions to food-related behaviors, it is first important to highlight functional properties that do not differ between subregions. For example, NAc neurons in both core and shell encode aspects of food reward. A subpopulation of NAc neurons encodes the palatability of sucrose, with greater increases in firing rate to more concentrated sucrose solutions (Taha and Fields, 2005). Much like the foods themselves, cues that signal the availability of food elicit approach behaviors aimed at food (Weingarten et al., 1983; Lovibond et al., 1983). This influence is likely realized within the NAc through the coincident NAc dopamine release and activation of NAc neurons. Inactivation of the NAc slows cue-elicited responding for food (Yun et al., 2004), suggesting that the NAc is a critical site for the influence of food cues on food-seeking behavior. Food-predictive cues evoke phasic changes in the firing rate of NAc neurons (Nicola et al., 2004; Roitman et al., 2005; Day et al., 2006) and cue-evoked NAc activity has been directly linked to food-directed actions (Taha et al., 2007; McGinty et al., 2013; Roitman and Loriaux, 2014). In one study, the magnitude of cue-evoked NAc spiking was been directly linked to the intensity of approach behaviors (e.g., speed, latency; McGinty et al., 2013). Interestingly, correlations between NAc spiking and behavioral output hold when either core or shell neurons are considered separately, suggesting both subregions participate in the invigoration of food-motivated behavior. Taken together, the ability of food cues to evoke phasic changes in the activity of NAc neurons imbues food-related stimuli with influence over food approach and ultimately consumption.

Unlike the core, the NAc shell exerts robust effects on food intake and may make unique contributions to the perception of rewarding tastes. Pharmacological manipulations of discrete regions within the NAc shell can induce voracious feeding, even in fully sated animals (Bakshi and Kelley, 1993; Stratford and Kelley, 1997, 1999). Interestingly, the ability of NAc shell

manipulations to promote feeding requires NMDA receptor signaling in the LH, suggesting a link between the hypothalamus and mesolimbic system in the regulation of feeding behavior. Thus, the NAc shell likely exerts a tonic inhibition on food intake that is relieved through pharmacological interference. The NAc shell also makes individual contributions to hedonic processing. Modulating opioid signaling in the NAc shell increases positive orofacial responses to rewarding taste stimuli (Peciña and Berridge, 2000, 2005). Notably, this effect is largely absent in the NAc core (Peciña and Berridge, 2000). One hypothesis is that the change in orofacial responses reflects a potentiation of the positive affective properties of the food. In summary, the NAc shell contributes to food intake by encoding the hedonic properties of food as well as facilitating food consumption.

In contrast to the NAc shell, pharmacological manipulations of glutamatergic signaling in the NAc core has no effect on food intake (Kelley and Swanson, 1997). However, the NAc core has been strongly associated with facilitating goal-directed actions and learned aspects of feeding behavior. The NAc core is critical for learning cue-reward associations: interfering with NAc core NMDA receptors impairs associations between a cue and the impending delivery of food reward (Di Ciano et al., 2001). Moreover, the NAc core appears to be critical for the behavioral response to incentive cues. Lesions of the NAc core disrupt the ability of learned cues to invigorate food-seeking behavior (Corbit et al., 2001; Corbit and Balleine, 2011). Pharmacological manipulations of the NAc also support differential roles for the core and shell in conditioned responding. Much like the lesion data, interfering with NAc core signaling disrupts cue-evoked behavior, whereas NAc shell manipulations increase irrelevant behaviors during food-seeking tasks (Floresco et al., 2008; Blaiss and Janak, 2009; Ambroggi et al., 2011). Thus, the core appears to facilitate goal-directed behavior, whereas the shell may help to

suppress irrelevant behaviors. This hypothesis is consistent with electrophysiological recordings. NAc core neurons respond in greater numbers and with larger increases in activity to a reward-paired cue compared to NAc shell neurons (Ambroggi et al., 2011). Additionally, in contrast with the shell, fewer core neurons respond to unrewarded cues (Ambroggi et al., 2011). Taken together, the NAc core and shell make distinct, but equally important, contributions to food intake and food-related behaviors elicited by rewarding food and cues associated with their availability.

K. The mesolimbic system as an interface between motivation and physiological state

While ghrelin can act in the mesolimbic system to augment food-directed behavior, ghrelin is but one member in a family of peripheral feeding hormones. Importantly, virtually all food-related signals have been demonstrated to have actions in the mesolimbic system. Thus, **the mesolimbic system integrates information from peripheral and central feeding signals to link physiological state with food-motivated behavior.**

Food consumption activates the human ventral striatum (Stice et al., 2013a). Interestingly, the striatal response to food receipt is sensitive to physiological state, and the duration of caloric restriction positively correlates with the striatal BOLD response to food (Stice et al., 2013b). Conversely, feeding to satiety diminishes the striatal response to chocolate (Small et al., 2001). Satiation also decreases cerebral blood flow, an indirect measure of neural activity, in the caudate and putamen (Gautier et al., 2000). Moreover, satiety attenuates the midbrain response to a calorically dense milkshake (Sun et al., 2014). Much like the response to food, the striatal response to food cues is also diminished following satiety (Dimitropoulos et al., 2012), while administration of leptin (which signals positive energy balance) decreases the striatal

response to images of food (Farooqi et al., 2007). Thus, the striatal response to the sight and taste of food is sensitive to energy status, which under normal conditions could help regulate intake.

As highlighted in an earlier section, the arrival of nutrients is relayed to the brain by an array of post-ingestive hormones, including blood glucose, insulin, amylin, and GLP-1. Virtually all of these signals can act in the VTA to regulate food-seeking behavior. VTA neurons express receptors for glucose (Levin et al., 2006), insulin (Figlewicz et al., 2003), amylin (Mietlicki-Baase et al., 2014), leptin (Figlewicz et al., 2003), and GLP-1 (Merchenthaler et al., 1999). Intra-VTA infusions of agonists for these receptors suppress food intake (Hommel et al., 2006; Alhadeff et al., 2012; Mebel et al., 2012; Mietlicki-Baase et al., 2013). Notably, viral interference with either leptin or amylin receptors specifically within the VTA results in hyperphagia and weight gain (Matheny et al., 2014; Mietlicki-Baase et al., 2014). This suggests that VTA actions of peripheral feeding hormones contribute to long-term body weight regulation. In addition to augmenting food intake, peripheral hormones exert a range of effects on VTA and NAc activity. Peripheral injections of leptin decrease the firing rate of VTA neurons (Hommel et al., 2006). Leptin also modulates the striatal response to food images (Farooqi et al., 2007). Bath application of leptin onto VTA slices suppresses presynaptic excitatory input onto VTA dopamine neurons (Thompson and Borgland, 2013), whereas insulin induces long-term depotentiation in VTA dopamine neurons (Labouèbe et al., 2013). Insulin and leptin both regulate aspects of NAc dopamine signaling, including release and reuptake (Perry et al., 2010; Speed et al., 2011; Mebel et al., 2012), whereas intra-VTA amylin suppresses phasic dopamine release in the NAc (Mietlicki-Baase et al., 2014). These data demonstrate that in addition to ghrelin, the mesolimbic system integrates information from a wide variety of metabolic signals and produces the corresponding change in food-seeking behavior.

VTA dopamine neurons also receive input from central nuclei that regulate food intake and body weight, including the LH. LH neurons respond to most metabolic factors (Adamantidis and de Lecea, 2009; Berthoud and Münzberg, 2011) and LH orexin neurons in particular have been strongly implicated in reward-directed behavior (Aston-Jones et al., 2010). The LH is one of the densest direct inputs to VTA dopamine neurons (Watabe-Uchida et al., 2012). Importantly, some of the VTA projecting neurons are orexinergic (Peyron et al., 1998; Fadel and Deutch, 2002). Orexin neurons broadly respond to hormones associated with physiological state (Adamantidis and de Lecea, 2009). Thus, innervation of the VTA by LH orexin neurons is one pathway by which the central response to physiological state could influence food-seeking behavior. Indeed, blocking VTA orexin receptors attenuates sucrose self-administration in rats (España et al., 2010) and impairs hedonic feeding induced by pharmacological manipulations of the NAc (Zheng et al., 2007). Orexin receptors are expressed in VTA dopamine neurons and increase dopamine neuron excitability by augmenting NMDA currents and presynaptic glutamate release (Korotkova et al., 2003; Borgland et al., 2006, 2008). *In vivo*, VTA orexin gates excitatory inputs from prefrontal cortex onto VTA dopamine neurons (Moorman and Aston-Jones, 2010) and augments phasic dopamine release evoked by electrical stimulation of the VTA (España et al., 2011). Thus, the LH to VTA projection represents a potential circuit by which physiological state could influence dopamine release evoked by food or related stimuli. However, this remains unknown.

L. Ghrelin acts in the mesolimbic system to augment food-directed behavior

In addition to hypothalamic sites, ghrelin also regulates food seeking via actions in the mesolimbic system. Injections of ghrelin directly into the VTA or the NAc increases food intake

(Naleid et al., 2005; Abizaid et al., 2006; Skibicka et al., 2011, 2013; Cone et al., 2014). Importantly, the VTA is a critical site for ghrelin's actions on feeding behavior, as blocking VTA ghrelin receptors attenuates feeding induced by systemic ghrelin administration (Abizaid et al., 2006). VTA ghrelin likely contributes to the long-term regulation of energy balance, as chronic injections of ghrelin into the VTA promote increased intake and weight gain, whereas blocking VTA ghrelin receptors have the opposite effect (King et al., 2011). Ghrelin increases the firing rate of VTA dopamine neurons (Abizaid et al., 2006) while peripheral as well as intra-VTA administration of ghrelin increase tonic dopamine levels in the NAc (Jerlhag et al., 2007; Jerlhag, 2008; Quarta et al., 2009). However, as discussed above, altering NAc dopamine may have little to do with food intake and therefore is unlikely to be the mechanism underlying the feeding effects of VTA ghrelin. Indeed, food intake induced by intra-VTA ghrelin does not require dopamine receptor signaling in the NAc (Skibicka et al., 2013).

While ghrelin's ability to increase NAc dopamine release does not contribute to food intake, it does regulate food-seeking behavior. Intra-VTA ghrelin increases operant responses for food-reward in a progressive ratio task, suggesting that ghrelin acts in the VTA to increase motivation for food (Skibicka et al., 2011). Importantly, the ability of intra-VTA ghrelin to promote motivation was attenuated by NAc dopamine receptor blockade (Skibicka et al., 2013). In a related study, 6-Hydroxy dopamine lesions of the VTA attenuated the ability of intra-VTA ghrelin to increase responding in a similar task (Weinberg et al., 2011). Together, these data strongly suggest that intra-VTA ghrelin promotes motivation for food by increasing NAc dopamine release. In addition to motivation, intra-VTA administration of ghrelin is sufficient to stimulate locomotor activity (Jerlhag et al., 2007). The effects of ghrelin on locomotion could

contribute to exploratory behaviors designed to locate new food sources, or at the very least help regulate energy expenditure.

Critically, recent human data demonstrates that ghrelin augments the mesolimbic response to food and food cues. In humans, the midbrain response to an intra-oral delivery of a calorie rich milkshake is correlated with circulating ghrelin levels in humans (Sun et al., 2014). Likewise, the striatal response to food pictures is also correlated with circulating ghrelin levels (Kroemer et al., 2013). Moreover, intravenous ghrelin increases the BOLD response to images of food in many brains areas linked to appetitive behavior, including the striatum (Malik et al., 2008). Interestingly, cue-induced feeding is blunted by GHS-R antagonism (Walker et al., 2012). This suggests that ghrelin levels act as a gate on the ability of food cues to promote food-seeking behavior. Given the role of mesolimbic circuitry in cue-driven behavior, ghrelin may influence how dopamine and the NAc encode food-predictive cues. However, this possibility remains unexplored.

M. <u>Ghrelin is dysregulated in obesity</u>

Accumulating evidence suggests that almost all metabolic hormones, as well as their respective signaling pathways, are in some way disrupted in obese individuals. Importantly, ghrelin is no exception. Ghrelin levels are reduced in obese individuals (Tschöp et al., 2001) and are negatively correlated with visceral and subcutaneous fat mass (Katsuki et al., 2004). Interestingly, low resting ghrelin levels are associated with insulin resistance and type-2 diabetes (Pöykkö et al., 2003). Not only are ghrelin levels reduced, obese individuals do not exhibit phasic spikes in relation to meal-time, and the post meal suppression of ghrelin is absent (English et al., 2002; Le Roux et al., 2005b). In rodent models, 12 weeks of high fat diet decreases peripheral ghrelin and GOAT mRNA in the stomach and decreases GHS-R expression in the

hypothalamus (Briggs et al., 2010). Moreover, high fat diet decreases NPY and AgRP expression and attenuates NPY and AgRP expression and secretion from hypothalamic tissue cultures in response to ghrelin administration (Briggs et al., 2010). Ghrelin fails to induce feeding in obese mice (Perreault et al., 2004), which could mean that an unhealthy diet induces ghrelin resistance. Interestingly, mice lacking GHS-R are resistant to diet-induced obesity, develop less adiposity, and expend more calories than wild type littermates (Wortley et al., 2005; Zigman et al., 2005). There is also evidence that ghrelin's influence on motivation is also disrupted in obesity. The ability of ghrelin to increase responding on a progressive ratio schedule is blunted in high fat diet fed mice (Finger et al., 2012). While leptin and insulin insensitivity have received the bulk of the attention in connection with obesity, it is clear that ghrelin secretion and signaling pathways also become pathological in obese subjects.

Importantly, these insults are reversible. Exercise and weight loss increases circulating ghrelin (Leidy et al., 2004). Ghrelin levels increase following gastric bypass surgery (Cummings et al., 2002) and GHS-Rs are upregulated in response to caloric restriction (Tups et al., 2004). Weight loss rescues reductions in arcuate NPY and AgRP expression, and restores the ability of ghrelin to promote food intake (Perreault et al., 2004; Briggs et al., 2013). Perhaps the recovery of ghrelin signaling, and the subsequent effects on appetite, contributes to the meager (15%) long-term dieting success rate in obese individuals (Ayyad and Andersen, 2000). Nonetheless, the suppression of ghrelin and its neurobiological effects by obesity, and their rescue by weight loss, strongly suggest that the ghrelin system is sensitive to body adiposity and disruption by an unhealthy lifestyle. However, how ghrelin dysregulation contributes to the maintenance of the obese phenotype is unknown.

N. Implications for current studies

The obesity epidemic is partially driven by the widespread prevalence of highly palatable and energy dense foods that are often aggressively marketed to consumers. This is because rewarding food and their associated cues can promote food seeking independent of caloric need, thereby contributing to overconsumption. The mesolimbic system is a critical output structure linking food reward and their associated cues with motivated behavior. Moreover, multiple components of the mesolimbic system are compromised in obesity, and these insults may contribute to obesity development (for review see Kenny, 2011; Stice et al., 2013c; Volkow et al., 2013). In recent years, a wealth of data has emerged highlighting how food and food cues evoke patterns of activity in mesolimbic circuitry and how these signals contribute to food-directed behavior. Concomitantly, numerous hormones have been discovered that relay information about physiological state (e.g., hunger, satiety, body adiposity) and act in a variety of brain sites to control feeding behavior, including the mesolimbic system.

The central idea of this thesis is that the mesolimbic system integrates diverse information about physiological state to regulate food-directed behavior. **Phasic increases in NAc dopamine and in the firing rate of NAc neurons critically participate in food directed actions. To be adaptive, these signals should be sensitive to physiological state. Augmenting mesolimbic signaling could be a novel mechanism by which physiological state influences food-related behavior. However, this possibility remains unexplored.** Given that ghrelin is unique amongst other feeding hormones for its ability to promote food intake, it is an attractive target for obesity treatment. However, attempts to pharmacologically target the GHS-R or neutralize the bioavailability of circulating ghrelin have yielded mixed results as potential therapeutic interventions for obesity (Allas and Abribat, 2013). The failure of these attempts

could be due to a number of factors. One is likely due to the diffuse nature of GHS-R expression throughout the brain. Another is ghrelin's involvement in other biological processes that have little to do with food intake, and therefore present a high potential for side effects. Lastly, the ghrelin system is compromised in obese subjects, which could nullify the effects of pharmacological treatments. Rather than globally targeting ghrelin receptors, a more pragmatic approach would first involve identifying the neural signature of ghrelin's influence on food-directed behavior. Once this understanding is developed, interventions could be designed to selectively offset or inhibit only the actions of ghrelin that contribute to intake. **While we know where in the brain ghrelin acts increase feeding, we know virtually nothing about how this influence is realized at a systems neuroscience level. What are the neural correlates of ghrelin's influence on food-seeking behavior? Does ghrelin influence food related behaviors by modulating phasic mesolimbic signaling evoked by food and their associated cues?**

Chapter II

Ghrelin acts as an interface between physiological state and phasic dopamine signaling[1]

A. Introduction

The prevalence of inexpensive, highly palatable foods is a major contributor to the soaring rate of obesity (Drewnowski and Specter, 2004). The positive reinforcing nature of these foods has led to a focus on mesolimbic circuitry. Feeding broadly increases dopamine release and turnover in the NAc (Hernandez and Hoebel, 1988; Taber and Fibiger, 1997) and the magnitude of dopamine release is dependent on physiological state (i.e., hunger vs. satiety; (Wilson et al., 1995; Ahn and Phillips, 1999; Bassareo and Di Chiara, 1999). In addition to broad increases in dopamine concentration, food reward also evokes phasic spikes in striatal dopamine concentration (Day et al., 2007; Brown et al., 2011; Beeler et al., 2012), which are the result of burst firing of midbrain dopamine neurons (Sombers et al., 2009; Zweifel et al. 2009; Owesson-White et al., 2012). These brief dopamine spikes play a critical role in reinforcement learning (Day et al., 2007; Stuber et al., 2008; Steinberg et al., 2013) and are thought to drive food-seeking behaviors (Roitman et al., 2004; Wanat et al., 2010). Food restriction increases burst firing of dopamine neurons in anesthetized mice (Branch et al., 2013). However, the mechanisms by which physiological state change, induced by food restriction, increases dopamine system activity remain unknown.

Ghrelin, a peptide released by the stomach, is elevated by food restriction (Tschöp et al., 2000b) and is the only known gut peptide associated with hunger (Cummings et al., 2004). Peripheral ghrelin crosses the blood-brain barrier (Banks et al., 2002) and ghrelin receptors are expressed throughout the brain (Zigman et al., 2006) including the VTA– the site of striatal-

[1] Previously published as "Ghrelin acts as an interface between physiological state and phasic dopamine signaling" in the Journal of Neuroscience 34(14): 4905-4913.

projecting dopamine neurons. Ghrelin promotes food seeking through multiple brain circuits, including the arcuate nucleus of the hypothalamus (Nakazato et al., 2001), the LH (Olszewski et al., 2003b), ventral hippocampus (Kanoski et al., 2012) as well as the mesolimbic system (Abizaid et al., 2006). In addition to the VTA, many of these sites directly interact with the mesolimbic system. Indeed, both peripheral and central administration of ghrelin increases dopamine concentration in the NAc, as measured by microdialysis (Jerlhag et al., 2006; Jerlhag, 2008). Thus, ghrelin receptors pose a likely target for modulation of phasic dopamine signaling evoked by food.

I measured dopamine signaling in the NAc core using FSCV while rats (fed *ad libitum* or food restricted) retrieved sugar pellets delivered with a variable and randomly selected inter-trial interval. Retrieval of each pellet was associated with a spike in dopamine concentration. I hypothesized that within-session central ghrelin manipulations would modulate these dopamine spikes and sought to determine site specificity for central ghrelin effects on phasic dopamine signaling.

B. Experimental Methods

1. Subjects

Male Sprague–Dawley rats (n=47; Charles River) weighing 325–425 g at the time of testing were used. Rats were individually housed with lights on from 7 AM to 7 PM. All training and experimental sessions took place during the light phase in standard operant chambers (Med Associates; St. Albans, VT) with a food receptacle and magazine for the delivery of single 45 mg sugar pellets (3.58 kcal/g; BioServ, Frenchtown, NJ). Rats were trained to retrieve sugar pellets that were delivered with a random inter-trial interval (delivery interval range: 30-90 s; mean: 60

+/- 8.2 s). Following 5 days of training, rats were surgically prepared for FSCV. After returning to pre-surgery body weight, rats were retrained for 2 days prior to the experimental session. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

2. Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.). All implants were targeted relative to bregma using the rat brain atlas of Paxinos and Watson (2006). A guide cannula (Bioanalytical Systems; West Lafayette, IN) was implanted dorsal to the right NAc core (+1.3 mm AP, +1.5 mm ML, -2.5 mm DV). An infusion cannula (Plastics One, Roanoke, VA) was also implanted [lateral ventricle (LV): 22-gauge, 11 mm cannula (GC313), -0.8 mm AP, -2.1 mm ML, -3.7 mm DV, angled 10° away from the midline; (VTA): 26-gauge 11 mm cannula (C315), -5.8 mm AP, +2.9 mm ML, -6.5 mm DV, angled 15° away from the midline; (LH): 22-gauge 11 mm cannula, -3.1 mm AP, +1.7 mm ML, -7.1 mm DV]. LH were coordinates were selected to target orexin neurons (Fadel and Deutch, 2002), and VTA coordinates were chosen to maximize the likelihood of affecting VTA neurons that project to the NAc core (Ikemoto, 2010). A chlorinated silver reference electrode was placed in left forebrain. Stainless steel skull screws and dental cement secured implants to the skull.

3. Experimental Protocol

During an experimental session, rats were placed into operant chambers as above. FSCV in awake and behaving rats and analyte identification and quantification have been extensively

described previously (Phillips et al. 2003; Cone et al., 2013). Briefly, a micromanipulator containing a glass-insulated carbon fiber (~75 µm; Goodfellow USA, Coraopolis, PA) (recording) electrode was inserted into the NAc guide cannula. The recording electrode was then lowered into NAc and locked into place. A FSCV headstage (University of Washington EME Shop) was used to tether the rat, apply voltage changes and measure resultant current changes. The electrode voltage was held at -0.4 V and ramped in a triangular fashion (-0.4 to +1.3 to -0.4 V; 400 V/s) at 10 Hz. In addition, an injector connected to a 10 µL Hamilton syringe was inserted into the infusion cannula. To verify that food reward reliably evoked phasic dopamine release, a single sugar pellet was delivered. If this failed to evoke dopamine release, the electrode was advanced 0.16 mm and the process was repeated.

Once a stable release site was confirmed, the experimental session began. Electrochemical data was synced with video and recorded during the entire session. After 10 pellets (mid-session), an infusion pump was activated to deliver an intra-cranial infusion. For LV experiments, n-octanoylated ghrelin (1 µg in 1 µL 0.9% saline; American Peptide, Sunnyvale CA), D-[Lys]-GHRP (1 µg in 1 µL 0.9% saline; Tocris Bioscience, Bristol UK) or vehicle was infused at a rate of 2 µL/min through a 28 gauge injector (1 mm projection beyond the infusion cannula). Both LH and VTA rats received either n-octanoylated ghrelin (0.4 µg in 0.3 µL aCSF) or vehicle at a rate of 0.15 µL/min. LH injectors were 28 gauge (1 mm projection) and VTA injectors were 33 gauge (2 mm projection). After the recording session, electrodes were removed; rats were disconnected from the headstage and returned to their home cage. *Ad libitum* chow intake was then monitored for the next 30 min. Following experiments, all recording electrodes were calibrated in a flow-cell as described previously (Sinkala et al., 2012) to convert

detected current to concentration. The average calibration factor for all electrodes used in these experiments was 43.47 +/- 1.9 nM/nA.

4. Data Analysis

All rats retrieved and consumed all pellets before and after pharmacological manipulations. Individual trials were background subtracted and dopamine concentration during the 10 seconds before to 10 seconds after pellet retrieval was extracted from voltammetric data using principal component analysis (Heien et al., 2004; Day et al., 2007). I calculated peak dopamine concentration evoked by pellet retrieval by finding the maximum dopamine concentration during the 1-second before to 1-second after retrieval of each individual pellet. These values were averaged and compared before and after LV, LH or VTA infusions.

5. Verification of Cannula Placements and Recording Sites

LV cannula placements were verified by injecting Angiotensin II into the LV (50 ng in 2 μL saline; Sigma-Aldrich, St. Louis, MO) and measuring home cage water intake. Angiotensin II caused drinking (at least 7 mL in 30 min) in all rats. Following completion of experiments, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg) and a small electrolytic lesion was made at the voltammetry recording site using a polyimide-insulated stainless steel electrode (A-M Systems, Inc.). Rats were then transcardially perfused with cold 0.01 M phosphate-buffered saline followed by 10% buffered formalin solution (Sigma, Inc). Brains from rats with LV cannula were removed and stored in formalin. Brains from LH and VTA cannulated rats were removed and stored in formalin for 24 h and transferred to 30% sucrose in 0.1 M phosphate buffer (PB). All brains were sectioned at 40 μm on a cryostat. NAc sections were mounted and

lesion locations were verified using light microscopy in conjunction with the rat brain atlas of Paxinos and Watson (2006). All recordings were confirmed to be from the NAc core (Fig. 2.1 A-D). Sections containing LH or VTA were stored in 5% sucrose/0.01% NaN_3 in 0.1 M PB until immunohistochemical staining. Of the rats that received LH infusions, a subset were immunohistochemically stained for orexin-A, and in all 6 rats (aCSF: n=3; ghrelin: n=3), cannula tips were confirmed to be in close proximity (approx. 100 µm) to orexin-A positive cell bodies. Of the rats that received VTA infusions, a subset were immunohistochemically stained for tyrosine hydroxylase (TH), and in all 10 rats (aCSF: n=3; ghrelin: n=3; orexin-A: n=4), cannula tips were confirmed to be in tyrosine hydroxylase (TH)-positive regions of the VTA. Cannula tips from all remaining animals were confirmed to be in their targeted locations (LH or VTA) using light microscopy.

6. Immunohistochemistry

Standard methods were adapted for orexin-A and TH immunohistochemistry (Mahler and Aston-Jones, 2012; McCutcheon et al., 2012b). Briefly, sections containing either LH or VTA were placed in 0.3% H_2O_2 for 10 min and then blocking solution containing either 3% normal donkey serum (Orexin-A) or 3% normal goat serum (TH) and 0.3% Triton-X for 2 h. Sections were incubated overnight at room temperature in blocking solution with primary antibody (goat anti-Orexin-A; 1:2000; SC-8070; Santa Cruz Biotechnology, Dallas TX; rabbit anti-TH; 1:1000; AB-152; Millipore). Next, sections were incubated in biotinylated secondary antibody (Orexin-A: 2 h, 1:500, Santa Cruz Biotechnology; TH: 1.5 h, 1:500, Vector Labs). Sections were then transferred to an avidin–biotin complex (1:500; Vector Labs) for 1.5 h (Orexin-A) or 45 min

(TH) before being reacted with diaminobenzene DAB (0.02%; Vector Labs) for 3-7 min. Finally, sections were mounted, dehydrated, and cover slipped.

7. <u>Assessing the role of VTA orexin receptors in food intake induced by LV ghrelin</u>

Rats were fed *ad libitum* during all phases of this experiment. Naïve rats (n=6) were anesthetized and prepared for stereotaxic surgery as described above. A single cannula was targeted to the left LV and bilateral cannulae were directed at the VTA. We used the same cannula, coordinates and procedures as described above. Following recovery, rats had 2 nights of pre-exposure to 20 of the same sugar pellets that were used in the voltammetry sessions. Next, rats were habituated to the testing procedure. The test chamber was a standard clear plastic rodent cage with a wire top, which was identical to the home cage except without bedding to help account for spillage. A small receptacle was filled with 10 g of sugar pellets, placed in the corner of the cage, and secured to the floor. Rats were allowed to freely consume sugar pellets for 1 h before they were removed from the testing chamber.

On test days, I infused either the orexin receptor antagonist SB-334867 (SB; 1 µg in 0.3 µL 50% DMSO in sterile water) or vehicle bilaterally into the VTA, followed by ghrelin (1 µg in 1 µL 0.9% saline) or vehicle into the left LV. Infuser gauge and infusion speeds were identical to those used above. Following infusions, animals were placed in the test chamber and allowed to freely consume sugar pellets for 1 h. After the session, rats were returned to their home cage and total consumption was recorded. Thus, the four treatments were: LV-vehicle, VTA-vehicle; LV-vehicle, VTA-SB; LV-ghrelin, VTA-SB; LV-ghrelin, VTA-vehicle. Each rat received all treatments, which were counterbalanced across days. Testing sessions occurred once every other day. Following completion, LV cannula locations were confirmed with Angiotensin II. Rats were

then transcardially perfused, brains removed, sliced on a cryostat, and tissue was prepared for light microscopy as described above for verification of VTA cannula locations.

8. Statistical Analysis

Peak dopamine concentration evoked during pellet retrieval was compared using a two-way [epoch (pre, post-infusion) X treatment (vehicle, ghrelin)] ANOVA, with Tukey's HSD *post-hoc* tests where appropriate. In the voltammetry experiments, food intake was compared using an unpaired t-test (two groups) or one-way ANOVA (>2 groups). Food intake following concurrent LV and VTA manipulations was compared using a two-way repeated measures ANOVA [LV treatment (vehicle, ghrelin) X VTA treatment (vehicle, SB)] ANOVA. Statistical analyses were performed using GraphPad 5.0 (Prism Inc.), Statistica 10 (Statsoft), or SPSS Version 20.0 (IBM).

C. Results

1. LV ghrelin potentiates food-evoked dopamine spikes in ad libitum fed rats

To determine whether ghrelin centrally regulates phasic dopamine signaling evoked by food, I used *ad libitum* fed rats with cannula in the lateral ventricle (LV) and infused vehicle (1 µL saline; n=6) or ghrelin (1 µg; n=6) into the LV mid-session. LV ghrelin increased the magnitude of dopamine spikes evoked during pellet retrieval (epoch X treatment interaction ($F(1,10)=15.96$, $p<.01$); *post hoc*: post-ghrelin $p<.01$ versus all comparisons; Fig. 2.2 A-C). Ghrelin significantly augmented post-recording session home cage chow intake relative to vehicle ($t(10)=4.816$, $p<.001$; Fig. 2.2 D).

2. Central ghrelin receptor antagonism suppresses food-evoked dopamine spikes in food restricted rats

I next sought to determine whether endogenous ghrelin modulates dopamine spikes evoked by food. Rats were food restricted and either vehicle (1 µL saline; n=5) or the ghrelin receptor antagonist D-[Lys]-GHRP (1 µg; n=5) was infused into the LV mid-session. Ghrelin receptor antagonism suppressed dopamine spikes evoked during pellet retrieval (epoch X treatment interaction ($F(1,8)=13.17$, $p<.01$); *post-hoc*: D-[Lys]-GHRP $p<.01$ versus pre-infusion; Fig. 2.3 A-C). LV D-[Lys]-GHRP also suppressed post-recording session home cage chow intake ($t(8)=2.346$, $p<.05$; Fig. 2.3 D).

3. Central ghrelin potentiates food-evoked dopamine spikes in a site-specific manner

To determine ghrelin's site of action on phasic dopamine signaling, I targeted the LH due to its sensitivity to ghrelin (Olszewski et al., 2003b) and strong link with the mesolimbic system (Peyron et al., 1998; Fadel and Deutch, 2002; Watabe-Uchida et al., 2012). The LH of *ad libitum* fed rats was infused, mid-session, with either vehicle (0.3 µL aCSF; n=6) or ghrelin (0.4 µg; n=5). LH ghrelin potentiated dopamine spikes evoked during pellet retrieval (epoch X treatment interaction ($F(1,9)=22.30$, $p<.01$); *post-hoc*: LH ghrelin $p<0.001$ versus pre-infusion; Fig. 2.4 A-C) – recapitulating the effects of intraventricular ghrelin. LH ghrelin also increased post-recording session home cage chow intake compared to vehicle ($t(9)=2.519$, $p<.05$; Fig. 2.4 D). All cannula tips were confirmed to be in the LH (Fig. 2.4 E) and immunohistochemistry indicated infusions were near orexin-A positive cell bodies (Fig. 2.4 F).

As ghrelin acts in the VTA (Abizaid et al., 2006) to influence feeding and food-directed motivation (Skibicka et al., 2013) I sought to determine if intra-VTA ghrelin modulates phasic

dopamine signaling. In addition, our data suggested, and considerable evidence supports, a link between ghrelin and LH orexin neurons (Olszewski et al., 2003; Toshinai et al., 2003; Perello et al., 2010). Given that orexin regulates the excitability of VTA dopamine neurons (Korotkova et al., 2003; Borgland et al., 2006), I also investigated whether the effects of LH ghrelin could be recapitulated by intra-VTA orexin-A. In *ad libitum* fed rats, I infused vehicle (0.3 µL aCSF; n=4), ghrelin (0.4 µg; n=5) or orexin-A (1 µg; n=5) into the VTA mid-session. Intra-VTA orexin-A, but not ghrelin, increased the magnitude of dopamine spikes evoked during pellet retrieval (epoch X treatment interaction ($F(2,11)=8.69$, $p<.01$); *post-hoc*: orexin-A $p<.01$ compared to before infusion; Fig. 2.5 A-D). Both intra-VTA ghrelin and orexin-A potentiated post-recording session home cage chow intake compared to vehicle ($F(2,11=5.18$, both $p<.05$); *post-hoc*: ghrelin, orexin-A $p<.05$ versus vehicle; Fig. 2.5 E). All cannula tips were confirmed to be in the VTA by light microscopy (Fig. 2.5 F) and immunohistochemistry for TH (Fig. 2.5 G).

4. <u>LV ghrelin-induced feeding is suppressed by VTA orexin receptor antagonism</u>

The results of our voltammetry experiments suggest that orexin action in the VTA is a possible mechanism by which LV and LH ghrelin could regulate dopamine signaling. Thus, I sought to provide evidence for a functional relationship between central ghrelin and VTA orexin. I tested whether VTA orexin signaling was critical for feeding induced by central ghrelin infusions in *ad libitum* fed rats (n=6). I found that bilateral blockade of VTA orexin receptors with SB significantly attenuated the ability of LV ghrelin to promote intake of rewarding food (LV treatment X VTA treatment interaction ($F(1,5)=8.59$, $p<.05$); Fig. 2.6 A). As before, cannula tips were confirmed to be in the LV by angiotensin-induced drinking and in the VTA by light microscopy (Fig. 2.6 B).

5. Physiological state modulates food-evoked dopamine spikes

Our pharmacological manipulations suggested that endogenous signaling related to physiological state influences phasic dopamine signaling. Thus, it was critical to determine if caloric restriction results in measurable differences in the phasic dopamine response to food. To investigate this possibility, I combined data from all *ad libitum* fed (n=37) and food restricted rats (n=10) used in this study and compared the trials that occurred before any pharmacological manipulations were made. Consistent with the notion that physiological state endogenously regulates phasic dopamine signaling, food restriction significantly augmented dopamine spikes evoked during pellet retrieval ($t(45)=2.61$, $p<.05$; Fig. 2.7 A-D).

D. Discussion

I found that the gut hormone ghrelin acts centrally to regulate phasic dopamine spikes evoked by food reward via action in the LH, but not VTA. The effect of LH ghrelin was recapitulated by intra-VTA orexin-A, and food intake induced by LV ghrelin was attenuated following blockade of VTA orexin receptors, suggesting a potential mechanism of action for LV and LH ghrelin. Importantly, food restriction augmented dopamine spikes evoked by food independent of any pharmacological manipulations, implying that endogenous signaling related to physiological state can influence the phasic dopamine response to food reward. Indeed, food restriction increases burst firing of dopamine neurons in anesthetized mice (Branch et al., 2013) and potentiates dopamine release evoked during feeding (Wilson et al., 1995). Food-restriction could broadly affect multiple signals related to physiological state, including blood glucose levels, leptin secretion, and vagal tone. However, given that ghrelin is elevated by food restriction (Tschöp et al., 2000; Cummings et al., 2004), these data suggest that one way

physiological state dynamically tunes the phasic dopamine response to food reward is through central ghrelin signaling.

The LH has long been known to link energy status and food-motivated behavior (Margules and Olds, 1962; Berthoud and Münzberg, 2011). The results of our LH manipulations suggest that metabolic hormones can act in the LH to modulate the phasic dopamine response to food reward. In addition, ghrelin affects food intake and food-related reinforcement through actions on LH orexin neurons (Olszewski et al., 2003b; Toshinai, 2003; Perello et al., 2010). Thus, the effect of intra-VTA orexin-A on phasic dopamine release and the ability of intra-VTA orexin receptor antagonism to blunt feeding in response to LV ghrelin suggests a potential mechanism by which LV and LH ghrelin could enhance food-evoked dopamine spikes. Indeed, intra-VTA orexin-A increases, while intra-VTA orexin receptor antagonism decreases, the magnitude of phasic dopamine evoked by electrical stimulation of the VTA (España et al., 2010, 2011). I extend these findings by showing that orexin-A in the VTA increases the magnitude of phasic dopamine evoked by behaviorally relevant natural rewards. Additionally, food intake induced by LV ghrelin is blunted in orexin knockout mice (Toshinai et al., 2003). The results of our concurrent LV and VTA manipulations highlight a critical role for VTA orexin in feeding induced by central ghrelin. Taken together, these data suggest that ghrelin acts through the LH as an interface between physiological state and motivational circuitry and highlights a mechanism by which peripheral feeding hormones influence food-specific signaling and food-directed behavior, particularly in areas of the brain linked to reward-seeking.

It is noteworthy that I did not observe an effect of intra-VTA ghrelin on phasic dopamine signaling. Ghrelin receptors are present on VTA dopamine neurons (Abizaid et al., 2006) and previous evidence suggests that administration of ghrelin into the VTA increases dopamine in

the NAc shell (Jerlhag et al., 2006). However, the present study measured dopamine in the NAc core, suggesting that there could be regional differences in ghrelin's effects on dopamine signaling. It is possible that ghrelin receptors are expressed on distinct pools of VTA dopamine neurons that selectively project to subterritories other than the core. Indeed, VTA dopamine neurons respond differently to pharmacological challenges based on their projection targets (Lammel et al., 2008). Another possibility is that intra-VTA ghrelin could generally increase the activity of VTA dopamine neurons without altering phasic, event-specific dopamine signals, a phenomenon that would not be well captured using FSCV. Future studies that involve tracing the projections of ghrelin receptor expressing VTA dopamine neurons in addition to comparing the effects of VTA ghrelin on dopamine fluctuations in distinct striatal subregions will speak to these discrepancies.

I have identified one region where ghrelin regulates (LH) and another region where ghrelin does not regulate (VTA) phasic dopamine signaling. However, ghrelin receptors are expressed throughout the brain (Zigman et al., 2006) and central ghrelin diffuses to a variety of brain nuclei, including the LH and VTA but also the arcuate nucleus of the hypothalamus and nucleus of the solitary tract (NTS; Cabral et al., 2013). Given that recent work has identified previously unknown projections from the NTS to the VTA (Alhadeff et al., 2012) additional sites abundant in ghrelin receptors require further study. Many ghrelin receptor expressing brain regions could either directly or indirectly regulate dopamine neurons. When given to humans, ghrelin increases the BOLD response to images of food across multiple brain regions linked to appetitive behavior (striatum, amygdala, orbitofrontal cortex; Malik et al., 2008). Thus, the LH is unlikely to be the only brain site where ghrelin influences phasic dopamine signaling.

While VTA ghrelin did not affect phasic dopamine signaling, it did increase home cage food intake suggesting that phasic dopamine signaling may not be a reliable predictor of consummatory behavior (for review see Salamone and Correa, 2012). This result is consistent with previous research indicating that manipulations of NAc dopamine do not alter free-feeding intake (Koob et al., 1978; Taber and Fibiger, 1997; Baldo et al., 2002). Dopamine may play a more critical role in motivating behavior towards high-effort, high-value options. Blocking NAc dopamine shifts choices away from operant responses in favor of freely available chow, but leaves total caloric intake unchanged (Salamone et al., 1991; Koch et al., 2000). The converse is true of hyperdopaminergic mice, as these animals will exert more effort to obtain food, but do not consume more food overall (Beeler et al., 2010). Furthermore, a recent report indicates that feeding elicited by intra-VTA ghrelin does not require NAc dopamine receptor activation (Skibicka et al., 2013). Thus, that VTA ghrelin promotes food intake in the absence of changes in phasic dopamine signaling is consistent with the notion that NAc dopamine is not required for normal food intake and VTA ghrelin can regulate feeding in a dopamine independent manner. Moreover, it speaks to VTA cell groups other than NAc core-projecting dopamine neurons as playing a critical role in feeding.

Even though changes in phasic dopamine release may not directly contribute to food consumption, our data have broad implications for food-related behaviors. Recent optogenetic experiments have revealed that phasic dopamine signaling is sufficient for aspects of behavioral reinforcement (Tsai et al., 2009; Witten et al., 2011) and reinforcement learning (Steinberg et al. 2013). Cues that signal the availability of food or the opportunity to respond for food evoke phasic dopamine release in the NAc core (Roitman et al. 2004; Day et al. 2007; Stuber et al. 2008), where dopamine is critical for pavlovian and instrumental learning (Smith-Roe and

Kelley, 2000; Di Ciano et al., 2001) as well as performance (Gore and Zweifel, 2013). Genetic manipulations that attenuate phasic dopamine release impair cue-mediated learning motivated by food reward (Zweifel et al., 2009). As mentioned above, NAc dopamine may direct food-seeking behavior according to cost-benefit relationships amongst available food sources (Salamone et al., 1991; Koch et al., 2000; Beeler et al., 2010). Phasic dopamine signaling may also play a role in these processes, as it has been shown to encode effort and cost variables related to food seeking (Day et al., 2010; Gan et al., 2010; Wanat et al., 2010; Sugam et al., 2012). Physiological state may also factor into such decisions, as ghrelin shifts food preferences towards higher calorie options (Perello et al., 2010). Taken together, our data suggest that modulation of phasic dopamine signaling by ghrelin and physiological state may play a role in a wide range of food-directed behaviors.

Energy-related signals, such as leptin and ghrelin, have been shown to alter the activity of dopamine neurons *in vitro* (Abizaid et al., 2006) and in anesthetized animals (Hommel et al., 2006). Additionally, ghrelin broadly increases dopamine levels in the NAc (Jerlhag et al., 2006; Jerlhag, 2008). Thus, in addition to the previously reported changes in general excitatory drive, our data indicate that the hunger signal ghrelin influences the dopamine response to events temporally linked to food approach and consumption. Regulation of phasic dopamine signaling by ghrelin is thus a means by which physiological state could influence a wide range of behavioral phenomena, including learning, reinforcement, and incentive motivation. Given that obesity is fueled, in part, by a failure to limit approach and consumption in response to food reward, our data suggest that LH ghrelin and VTA orexin receptors represent targets for therapeutic interventions.

Figure 2.1

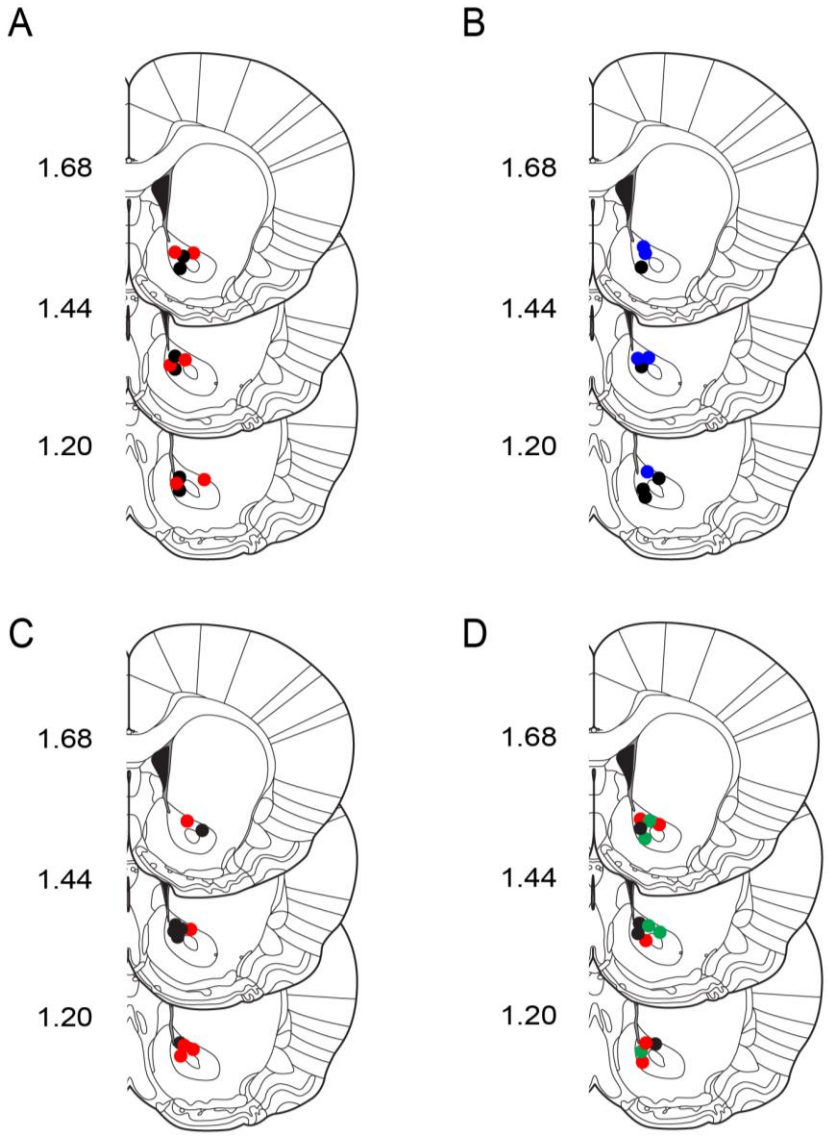

Figure 2.1: Summary of NAc recording sites. Coronal brain sections modified from Paxinos and Watson (2007). Colored circles indicate approximate recording locations. Numbers at left indicate approximate distance from bregma ***A,*** Recording locations from *ad-libitum* fed rats that received LV saline (n=6) or ghrelin (n=6). ***B,*** Recording locations from food-restricted rats that received LV saline (n=5) or D-[Lys]-GHRP (n=5). ***C,*** Recording locations from *ad libitum* fed rats that received LH aCSF (n=6) or ghrelin (n=5). ***D,*** Recording locations from *ad libitum* fed rats that received intra-VTA aCSF (n=4), ghrelin (n=5), or orexin-A (n=5). [black = vehicle (saline or aCSF); red = ghrelin; blue = D-[Lys]-GHRP, green = orexin-A].

Figure 2.2

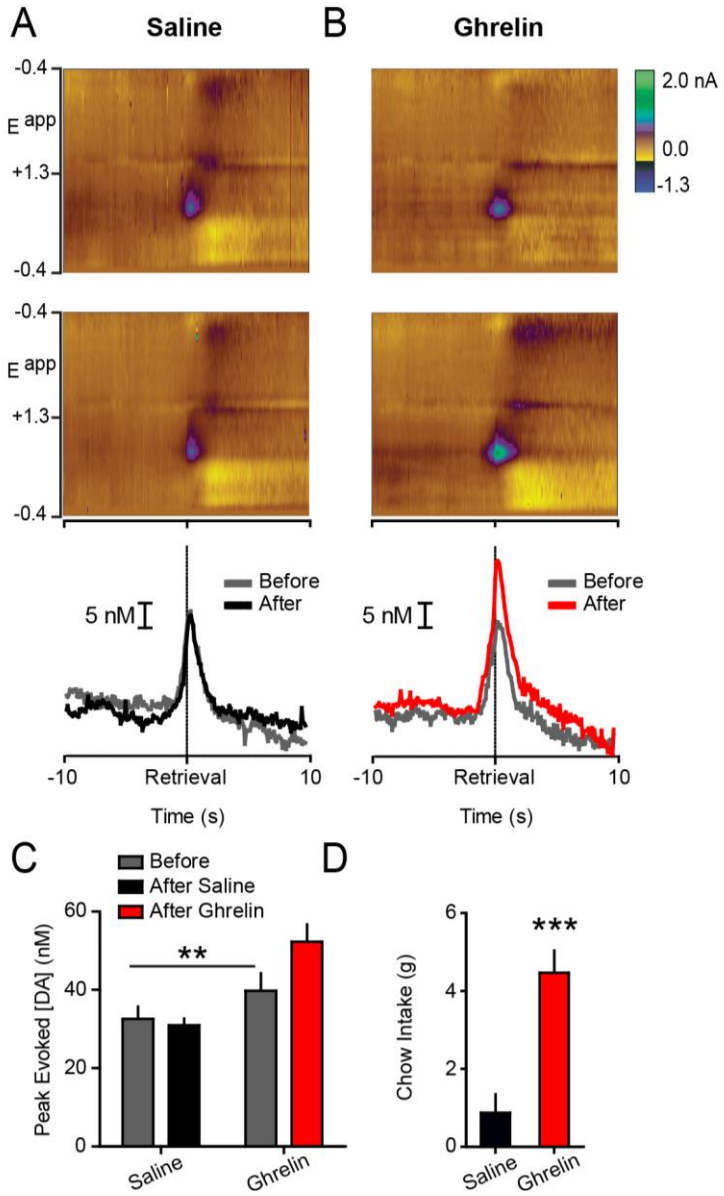

Figure 2.2: Ghrelin increases phasic dopamine evoked by food reward in *ad libitum* fed rats. ***A,*** (top) Average colorplot (10 trials/rat; n=6) depicting current changes (color) as a function of electrode potential (y-axis) in the 10 s before and after (x-axis) pellet retrieval. Dopamine [identified by its oxidation (~+0.6 V; green) and reduction (~-0.2 V; light yellow) features] was transiently evoked during pellet retrieval, prior to vehicle infusion. (middle) Average colorplot after LV vehicle infusion. (bottom) Average dopamine concentration aligned to retrieval before and after LV saline extracted from individual colorplots using chemometric analysis (Heien et al., 2004). ***B,*** Average colorplots prior to (top) and after LV ghrelin (middle; 10 trials/rat; n=6). Average dopamine concentration aligned to pellet retrieval before and after LV ghrelin (bottom). ***C,*** Peak dopamine evoked during pellet retrieval, before and after LV vehicle or ghrelin. ***D,*** Post-session chow intake following LV vehicle or ghrelin. Bar data are mean±SEM; **p<.01, ***p<.001.

Figure 2.3

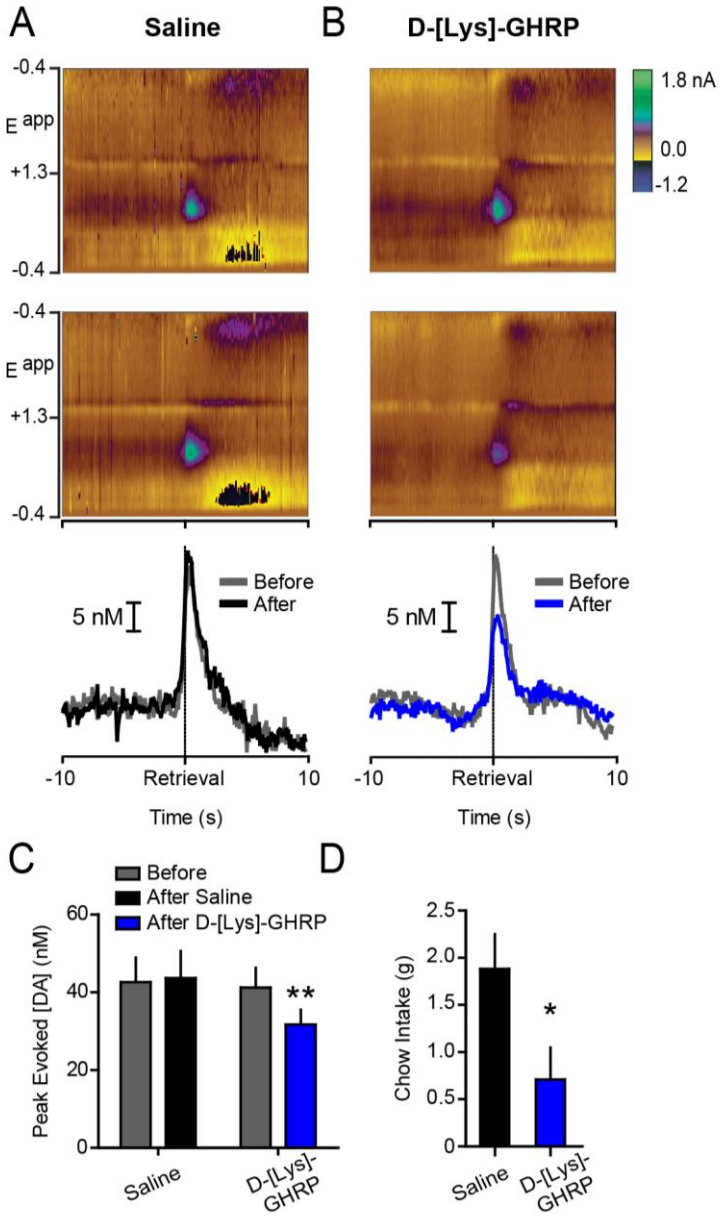

Figure 2.3: Ghrelin receptor antagonism attenuates phasic dopamine evoked by food reward in food-restricted rats. ***A,B,*** (top, middle) Conventions are identical to those in Figure 2 except before and after vehicle (10 trials/rat; n=5) or D-[Lys]-GHRP (10 trials/rat; n=5). (bottom) Average dopamine concentration aligned to pellet retrieval before and after LV saline or D-[Lys]-GHRP. ***C,*** Peak dopamine evoked during pellet retrieval, before and after LV saline or D-[Lys]-GHRP. ***D,*** Post-session chow intake following LV saline or D-[Lys]-GHRP. Bar data are mean±SEM; *p<.05, **p<.01.

Figure 2.4

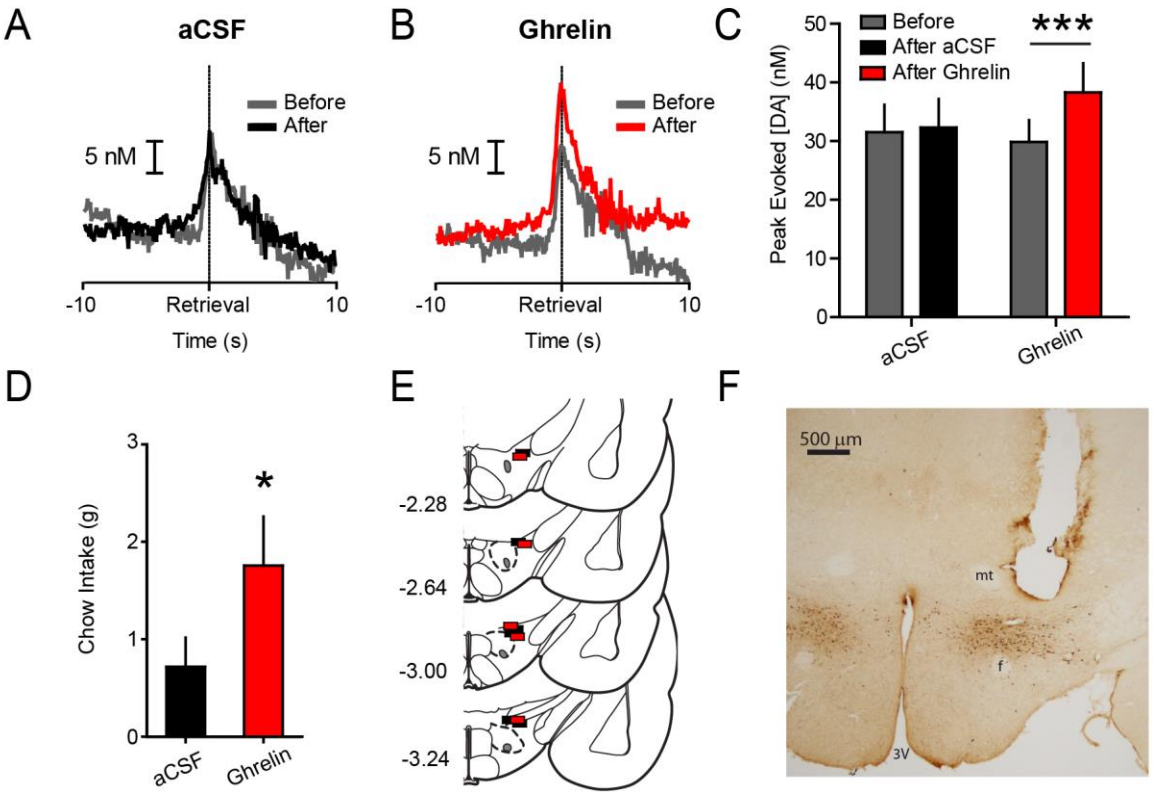

Figure 2.4: Phasic dopamine evoked during pellet retrieval is potentiated by intra-LH ghrelin. ***A,*** Average dopamine concentration evoked during pellet retrieval before (gray) and after (black) intra-LH aCSF infusion (n=6). ***B,*** Average dopamine concentration evoked during pellet retrieval before (gray) and after (red) intra-LH ghrelin infusion (n=5). ***C,*** Peak dopamine evoked during pellet retrieval before and after intra-LH infusion of aCSF or ghrelin. ***D,*** Intra-LH ghrelin increased post-session chow intake, relative to vehicle. ***E,*** Coronal brain sections modified from Paxinos and Watson (2007). Colored squares indicate approximate locations of cannula tips from all rats used in LH infusion experiments [black = aCSF; red = ghrelin]. Numbers at left indicate approximate distance from bregma in mm. ***F,*** Representative coronal brain slice with LH cannula stained for orexin-A. 3V=third ventricle, f=fornix, mt=mammillothalamic tract. Bar data are mean±SEM; *p<.05, *** p<.001.

Figure 2.5

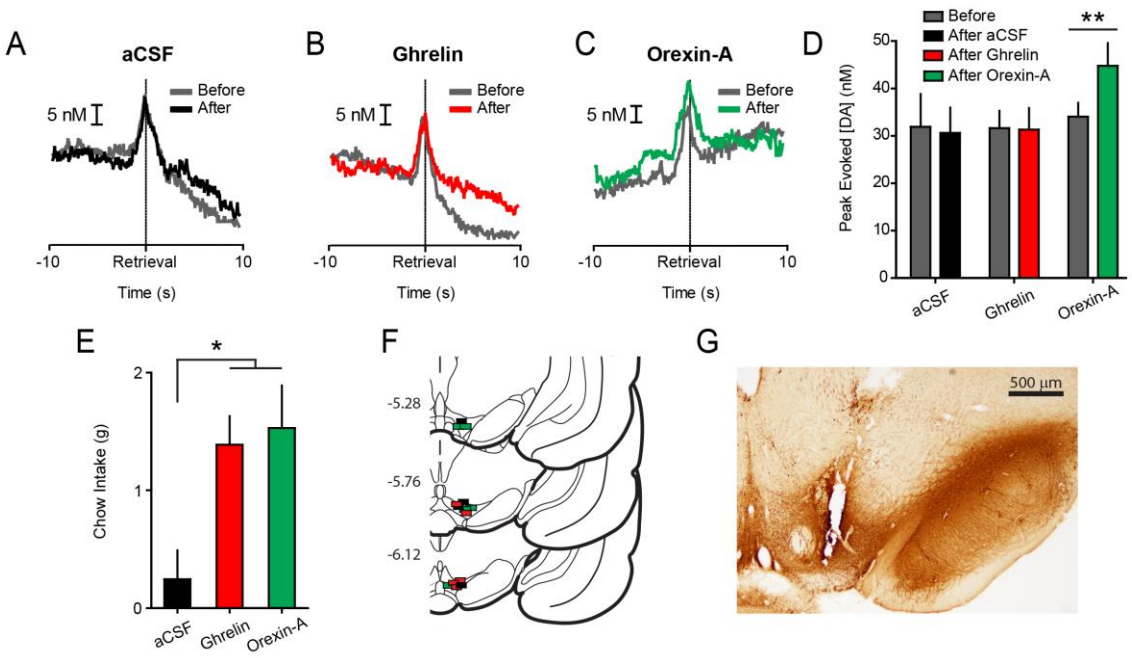

Figure 2.5: Intra-VTA orexin-A, but not ghrelin, potentiates phasic NAc dopamine evoked during pellet retrieval. ***A,*** Average dopamine concentration evoked during pellet retrieval before (gray) and after (black) intra-VTA aCSF infusion (n=4). ***B,*** Average dopamine concentration evoked during pellet retrieval before (gray) and after (red) intra-VTA ghrelin infusion (n=5). ***C,*** Average dopamine concentration evoked during pellet retrieval before (gray) and after (green) intra-VTA orexin-A infusion (n=5). ***D,*** Peak dopamine evoked during pellet retrieval before and after intra-VTA infusion of aCSF, ghrelin, or orexin-A. ***E,*** Intra-VTA ghrelin and orexin-A significantly increased post-session chow intake, relative to vehicle. ***F,*** Coronal brain sections modified from Paxinos and Watson (2007). Colored squares indicate approximate locations of cannula tips from all rats used in VTA infusion experiments [black = aCSF; red = ghrelin; green = orexin-A]. Numbers at left indicate approximate distance from bregma in mm. ***G,*** Representative coronal brain slice with VTA cannula stained for TH. Bar data are mean±SEM; * p<.05, ** p<.01.

Figure 2.6

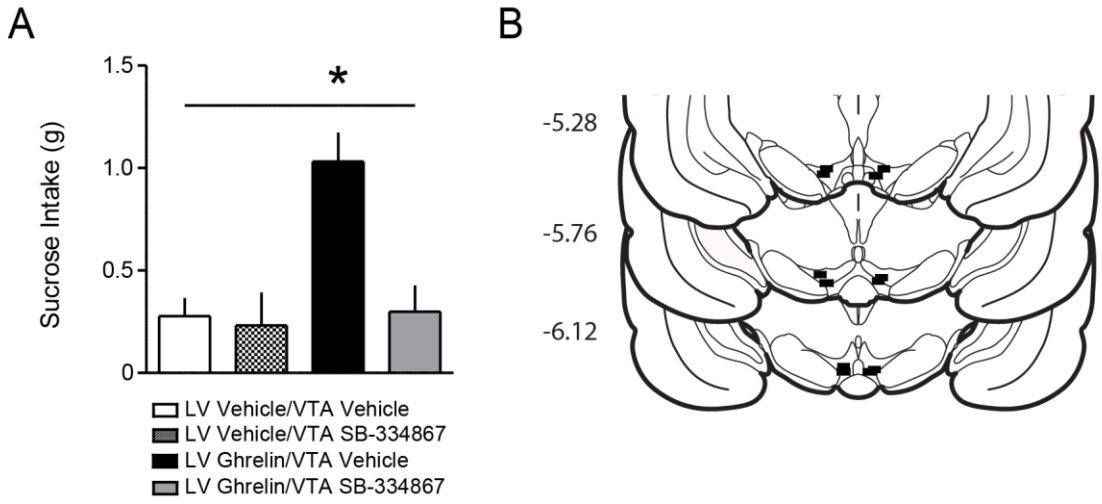

Figure 2.6: Bilateral intra-VTA orexin antagonism attenuates feeding induced by LV ghrelin. ***A,*** Sucrose pellet consumption following LV ghrelin is suppressed by bilateral VTA infusions of the orexin receptor antagonist SB-334867 but not vehicle (n=6). ***B,*** Coronal brain sections modified from Paxinos and Watson (2007). Black rectangles indicate approximate VTA infusion locations. Numbers at left indicate approximate distance from bregma. Bar data are mean±SEM; *LV X VTA interaction $p<.05$.

Figure 2.7

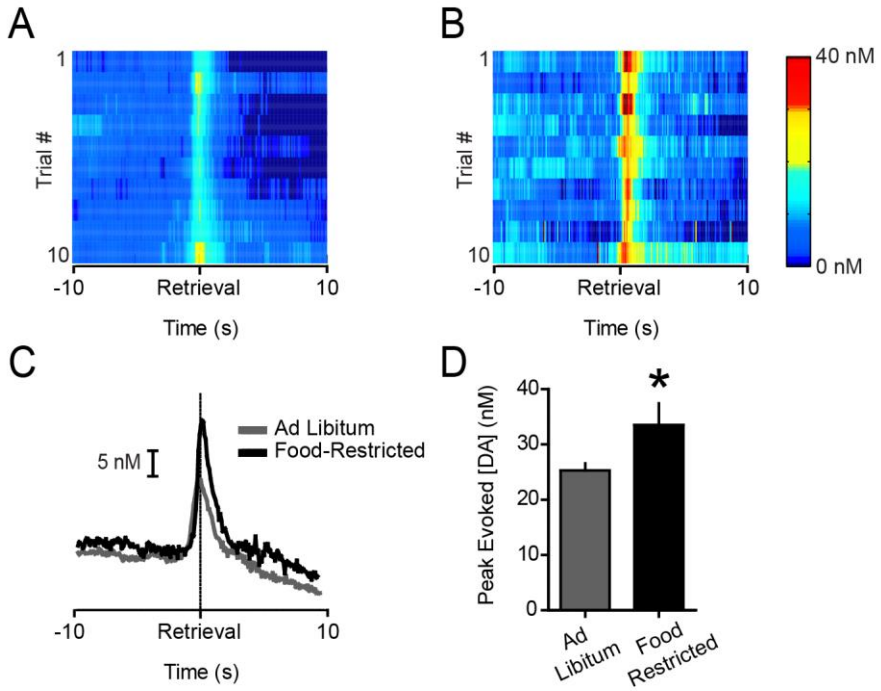

Figure 2.7: Phasic dopamine evoked during pellet retrieval is augmented by food restriction. ***A,*** Heatplot depicting average dopamine concentration per trial for all *ad libitum* fed rats from the experiments presented in this manuscript (n=37). Each row represents the average dopamine concentration for each of the 10 pellet retrievals before any infusions were made. ***B,*** Same conventions as in (a) but for all food-restricted rats used in the experiments presented in this manuscript (n=10). ***C,*** Average dopamine concentration evoked during pellet retrieval in *ad libitum* (gray) versus food-restricted (black) rats. ***D,*** Peak dopamine concentration evoked during pellet retrieval is augmented by food-restriction. Bar data are mean±SEM; *p<.05.

Chapter III

Ghrelin regulates phasic mesolimbic signaling evoked by food-predictive stimuli

A. Introduction

Beginning with the seminal work of Pavlov, it has been well established that food-predictive stimuli engage physiological processes that prepare the body for the ingestion of calories (Woods, 1991; Woods and Ramsay, 2000). The sights, smells, and environmental stimuli associated with food can also trigger approach and ultimately food consumption, even in sated animals (Lovibond, 1983; Weingarten, 1983; Roitman et al., 2001). This is especially problematic in modern cultures where humans are bombarded with promotions for food (Mink et al., 2010), which can 'prime' eating behavior (Harris et al., 2009). Thus, food cues and the neural circuits they engage are likely key contributors to the recent dramatic rise in overeating and incidence of obesity.

Among other circuits (Petrovich, 2013), food-predictive cues activate the mesolimbic system (Tang et al., 2012) including the midbrain dopamine neurons and one of their major targets, the nucleus accumbens (NAc). Pharmacological modulation of the NAc, including that of NAc dopamine receptors, affects cue-elicited responding for food (Di Ciano et al., 2001; Wyvell and Berridge, 2001; Yun et al., 2004; Lex and Hauber, 2008; Blaiss and Janak, 2009; Corbit and Balleine, 2011). Food-paired cues evoke brief (phasic) increases in NAc dopamine concentration (Day et al., 2007; Stuber et al., 2008; Brown et al., 2011) and in the firing rate of NAc neurons (Nicola et al., 2004; Day et al., 2006; Wan and Peoples, 2006). These cue-evoked mesolimbic signals have been directly linked to food-directed actions that culminate with food consumption (Roitman et al., 2004; Day et al., 2007; Taha et al., 2007; Stuber et al., 2008; Cacciapaglia et al., 2011; Flagel et al., 2011; McGinty et al., 2013; Roitman and Loriaux, 2014).

Taken together, phasic mesolimbic signaling evoked by food-predictive cues strongly contributes to cue-evoked food approach and food intake.

To be adaptive, the neural signals that participate in food-directed behavior should be sensitive to physiological state (i.e., hunger vs. satiety). One candidate that may link physiological state with mesolimbic signaling is the stomach hormone ghrelin; the only known peripheral peptide associated with hunger (Cummings et al., 2004). Ghrelin crosses the blood-brain barrier (Banks et al., 2002), and interacts with a distributed network of ghrelin receptor expressing nuclei (Zigman et al., 2006; Cabral et al., 2013), including the mesolimbic system (Naleid et al., 2005; Abizaid et al., 2006; Skibicka et al., 2013). Ghrelin augments the BOLD signal response to food cues in multiple limbic areas linked to appetitive behaviors (Malik et al., 2008). The ability of food-predictive cues to promote food-directed behavior is similarly blunted by both satiety and ghrelin antagonism (Corbit et al., 2007; Walker et al., 2012). These studies suggest that physiological state in general and ghrelin specifically could regulate neural responsivity to food-predictive cues. Indeed, we recently demonstrated that central ghrelin enhances phasic dopamine release evoked by food reward (Cone et al., 2014). However, how ghrelin modulates mesolimbic encoding of food-predictive cues remains uninvestigated. To address this, we measured dopamine signaling in the NAc using fast-scan cyclic voltammetry (FSCV) and recorded spiking activity of individual NAc neurons using *in vivo* electrophysiology in separate groups of rats in response to Pavlovian conditioned cues. We hypothesized that central manipulation of ghrelin would modulate phasic mesolimbic signaling evoked by food-predictive stimuli.

B. Experimental Methods

1. Subjects

Male Sprague-Dawley rats (n=23; 350–400 g) were housed individually and maintained on a 12:12-h light-dark cycle (on at 7:00 AM). Laboratory chow (LabDiet 5012; Richmond, IN) and water were provided *ad libitum*. Animals were treated in accordance with the guidelines put forth by the National Institutes of Health and under the approval of the Animal Care Committee of the University of Illinois at Chicago.

2. Behavioral Session

A Pavlovian procedure was used in which the delivery a single 45 mg sugar pellet (3.58 kcal/g; BioServ, Frenchtown, NJ) was preceded by a distinct compound stimulus that occurred 3 s earlier (CS+). A second compound stimulus was presented but terminated after 3 s and predicted no pellet delivery (CS-). The two possible cues were a 60 dB white noise or 50 dB tone, which turned off after 1 s, and left or right cue light, which remained lit for 3 s. Cue identities (CS+ vs. CS-) were counterbalanced across rats. Trial types were randomly selected, as was the inter-trial interval (range: 30–90 s) between cue presentations. Rats were trained for 10 days prior to surgery. Training sessions consisted of 30 and 60 trials for voltammetry and electrophysiology experiments, respectively. All training and experimental sessions took place during the light phase in standard operant chambers (Med Associates; St. Albans, VT). The same behavioral procedure was used in both voltammetry and electrophysiology experiments. All rats were fed *ad libitum* throughout the duration of training and testing.

3. FSCV Surgical Procedures

Rats (n=10) were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.). All implants were targeted relative to bregma using the rat brain atlas of Paxinos and Watson (2007). A guide cannula (Bioanalytical Systems; West Lafayette, IN) was implanted dorsal to the right NAc core (+1.3 mm AP, +1.5 mm ML, -2.5 mm DV). A chlorinated silver reference electrode was placed in left forebrain. Additionally, a 22-gauge, 11 mm infusion cannula (GC313; Plastics One, Roanoke, VA) was implanted in the lateral ventricle (LV; -0.8 mm AP, -2.1 mm ML, -3.7 mm DV, angled 10° away from midline). Stainless steel skull screws and dental cement were used to secure the guide and infusion cannulae and reference electrode to the skull. Once dry, the obdurator was removed from the guide cannula, and a micromanipulator containing a carbon fiber electrode was attached. The electrode was then lowered into the NAc. A bipolar-stimulating electrode (0.20 mm diameter; Plastics One, Roanoke, VA) was positioned dorsal to the VTA (5.2 mm posterior to bregma, +1.0 mm lateral to midline, and 7.0 mm ventral to skull surface) and lowered in 0.2-mm increments until electrically (60 pulses, 60 Hz, 120 µA, 4 ms/phase) evoked dopamine release was detected via the carbon fiber electrode (see below for details). After optimizing evoked dopamine release, the stimulating electrode was cemented in place and the carbon fiber electrode was removed and replaced with the obdurator. After 5–7 days recovery, rats were retrained for two days. Retraining sessions were identical to earlier sessions with the exception that rats were tethered to the recording headstage for habituation. The test session occurred the day after the second re-training session.

4. <u>Voltammetry Recordings</u>

FSCV in awake and behaving rats and analyte identification and quantification have been extensively described previously (Phillips et al., 2003; Heien et al., 2004; Cone et al., 2013).

Briefly, a micromanipulator containing a glass-insulated carbon fiber (~75 µm; Goodfellow USA, Coraopolis, PA) (recording) electrode was inserted into the NAc guide cannula. The recording electrode was then lowered into NAc and locked into place. A FSCV headstage (University of Washington EME Shop) was used to tether rats, apply voltage changes and measure resultant current changes. The electrode voltage was held at -0.4 V, relative to the Ag/AgCl reference electrode and ramped in a triangular fashion (-0.4 to +1.3 to -0.4 V; 400 V/s). This voltage ramp was repeated at 10 Hz. In addition, an injector connected to a 10 µL Hamilton syringe was inserted into the infusion cannula. To verify that the NAc recording site could capture phasic dopamine release events, electrical stimulation (120 µA, 60 Hz, 24p) was delivered to the VTA/SN. If stimulation failed to evoke phasic dopamine release, the recording electrode was advanced 0.16 mm and the process was repeated.

Once detectable and stable electrically-evoked release was confirmed, electrical stimulations ceased and the experimental session, as described above, began. Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each event to the FSCV interface to be time-stamped along with the electrochemical data. Electrochemical data was also synced with time-stamped video that was recorded during the entire session. After 10 CS+ and CS- presentations (mid-session), an infusion pump was activated to deliver an intra-cranial infusion. n-octanoylated ghrelin (1 µg in 1 µL 0.9% saline; American Peptide, Sunnyvale CA) or vehicle was infused at a rate of 2 µL/min through a 28 gauge injector (1 mm projection beyond the infusion cannula). We previously reported that this dose of ghrelin, when delivered to the LV is sufficient to increase the phasic dopamine response to food reward in the NAc and promote intake of both chow and sucrose (Cone et al., 2014). We presented an additional 10 CS+ and CS- trials following LV

infusion. After the recording session, electrodes were removed; rats were then disconnected from the headstage and returned to their home cage. Following experiments, all recording electrodes were calibrated in a flow-cell as described previously (Sinkala et al., 2012) to convert detected current to concentration. The average calibration factor for all electrodes used in these experiments was 40.20 +/- 3.3 nM/nA.

5. Voltammetry Data Analysis

All rats retrieved and consumed all delivered pellets before and after pharmacological manipulations. Individual CS+ and CS- trials were background subtracted and dopamine concentration during the 5 seconds before to 10 seconds after the event of interest was extracted from voltammetric data using principal component analysis (Heien et al., 2004; Day et al., 2007). We averaged the dopamine concentration during the entire CS+ or CS- (3 second window) for each trial. These values were compared before and after LV infusions (see below).

6. Electrophysiology Surgical Procedures

Under ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia, rats (n=13) were implanted with two microwire electrode arrays and an infusion cannula aimed at the LV. The electrode arrays were chronically implanted bilaterally at AP +1.5, ML ±1.1-1.3 mm relative to bregma, and −6.5 mm relative to brain surface using the rat brain atlas of Paxinos and Watson (2007). Each array was organized into two columns of four microwires (50 μm diameter, tip separation 0.25 mm; MicroProbes for Life Science, Gaithersburg, MD). Ground wires from each array were wrapped around a skull screw and implanted at a remote location ~1 mm into the brain. The 22-gauge, 11 mm LV cannula (GC313; Plastics One, Roanoke, VA) was implanted at AP -0.8, ML -2.4, and -3.75 mm relative to skull surface angled 15◦ away from midline. LV

coordinates were adjusted from above to accommodate the left electrode array. Electrode arrays and the cannula were fixed in place with dental acrylic adhered to skull screws. All rats were given a 10-day recovery period before retraining for 2 days prior to recordings. During the two retraining sessions, rats were tethered to a headstage for habituation, but no data were collected.

7. Electrophysiological Recordings

Before the start of each recording session, rats were connected to a flexible recording cable (Plexon, Dallas, TX, USA) attached to a commutator (Crist Instrument Company, Hagerstown, MD, USA). The headstage of each recording cable contained 16 miniature unity-gain field effect transistors. The activity of single neurons was recorded differentially between each active and an inactive (reference) electrode from the permanently implanted microwire arrays. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation and discrimination of neuronal activity was accomplished using a commercially available neurophysiological system (multichannel acquisition processor; MAP System, Plexon). Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each event to the MAP system to be time-stamped along with the neural data. Principal component analysis (PCA) of continuously recorded waveforms was performed prior to each session and aided in the separation of multiple neuronal signals from the same electrode. During the recording session, waveforms that matched the templates generated by PCA were collected as the same neuron.

All rats underwent two separate recording sessions with one day in between. In the first session, rats received an LV infusion of either n-octanoylated ghrelin (1 µg in 1 µL 0.9% saline; American Peptide, Sunnyvale CA) or vehicle infused at a rate of 2 µL/min through a 28-gauge

injector (1 mm projection beyond the infusion cannula). During the second session, the other infusion was made. Treatments were counterbalanced across days. The LV infusion was delivered after 20 CS+ and CS- presentations (mid-session), and we presented an additional 20 CS+ and CS- trials following infusion.

After the experimental sessions, cell recognition and sorting was finalized using an offline sorter program (Plexon), which assessed neuronal data based on PCA of the waveforms, cell firing characteristics and inter-spike intervals. Data were then exported to Neuroexplorer (Nex Technologies, Madison, AL) and Matlab (Mathworks, Natick, MA, USA) for analyses.

8. Electrophysiological Data Analysis

For electrophysiological recordings, we first identified neurons that increased their firing rate in response to the CS+ during first half of the recording session (before LV infusion). Individual neurons were assessed for their response to the CS+ or CS- using a Wilcoxon signed-rank test. Briefly, the response window for CS trials (0.1 – 1 s following CS onset) was tested against a baseline period from -10 to -5 s before the onset of the CS. For both the CS+ and CS-, individual cells were classified as responsive cells if the average firing rate during the entire 1 s following CS onset was significantly different than baseline. Importantly, neurons were classified for their response to session events (CS+, CS-) based only on trials that occurred before LV infusions. This approach allowed us to assess how the CS populations were modulated by ghrelin or vehicle without biasing our analysis due to infusion-related changes in response properties.

We furthered analyzed cue-responsive neurons for the effect of LV infusions on neural responses. First, we assessed changes in cue-evoked activity resulting from LV infusion across

all CS+ trials. To control for changes in baseline activity, we normalized each neuron's firing rate to its' average activity from -10 to -5 seconds before CS+ onset (the same baseline period used to identify responsive cells). Next, we calculated the average normalized firing rate (100 ms bins) during the 3 s CS+ for all trials before and after infusion. We then used these values to calculate the % change in average normalized firing rate during the CS+ (after vs. before LV infusion) for each neuron in 5 trial blocks (4 blocks total from 20 post-infusion trials).

To assess how discriminable CS+ neural responses were following LV infusion, we applied a Receiver Operating Characteristic (ROC) analysis (Green and Swets, 1966). For each neuron, we calculated the trial-by-trial firing rate during the 3 s CS+ for each neuron in 500 ms bins. To determine how discriminable responses were after the LV infusion compared with before, we computed the area under the ROC curve (auROC) for the CS+ firing rate distribution of each neuron during the final 10 trials of the session (after infusion) to the CS+ firing rate distribution during all 20 pre-infusion trials. A neuron with an auROC value above 0.50 indicated greater CS+ evoked activity following LV infusion, while an auROC value below 0.50 indicated reduced CS+ evoked activity after infusion. We also examined the proportions of neurons that had significant auROC values in positive (auROC>0.5) versus negative (auROC<0.5) directions. As a control, we also performed a second ROC analysis, but instead compared only trials that occurred before infusion. For this analysis, we compared the firing rate distributions during the 20 trials before infusion in 10 trial blocks (i.e firing rate on trials 11-20 vs. trials 1-10). In this case, an auROC value above 0.50 indicates greater activity in later pre-infusion trials, whereas auROC values less than 0.50 indicated greater activity in earlier trials.

9. <u>Verification of Cannula Placements and Recording Sites</u>

Cannula placements were verified by measuring home cage water intake immediately following the injection of Angiotensin II into the LV (50 ng in 2 μL saline; Sigma-Aldrich, St. Louis, MO) and. The Angiotensin II test took place after all recording sessions were complete. Angiotensin II caused drinking (at least 7 mL in 30 min) in all rats. Following completion of experiments, rats were deeply anesthetized with sodium pentobarbital (100 mg/kg). For the voltammetry experiments, a small electrolytic lesion was made at the voltammetry recording site by passing a 10-μA current through a polyimide-insulated stainless steel electrode (A-M Systems, Inc.) 4 times for 4 s each. For electrophysiology experiments, a 100-μA current was passed through each electrode of the microelectrode array for 4 s. In both experiments, current was administered using a lesion-making device (UGO Basile S.R.I.; Comerio, Varse, Italy). Rats were then transcardially perfused with cold 0.01 M phosphate-buffered saline followed by 10% buffered formalin solution (Sigma, Inc). Brains were removed and stored in 10% formalin (voltammetry experiments) or 10% formalin in 3% potassium ferrocyanide (electrophysiology experiments) for 24 h, after which all brains were transferred to 30% sucrose in 0.1 M phosphate buffer (PB) for 7 days. The potassium ferrocyanide reacts with deposited iron from the electrodes to reveal a blue reaction product corresponding with the location of an electrode tip. After post-fixing, 50-μm coronal brain sections were taken using a cryostat and NAc sections were mounted on glass slides. Lesion locations were verified using light microscopy in conjunction with the rat brain atlas of Paxinos and Watson (2007). All voltammetry recordings were confirmed to be from the NAc core. All electrophysiology recordings were confirmed to be in the NAc core or shell.

10. <u>Statistical Analysis</u>

CS+ selectivity was assessed within animal (voltammetry) or within neuron (electrophysiology). For voltammetry, average dopamine concentration evoked by the CS+ was compared to that evoked by the CS- using a paired t-test. For electrophysiology experiments, the average firing rate during the CS+ and CS- within the population of neurons that were CS+ responsive was compared with a paired t-test. The effects of LV infusion were tested using two-way repeated measures ANOVA with Tukey's HSD *post-hoc* tests where appropriate. In voltammetry experiments, we compared the average dopamine concentration evoked during the 3 s cue period (CS+ or CS-) with main effects of epoch (pre, post-infusion) X treatment (saline, ghrelin). In electrophysiology experiments, we compared the post-infusion change (%) in the average normalized firing rate within neuron during the 3 s CS period in 5 trial blocks. We tested for main effects of block (1, 2, 3, 4) X treatment (saline, ghrelin). In both voltammetry and electrophysiology experiments, the effects of LV infusion on retrieval latency were analyzed using a two-way repeated measures ANOVA [epoch (pre, post-infusion) and treatment (vehicle, ghrelin)]. Proportions of neurons that responded to task parameters as well as proportions of neurons with significant auROC values were compared between groups using a two-sample Z-test. Distributions of ROC values were compared using a two-sample Komolgorov-Smirnov test. Statistical analyses were performed using GraphPad 5.0 (Prism Inc.), MATLAB (Mathworks), Statistica 10 (Statsoft).

C. Results

1. Food-predictive cues evoke greater dopamine release than neutral cues

CS+ and CS- cues elicited differential DA release in the NAc during the block of trials preceding LV infusion. Figure 1A shows robust dopamine release in response to a food-

predictive cue during a single representative trial. (Fig. 3.1 A). While non-food predictive cues also evoked dopamine release, the magnitude was smaller in comparison to reward-predictive stimuli (Fig. 3.1 B-C). For all pre-LV infusion trials, we compared the average dopamine concentration during the 1-second following the CS+ and CS- onset within rats. Consistent with previous reports, the CS+ evoked greater dopamine release than the CS- ($t(9)=5.67$, $p<.001$; Fig. 3.1 D). The results were unaffected when groups were analyzed separately based on the identity of the LV infusion they would later receive (Saline and Ghrelin CS+ vs. CS- both $p<.05$; data not shown). All FSCV recordings were made in the NAc core (Fig. 3.1 E).

2. Food-predictive cues activate more NAc neurons and more effectively drive NAc activity than neutral cues

We recorded from a total of 199 NAc neurons from 13 rats across the two sessions (ghrelin sessions: 95 cells; vehicle sessions: 104 cells). Consistent with previous studies, we found a subpopulation of NAc neurons that responded to the onset of the CS+. Figure 3.2 A shows an example perievent raster of a neuron that responded to the CS+ with a significant increase in firing rate. This same neuron had a much weaker response to the CS- (data not shown). Previous studies have shown that NAc core and shell neurons encode outcome-predictive cues compared to cues not associated with any consequences. Thus, we first investigated whether the population of NAc neurons that we sampled differentially encoded the CS+ and CS-. We examined whether each neuron ($n = 199$ cells) responded to the CS+ or CS-, relative to baseline, in the trials before pharmacological manipulations. For this analysis, cells could respond to the CS+, CS-, or both. Across all recorded cells, there were significantly more cells that responded to the CS+ (34%; 67/199) than the CS- (12%: 24/199) ($Z=5.13$, $p<.00001$). The results were unaffected when the groups were analyzed separately according to subsequent

infusion (Ghrelin: $Z = 4.49$, $p<.05$; Saline: $Z = 2.88$, $p<.05$). Thus, a greater proportion of NAc neurons encoded the CS+ compared to the CS-.

We next sought to determine whether the neurons that increased in firing rate in response to the CS+ selectively encoded the food-predictive cue. We compared the average firing rate during the 1 second following the onset of the CS+ and CS- for the 67 neurons that responded to the CS+ before LV infusions. Within this subset of neurons, we found that the firing rate during the first second following cue onset was significantly greater for the CS+ than the CS- ($t(64)=7.45$, $p<.0001$; Fig. 3.2 B-C). The results were unchanged if we analyzed the entire 3 s CS period ($p<.0001$; data not shown). Thus, neurons that responded to the CS+ selectively encoded the motivationally relevant cue, which is consistent with previous reports. All neurons included in our analysis were confirmed to come from either the NAc core or shell (Fig. 3.2 D).

3. <u>Central ghrelin selectively increases the phasic dopamine response to food-predictive cues</u>

To determine whether central ghrelin regulates phasic dopamine signaling evoked by a food-predictive cue, we used *ad libitum* fed rats with cannula in the LV and infused vehicle (1 µL saline; n=5) or ghrelin (1 µg; n=5) into the LV mid-session. LV ghrelin increased the magnitude of dopamine evoked by the CS+ compared to baseline values (epoch X treatment interaction ($F(1,8)=11.00$, $p<.05$); *post hoc*: post-ghrelin $p<.01$ versus pre-infusion values; Fig. 3.3 A: before/after LV saline; Fig. 3.3 B: before after LV ghrelin; Fig. 3.3 C: average dopamine evoked by CS+). To determine whether ghrelin selectively augments the phasic dopamine response to motivationally significant stimuli or merely increases phasic dopamine release in a non-specific manner, we also analyzed the effects of ghrelin on the magnitude of dopamine evoked by CS- presentation. As above, we compared the average dopamine concentration during

the 3 s following CS- onset before compared to after LV infusion. Consistent with the notion that ghrelin selectively modulates phasic dopamine release to motivationally significant stimuli, we found that LV ghrelin did not alter the magnitude of dopamine evoked by the CS- compared to LV saline as there was no effect of either epoch (before vs. after; $F(1,8)=0.38$, $p=.55$) nor treatment (saline vs. ghrelin; $F(1,8)=1.13$, $p=.31$) and no interaction between the main effects ($F(1,8)=2.06$, $p=.18$; data not shown).

4. <u>Central ghrelin does not affect the baseline firing rate of CS+ responsive NAc neurons</u>

Prior to examining the effects of ghrelin on neural responses to cues, we examined whether LV ghrelin affected baseline activity of NAc neurons that responded to the CS+. Indeed, an increased CS+ response could reflect an increased response to the CS+ specifically, or alternatively, could have resulted from an overall increase in spiking activity for a given neuron. To partially determine this, we calculated the average firing rate for each neuron during the baseline epoch we used to identify CS responsive neurons (-10 to -5 s prior to CS+ onset). LV infusion significantly altered the baseline firing rate of the CS+ population (Saline before: 5.55±0.9; Saline after: 4.86±0.8; Ghrelin before: 5.63±0.9; Ghrelin before: 5.81±0.9 spikes/s; epoch X treatment interaction ($F(1,65)=19.14$, $p<.001$). However, *post hoc* tests revealed that this was due to a significant decrease in the baseline firing rate of CS+ responsive neurons following LV saline (before vs. after: $p<.05$), as there was no effect of LV ghrelin on the baseline firing rate of CS+ responsive cells (before vs. after: $p=.22$). Thus, LV ghrelin infusion did not affect the baseline activity of NAc neurons that respond to the CS+.

5. <u>Central ghrelin selectively influences NAc neuron responses to a food-predictive cue</u>

Examining the response of CS+ neurons across all pre and post-infusion trials revealed there was a decrease in the magnitude of the neural response to the cue (0-3s following CS+ onset) in the saline infusion condition (Fig. 3.4 A), whereas NAc responses to the CS+ increased following LV ghrelin infusion (Fig. 3.4 B). Thus, LV infusion appeared to have bi-directional effects depending on infusion type. To characterize the effects and time course of LV infusions on NAc activity evoked by the CS+ we compared the % change in normalized firing rate during the entire 3 s CS+ (after relative to before LV infusion) in 5 trial blocks. The enhancement of CS+ evoked activity following ghrelin administration depended on time since infusion (block X treatment ANOVA: $F(3,65)=4.62$, $p<.01$; Fig. 3.4 C). *Post-hoc* tests revealed this was driven by later blocks (block 3: $p=.07$; block 4: $p<.001$). Within groups there was no effect of saline across blocks; however ghrelin significantly increased normalized CS+ activity during the post-infusion period (block 1 vs. block 4: $p<.05$).

Across all CS+ responsive neurons, ghrelin significantly augmented responses during the later trials of the recording session. This effect may reflect subtle modulation in CS+ activity across the entire population, or robust effects on a smaller group of CS+ responsive cells. Thus, we next examined how LV ghrelin influences cue evoked activity in the NAc at the level of individual neurons. To accomplish this, we used ROC analysis to compare the firing rate of each neuron during the CS+ (0-3 s, 500 ms bins) for the last 10 trials after LV infusion compared to all pre-infusion trials. Consistent with the population level normalized firing rate changes, the distributions of auROC values were significantly different across groups. AuROC values following LV ghrelin were rightward shifted (i.e., greater activity in post-infusion trials) compared to LV saline (Komolgorov-Smirnov test; $p<.01$; Fig. 3.4 D-E). At the level of individual neurons, a greater proportion of cells had auROC values significantly above 0.50

following LV ghrelin (8/32) compared to LV saline (2/35; Z=2.21, p<.05). Additionally, a smaller proportion of cells had auROC values that were significantly below 0.50 following LV ghrelin (4/32) versus LV saline (14/35; Z=-2.53, p<.05; Fig 3.4 D-E). The results of the cell-by-cell ROC analysis indicate, compared to LV saline, LV ghrelin increases the CS+ response in a greater proportion of NAc neurons while potentially preventing a decrement in the response of other cells.

Changes in firing rate during the post-infusion period contrast with those observed pre-infusion. We compared firing rates during the CS+ for the first block of 10 trials before infusion compared to second block of 10 pre-infusion trials. The average auROC value across the CS+ population was approximately 0.50 for both groups indicating the neural response to the CS+ did not change during the pre-infusion epoch (Saline auROC: 0.48 ± 0.01; Ghrelin auROC: 0.50 ± 0.01; (t(65)=1.04, p=.30), and there was no difference in the proportion of cells that had significant auROC values across groups (Saline: 6/35; Ghrelin: 6/32). This was true for neurons with significant auROC values above 0.50 (Saline: 2/35 cells; Ghrelin: 3/32), as well as neurons with significant auROC values below 0.50 (Saline: 4/35; Ghrelin: 3/32). These data suggest that the auROC effects reported above are due specifically to LV infusions rather than intra-neuronal response variability during the session.

Only 12% (24/199) of all recorded neurons responded to the CS-. Notwithstanding the small sample size, we examined whether ghrelin altered the response of NAc neurons to motivationally neutral stimuli. As above, we compared the % change in normalized firing rate following LV infusion in 5 trial blocks. LV infusion did not alter the NAc neuron response to the CS-, as we found no effect of either epoch (pre vs. post; F(3,18)=2.29, p=.09) nor treatment

(saline vs. ghrelin; $F(1,18)=0.33$, $p=.57$) and no interaction ($F(3=3,18)=0.21$, $p=.88$; data not shown).

6. Central ghrelin does not recruit previously unresponsive NAc neurons

Above, we restricted our analyses to the subset of neurons that significantly responded to the CS+ prior to infusions. It is also possible that LV ghrelin recruits previously unresponsive neurons to respond to features of the behavioral paradigm. To examine this possibility, we examined the subpopulation of neurons that did not significantly respond to the CS+ or CS- during the 20 trials before infusion, to determine if any began to respond to the CS+ or CS- after LV infusion. If ghrelin infusion recruited previously unresponsive neurons, we would expect the proportion of cells that become responsive after infusion to differ between saline- and ghrelin-infused groups. While there was a small subpopulation of cells that began to respond to the CS+ after LV infusion, the proportions of these cells were not significantly different between groups (Saline: 6% (6/104); Ghrelin: 8% (8/95), $p =.46$). There were also no differences in the proportion of neurons that began to respond to the CS- (Saline: 7% (7/104); Ghrelin 7% (7/95); $p =.85$). Thus, compared to saline, LV administration of ghrelin did not augment the proportions of cells that encoded paradigm parameters after infusion.

D. Discussion

In humans, cues associated with food powerfully enhance both ratings of appetite and food consumption (Ferriday and Brunstrom, 2008). As food cues pervade our lives, understanding the neurobiological substrates that encode such cues and how these signals are modulated is of great clinical importance especially in light of the ongoing worldwide obesity problem. Among other circuits (Petrovich, 2013), food cues activate striatal circuitry. Food-cue

reactivity is also influenced by physiological state (e.g., hunger, satiety; (Corbit et al., 2007; D'Agostino and Small, 2012). The 'hunger hormone' ghrelin regulates aspects of both normal and cue-potentiated feeding (Nakazato et al., 2001; Walker et al., 2012) and modulates striatal food-cue responses as measured with fMRI (Malik et al., 2008; Kroemer et al., 2013). Here, we found that ghrelin modulates the response of specific striatal elements to food cues – namely cue-evoked phasic dopamine and neural activity in the NAc. Critically, ghrelin had no effect on the encoding of a neutral stimulus, nor did it affect the baseline activity of NAc neurons, suggesting that ghrelin's effects were specific to motivationally relevant cues. Given the extensive literature on the role of striatal signaling in cue-evoked behavior, ghrelin's ability to regulate phasic mesolimbic signals evoked by food cues likely has broad implications for cue-driven behavior and may represent a novel mechanism by which ghrelin and physiological state augment food seeking.

Ghrelin effects on mesolimbic circuitry have been shown previously (for review see Skibicka and Dickson, 2011). In animals not engaged in any feeding-related tasks, both systemic and central administration of ghrelin directly into the VTA increases dopamine concentration in the NAc (Jerlhag et al., 2007; Jerlhag, 2008). These results could explain how dopamine release during food consumption is enhanced in food-restricted animals (Wilson et al., 1995; Cone et al., 2014). Indeed, we recently showed that central administration of ghrelin specifically potentiates the phasic dopamine response to primary food reward (Cone et al., 2014). Phasic dopamine responses shift from food itself to cues that predict food (see Figures 1 and 3, but also Schultz, 1998; Day et al., 2007; Stuber et al., 2008). The shift and residual response at time of reward is thought to participate in associative learning (Schultz and Dickinson, 2000) and incentive motivation (Berridge and Robinson, 1998; Flagel et al., 2011). Indeed, the magnitude of cue-

evoked phasic dopamine release has been linked to approach behaviors (Day et al., 2007; Stuber et al., 2008) and genetic manipulations that attenuate phasic dopamine signaling disrupt learning about environmental cues associated with food (Zweifel et al., 2009). Recent work with optogenetics has provided a causal link for dopamine's involvement in these phenomena, as phasic activation of dopamine neurons promotes learning about cues paired with food reward (Steinberg et al., 2013) and invigorates reward-seeking behaviors (Ilango et al., 2014). Taken together, these results suggest that ghrelin's ability to modulate cue-evoked dopamine signaling could promote learning about environmental cues that signal food availability as well as gate the ability of food cues to drive food-seeking behavior.

In addition to phasic increases in NAc dopamine, food-predictive cues also evoke phasic changes in the activity of individual NAc neurons (Nicola et al., 2004; Roitman et al., 2005; Day et al., 2006; Wan and Peoples, 2006). Cue-evoked NAc activity has been directly linked to food-seeking actions in a variety of behavioral paradigms (Taha et al., 2007; McGinty et al., 2013; Roitman and Loriaux, 2014). Additionally, interfering with normal phasic NAc signaling disrupts food consumption following cue presentation (Krause et al., 2010). Thus, by augmenting phasic NAc spike output, ghrelin could gate the ability of food cues to elicit food-seeking behaviors and may regulate the intensity of behaviors aimed at food consumption (McGinty et al., 2013). Moreover, phasic activation of multiple sources of NAc input as well as phasic activation of NAc neurons is sufficient to reinforce instrumental behavior (Britt et al., 2012). These data suggest that modulation of NAc output by ghrelin could potentiate neural ensembles involved in food approach and consumption.

Given that our pharmacological manipulations were ventricular, the brain region or regions where ghrelin acts to regulate phasic mesolimbic signaling warrants consideration. We

recently demonstrated that intra-VTA ghrelin had no effect on phasic dopamine release evoked by primary food reward - and instead suggested a role for lateral hypothalamus (Cone et al., 2014). Thus the VTA is an unlikely site of action at least with respect to phasic dopamine signaling. Intra-NAc ghrelin promotes feeding (Naleid et al., 2005), however multiple aspects of our data discount the possibility that ghrelin was acting within the NAc. Firstly, we observed no effect of ghrelin on CS- evoked activity measured with both voltammetry and electrophysiology. Ghrelin also had no effect on the baseline activity of NAc neurons. Thus, the selectivity of ghrelin's effects on CS+ evoked activity reduce the likelihood of direct actions within the NAc or VTA, where we might have expected a general change in activity resulting from non-selective modulation.

A more probable mechanism is that ghrelin is acting outside of the NAc and VTA to increase food cue related input to these regions. Ghrelin increases the BOLD response to food cues in multiple brain regions linked to appetitive behavior, including the striatum, amygdala, hippocampus, and orbitofrontal cortex (Malik et al., 2008). Of these regions, previous data highlights a prominent role for the amygdala in regulating NAc activity and cue-evoked behavior. Basolateral amygdala (BLA) neurons respond to food-predictive cues (Tye and Janak, 2007) and BLA projections to the NAc regulate food seeking (Stuber et al., 2011). Inactivating the BLA impairs phasic NAc activity and NAc dopamine release evoked by food-paired stimuli (Ambroggi et al., 2008; Jones et al., 2010a, 2010b). Lesions of the BLA disrupt cue-potentiated feeding (Holland et al., 2002), and render animals insensitive to satiety based devaluation procedures (Johnson et al., 2009), suggesting that the BLA integrates information about physiological state to regulate food cue-evoked behavior. Critically, ghrelin alters the activity of neurons in the BLA complex (Alvarez-Crespo et al., 2012; Song et al., 2013). Thus, the BLA

represents a potential site where ghrelin could influence cue-evoked dopamine and NAc activity and will be a site of future investigation.

Dopamine has multifaceted effects on NAc neurons. Thus, the change in cue-evoked NAc spiking following LV ghrelin could have been a direct result of increased cue-evoked dopamine release. Coincident changes in NAc cell firing and phasic dopamine release have been observed at the same recording site (Cheer et al., 2007; Cacciapaglia et al., 2011) and pharmacological manipulations of the VTA diminish cue-evoked activity in the NAc (Yun et al., 2004; Cacciapaglia et al., 2011). Phasic dopamine release is thought to recruit the activation of dopamine type 1 receptors (Dreyer et al., 2010), which alter the excitability of striatal neurons through calcium channels (Surmeier et al., 1995) as well as AMPA and NMDA receptor trafficking (Snyder et al., 2000; Hallett et al., 2006). Our data show that while ghrelin increased cue-evoked dopamine immediately (i.e., within the first 10 post-infusion trials), effects on phasic NAc cue responses were not observed until later trials. This is consistent with the time course of dopamine-dependent GluR1 trafficking in the NAc (Chao et al., 2002; Mangiavacchi and Wolf, 2004; Sun et al., 2008). These data raise the intriguing possibility that ghrelin's enhancement of cue-evoked dopamine release renders subsets of NAc neurons more sensitive to food-cue related input.

Interestingly, we observed different patterns of neural responses on CS+ evoked NAc spiking following LV infusions of saline and ghrelin. Administration of saline was followed by a reduction in CS+ activity. However, the decrease in CS+ activity following LV saline was not apparent until the 4^{th} post-infusion block, suggesting that it is unlikely to have resulted from the infusion. Satiety signals like insulin, amylin, and GLP-1, all of which are released in response to food intake, can act at the level of the VTA as well as NAc to decrease food intake and food-

directed behavior (Baldo and Kelley, 2001; Dossat et al., 2011; Alhadeff et al., 2012; Mebel et al., 2012; Mietlicki-Baase et al., 2013), and intra-VTA amylin attenuates food-evoked phasic dopamine signaling (Mietlicki-Baase et al., 2014). Given that our animals were fed *ad libitum*, satiety is a probable source of this late-session modulation following LV saline.

In summary, we report that ghrelin, a peripheral hormone associated with hunger, augments cue-evoked activity in two distinct striatal elements that jointly participate in cue-related learning and cue-evoked behavior. Our results, therefore, implicate physiological state in general, and ghrelin specifically, as strong regulators of mesolimbic activity, and highlight novel mechanisms through which physiological state influences neural signals underlying food-directed behaviors that culminate in food consumption.

Figure 3.1

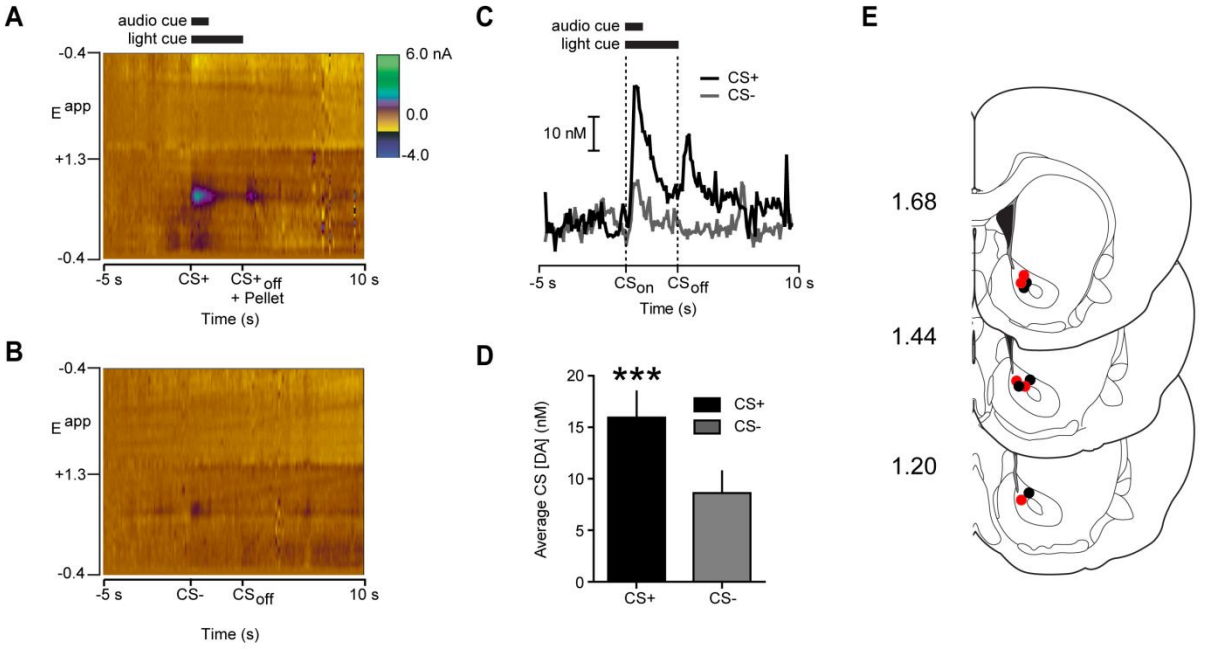

Figure 3.1: NAc dopamine release preferentially encodes food-predictive stimuli. ***A,*** Representative single trial colorplot depicting current changes (color) as a function of electrode potential (y-axis) in the 5 s before to 10 s after (x-axis) CS+ onset. Solid Bars at top denote duration of light/tone cue. Dopamine [identified by its oxidation (~+0.6 V; green) and reduction (~-0.2 V; light yellow) features] was transiently evoked by the CS+. ***B,*** Representative single trial colorplot from the same animal as in *A* but for a CS- trial. ***C,*** Dopamine concentration extracted from the electrochemical data in *A* and *B* using chemometric analysis (Heien et al., 2004). ***D,*** Average dopamine concentration evoked during the 1 s following CS onset for all pre-infusion CS+ and CS- trials (n=10 rats; 10 CS+ and CS- trials per rat). ***D,*** Coronal brain sections modified from Paxinos and Watson (2007). Numbers at left indicate approximate distance from Bregma. Colored circles indicate approximate voltammetry recording locations from *ad-libitum* fed rats that received LV saline (n=5; black) or ghrelin (n=5; red). ***p<.001.

Figure 3.2

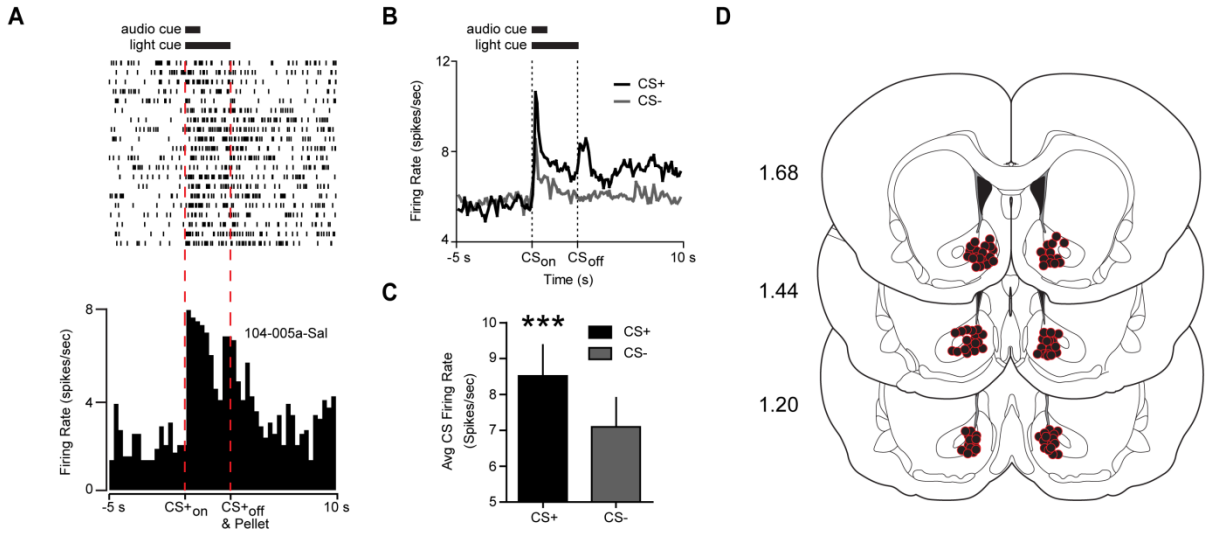

Figure 3.2: NAc neurons preferentially encode food-predictive stimuli. ***A,*** Raster plot (above) and perievent histogram (below) aligned to the CS+ for a representative NAc neuron that responded to the CS+ with a significant increase in firing rate. ***B,*** Average firing rate during the 5 s before to 10 s after CS onset for all pre-infusion CS+ and CS- trials across the population of neurons that responded to the CS+ (67/199 neurons from the two recording sessions; 20 CS+ and CS- trials). ***C,*** Average firing rate of the 67 neurons plotted in *B* during the initial 1 s following CS+ and CS- onset. ***D,*** Coronal brain sections modified from Paxinos and Watson (2007). Numbers at left indicate approximate distance from Bregma. Colored circles indicate confirmed electrode placements in the NAc (n=13 rats; 8 wires per hemisphere per rat). Note that some circles are obscured due to overlap. ***p<.001.

Figure 3.3

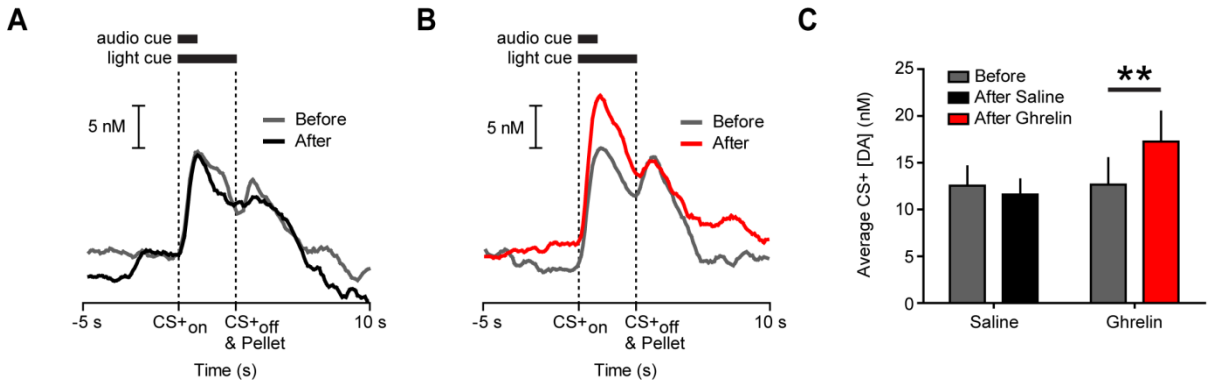

Figure 3.3: LV ghrelin increases the magnitude of dopamine evoked by the CS+. ***A,*** Average trace of dopamine concentration during the 5 s before to 10 s after CS+ onset before (gray) and after (black) LV infusion of saline (n=5 rats; 10 trials per rat per epoch). ***B,*** Average trace of dopamine concentration during the 5 s before to 10 s after CS+ onset before (gray) and after (red) LV infusion of ghrelin (n=5 rats; 10 trials per rat per epoch). ***C,*** Average dopamine concentration during the 3 s CS+ before and after LV saline or ghrelin. **p<.01.

Figure 3.4

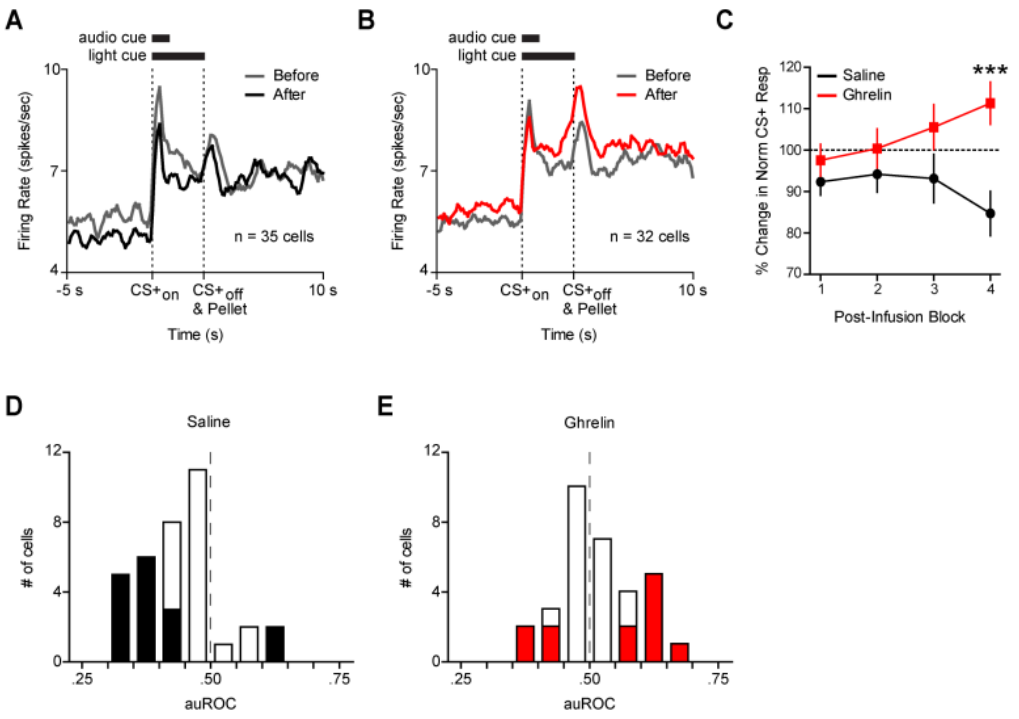

Figure 3.4: LV ghrelin increases CS+ evoked activity in NAc neurons. ***A,*** Average firing rate of CS+ responsive cells during the 5 s before to 10 s after CS+ onset before (gray) and after (black) LV infusion of saline (n=35 neurons; 20 trials per neuron per block). ***B,*** Average firing rate of CS+ responsive cells during the 5 s before to 10 s after CS+ onset before (gray) and after (red) LV infusion of ghrelin (n=32 neurons; 20 trials per neuron per block). ***C,*** Average % change in normalized firing rate during the 3 s CS+ following LV saline (black) or ghrelin (red) in 5 trial blocks. Pre-infusion firing rate is indicated by the dashed line at 100%. ***D,*** Population density histogram of differences in firing rate (plotted as auROC values) during the 3 s CS+ comparing the second block of 10 trials after LV saline to pre-infusion firing rate (auROC<0.5, after<before; 0.5, no difference; auROC>0.5, after>before). Cells with significant auROC values are filled black ($p<.05$). ***E,*** Population density histogram of auROC values during the 3 s CS+ comparing the second block of 10 trials after LV ghrelin to pre-infusion firing rate (auROC<0.5, after<before; auROC>0.5, after>before). Cells with significant auROC values are filled red ($p<.05$). ***$p<.001$ for ghrelin compared to saline.

Chapter IV

General Discussion

Rewarding food and environmental cues that signal food availability strongly influence motivated behavior and drive food consumption, in part, by activating the mesolimbic system. Food and food cues evoke phasic changes in the activity of mesolimbic circuitry, and these phasic fluctuations in mesolimbic signaling directly contribute to behavioral reinforcement, reward-related learning, and motivation. To be adaptive, the neural signals that participate in these processes should be sensitive to physiological state (i.e., hunger vs. satiety). Indeed, recent data suggests that peripheral hormones related to physiological state act within the mesolimbic system to regulate food intake and food-directed behavior. However, whether hormones that relay information about physiological state influence mesolimbic encoding of food and food-predictive stimuli was unknown. The goal of the present study was to examine whether ghrelin, the only known peripheral hormone associated with hunger, influenced two types of mesolimbic signals involved in food-directed behavior; namely phasic dopamine release and phasic increases in the activity of NAc neurons evoked by primary food reward and food-predictive cues. My results demonstrate that ghrelin acts centrally to regulate both phasic dopamine signaling and phasic increases NAc neural activity evoked by food and food cues. I also demonstrate that ghrelin acts in the LH, but not VTA to regulate phasic dopamine release evoked by food, and likely does so by activating LH orexin neurons. Taken together, these data represent novel mechanisms through which physiological state in general, and ghrelin specifically, augments neural signals underlying food-directed behavior, particularly in areas of the brain linked to reinforcement, reward learning and motivation.

A. Central ghrelin regulates phasic dopamine release evoked by food reward

I showed that the magnitude of phasic dopamine spikes evoked by food is increased by central administration of ghrelin and attenuated by antagonism of central ghrelin receptors. While the dose of ghrelin used in these studies was superphysiologic, attenuation of phasic dopamine release following GHS-R antagonism (which would block endogenous ghrelin) suggests that circulating ghrelin augments the phasic dopamine response to food. This is further supported by the fact that I also showed that food-restriction, which elevates endogenous ghrelin, (Tschöp et al. 2000; Cummings et al. 2004) augments phasic dopamine release independent of any pharmacological manipulations. Thus, the bi-directional modulation of phasic dopamine release by ghrelin demonstrated here suggests that natural fluctuations in ghrelin that occur throughout the day (Cummings et al., 2001) may tune how dopamine neurons respond to food reward. Moreover, many aspects of the ghrelin system are dysregulated in obesity (Tschöp et al. 2001; English et al. 2002), suggesting that ghrelin's modulation of phasic dopamine signaling would be correspondingly disrupted - a topic worthy of future study.

Previous work has demonstrated that exogenous factors related to food reward potently influence the magnitude of phasic dopamine release. These factors include caloric content (Beeler et al. 2012), temporal delays to reinforcement (Wanat et al. 2010), and the magnitude of the anticipated outcome (Gan et al. 2010). Here, I provide the first evidence that an *endogenous* signal related to hunger regulates phasic dopamine release evoked by food reward. Ghrelin's ability to augment food-evoked dopamine spikes has broad implications for food-related behavior. Phasic stimulation of dopamine neurons can promote behavioral reinforcement (Tsai et al. 2009; Witten et al. 2011; Steinberg et al. 2014) as well as motivation for food (Ilango et al. 2014). Moreover, the magnitude of dopamine release has been correlated with aspects of

approach behavior (Wassum et al. 2013) and motivation (Wassum et al. 2012). Thus, regulation of phasic dopamine release by ghrelin could augment food-seeking behavior and differentially reinforce food-directed actions in a state dependent manner. In natural foraging environments, these effects would be highly adaptive by serving to drive food seeking and strengthen behaviors that lead to food in times of caloric need. However, in an obesogenic environment, ghrelin's effects on phasic dopamine signaling could promote overconsumption.

Peripheral ghrelin crosses the blood brain barrier, (Banks et al. 2002) and can diffuse to virtually all GHS-R expressing nuclei (Zigman et al. 2006; Cabral et al. 2013). While later sections will discuss the results of ghrelin infusions directly into the LH and VTA, these two sites are unlikely to be the only sites where ghrelin could influence phasic dopamine signaling. In humans, ghrelin activates a wide range of nuclei involved in appetitive behavior, including the amygdala, hippocampus, and orbitofrontal cortex (Malik et al. 2008). Interestingly, both the amygdala and hippocampus are ghrelin sensitive (Alvarez-Crespo et al., 2012; Kanoski et al., 2012; Song et al., 2013) and influence mesolimbic activity (Lodge and Grace, 2006a; Grace et al., 2007; Jones et al., 2010a, 2010b; Stuber et al., 2011; Britt et al., 2012). Other potential sites of action also warrant consideration, including the PPTg and LDTg. Both the PPTg and LDTg regulate VTA burst firing (Lodge and Grace, 2006b; Chen and Lodge, 2013). Ghrelin excites PPTg and LDTg neurons (Kim et al., 2009; Takano et al., 2009), and LDTg ghrelin administration increases NAc dopamine in awake rats (Jerlhag et al., 2012). Thus, the PPTg and LDTg represent potential, but as yet uninvestigated, sites of action for ghrelin's influence on phasic dopamine signaling.

Physiological state influences motivation and reward-related behavior through actions in the mesolimbic system (Carr, 1996, 2007; Carr et al., 2001). This is likely due to peripheral

feeding peptides, as virtually all of the feeding related hormones have been shown to act in the VTA, including leptin (Fulton et al., 2006; Hommel et al., 2006), GLP-1 (Dossat et al., 2011; Alhadeff et al., 2012), ghrelin (Abizaid et al., 2006) and amylin (Mietlicki-Baase et al., 2013). The diversity of hormones with actions in the mesolimbic system suggests that the VTA integrates information related to physiological state to produce a corresponding change in behavior. Previous work demonstrated that leptin decreases the activity of VTA dopamine neurons in anesthetized animals (Hommel et al., 2006), while ghrelin broadly increases NAc dopamine concentration, as measured with microdialysis (Jerlhag et al., 2006). Moreover, food-restriction increases burst firing of dopamine neurons in anesthetized mice, suggesting that physiological state could alter phasic dopamine signaling (Branch et al., 2013). Overall, these studies suggest that peripheral hormones could alter reward-related behavior by acting on VTA dopamine neurons. My data extend previous findings to show that ghrelin, a hormone strongly associated with physiological state, augments phasic dopamine release in awake, behaving animals. Given that phasic dopamine signals are strongly tied to reward-directed behavior, these data suggest a novel mechanism underlying the influence of physiological state on goal-directed behaviors. Moreover, it demonstrates that a hormone related to food intake influences how the brain responds to food, specifically in areas of the brain linked to reward seeking. Future studies should seek to identify whether other feeding peptides also augment phasic dopamine signaling, as this could be a general mechanism for hunger and satiety to promote or suppress food-seeking behavior.

Lastly, LV ghrelin promotes food intake even in sated animals (Tschöp et al., 2000; Lawrence et al., 2002). Conversely, central blockade of ghrelin receptors attenuates feeding (Nakazato et al., 2001). Thus, that I observed similar effects on food intake in response to

stimulation and antagonism of central ghrelin receptors is consistent with previous reports. Moreover, the food intake data indicates the doses used here were sufficient to influence the activity of central GHS-Rs.

B. Central ghrelin regulates phasic dopamine release evoked by food reward via action in the LH

The LH integrates aspects of physiological state to alter motivated behavior (Adamantidis and de Lecea, 2009; Berthoud and Münzberg, 2011). Importantly, LH neurons have been shown to increase their firing rate in response to the receipt of food reward and this response diminishes with satiety (Rolls et al., 1986), suggesting that LH neurons phasically respond to the receipt of food reward in a state dependent manner. It is interesting to speculate whether LH neurons that respond to food reward excite the VTA, as this could be the population underlying LH ghrelin's effects on phasic dopamine release. However, the LH is home to an array of unique cell types, many of which have been shown to interact with VTA dopamine neurons to augment feeding and other reward-related behaviors, including neurotensin (Glimcher et al., 1987; Geisler and Zahm, 2006; Kempadoo et al., 2013), MCH (Domingos et al., 2013), as well as orexin (Muschamp et al., 2014). Thus, it remains to be determined what LH cells types were driving the increase in phasic dopamine release following LH ghrelin administration. However, when considered along with previous studies, LH orexin neurons likely underlie the effects reported here. Ghrelin increases the firing rate of LH orexin neurons (Yamanka et al., 2003), while the effects of ghrelin on food intake requires orexin, but not MCH (Toshinai et al., 2003). Moreover, LH ghrelin promotes food intake and induces fos expression in LH orexin neurons (Olszewski et al., 2003b). Lastly, intra-VTA infusions of neurotensin reduce feeding (Cador et al., 1986). Here,

LH ghrelin increased feeding, which is consistent with previous data (Olszewski et al., 2003b) and discounts the possibility that LH ghrelin was acting through neurotensin. Lastly, my data also demonstrate that intra-VTA orexin recapitulated the results of LH ghrelin infusions. Thus, ghrelin was likely activating orexin-expressing neurons in the LH to increase phasic dopamine release evoked by food. Importantly, my results suggest that signals related to physiological state can act in the LH to influence mesolimbic signals linked to reinforcement and goal-directed actions. Thus, these data further establish the LH as a critical link between metabolism and motivated behavior.

C. Intra-VTA ghrelin promotes food intake but does not augment phasic dopamine release evoked by food reward.

Notably, intra-VTA ghrelin did not alter phasic dopamine release in the NAc – an unexpected result given previous data demonstrating that ghrelin acts in the VTA to modulate dopamine dependent behaviors (Jerlhag et al., 2007; Skibicka et al., 2011; Skibicka et al., 2013) and increases NAc dopamine release (Jerlhag et al., 2007). However, in previous studies, dopamine measurements were made in the NAc shell, whereas here voltammetry measurements were made in the NAc core. Thus, there is a possibility that ghrelin selectively alters the activity of select VTA dopamine neurons depending on their projection targets, especially considering that VTA dopamine neurons differentially respond to pharmacological challenges depending on which brain areas they innervate (Lammel et al., 2008). This question could be resolved by labeling ghrelin receptor expressing VTA dopamine neurons coupled with injections of retrograde tracers in dopamine terminal regions to determine where ghrelin receptor VTA dopamine neurons are projecting. Another possibility is that ghrelin alters tonic activity in NAc

core projecting VTA dopamine neurons, but fast-scan cyclic voltammetry samples too frequently to optimally detect these changes.

The observation that intra-VTA ghrelin increased food intake without altering phasic dopamine signaling suggests that intra-VTA ghrelin promotes feeding in a dopamine-independent manner. Importantly, previous studies demonstrated food intake elicited by intra-VTA ghrelin was unaffected by NAc dopamine receptor antagonism (Skibicka et al., 2013). Whether the ability of intra-VTA ghrelin to increase food intake is mediated by non-dopaminergic neurons or by dopamine neurons that project to targets other than the NAc warrants further investigation. Future experiments should measure food intake following infusion of ghrelin into the VTA, while simultaneously blocking receptors for dopamine, glutamate or GABA in VTA target regions other than the NAc.

Notably, the ability of intra-VTA ghrelin to promote food motivation (as measured by increased breakpoint in a progressive ratio task) was blunted by intra-NAc dopamine receptor blockade (Skibicka et al., 2013). Thus, VTA ghrelin increases food intake and motivation through two distinct mechanisms. Responding on a progressive ratio is dopamine dependent (Salamone and Correa 2012). Thus, it is noteworthy that we did not observe an effect of intra-VTA ghrelin on phasic dopamine signaling. However, attenuating phasic dopamine signaling by genetically deleting NMDA receptors from dopamine neurons has no effect on breakpoint (Zweifel et al., 2009). Additionally, genetically knocking out the dopamine transporter, which tonically elevates NAc dopamine levels and potentially masks phasic dopamine release events, increases the amount of effort animals will exert to obtain food (Beeler et al., 2010). Conversely, phasic dopamine responses to the receipt of reward are enhanced in later stages of a progressive ratio paradigm (Wanat et al., 2010). However, control experiments in which the effort was fixed

and the delays between reinforcers were yoked demonstrated that the increase in phasic dopamine release observed in later trials could be explained by the delay to reinforcement and not effort (Wanat et al., 2010). While phasic dopamine release may invigorate goal-directed behavior (Wassum et al., 2012; Ilango et al., 2014), whether phasic dopamine signaling contributes to breakpoint measures still remains unclear. In sum, these data are consistent with the idea that VTA ghrelin could increase food-directed motivation without effecting phasic dopamine signaling.

Taken together, VTA ghrelin regulates dopamine dependent (motivation) and dopamine independent behaviors (freely available food intake), without altering phasic dopamine release in the NAc. Yet, intra-VTA ghrelin administration has been shown to increase dopamine levels in the NAc shell over the course of an hour, thus demonstrating that VTA ghrelin can influence dopamine release (Jerlhag et al., 2007). These data speak to the heterogeneity of VTA neurons and their diverse functions as well as projection targets (Lammel et al., 2008, 2011, 2012). Identifying the unique populations of dopaminergic as well as non-dopaminergic GHS-R expressing VTA neurons and their respective roles in food-related behavior could lead to selective targets for the treatment of obesity.

D. VTA orexin increases phasic dopamine release evoked by food reward

The orexin system has been strongly implicated in reward-directed behaviors for both food and drug rewards (Borgland et al., 2010; Cason et al., 2010; Choi et al., 2010). Importantly, the VTA appears to be a critical site of action for many of orexin's effects on motivated behavior. Intra-VTA orexin receptor blockade reduces ethanol and cocaine self-administration (James et al., 2011; Srinivasan et al., 2012). Conversely, intra-VTA infusions of orexin-A

promote responding for intravenous cocaine (España et al., 2011). Moreover, VTA orexin-A administration is sufficient to reinstate cocaine-seeking behavior (Harris et al., 2005; Wang et al., 2009), while blocking VTA orexin receptors attenuates reinstatement of cocaine seeking (James et al., 2011). With respect to food, blocking VTA orexin receptors blunts the dramatic increase in food intake following pharmacological manipulations of the NAc (Zheng et al., 2007). Thus, modulation of VTA orexin potently influences reward-directed behaviors.

VTA Orexin-A is likely modulating motivated behavior through actions on VTA dopamine neurons. Intra-VTA orexin-A infusions are sufficient to condition a place preference, but fail to do so when NAc dopamine receptors are blocked (Taslimi et al., 2012). Blocking VTA orexin receptors attenuates the dopamine response to cocaine (España et al., 2010) and impairs cocaine sensitization (Borgland et al., 2006). In slice, bath application of orexin-A increases the firing rate of VTA dopamine neurons (Korotkova et al., 2003) and increases excitatory synaptic transmission in VTA dopamine neurons (Borgland et al., 2006). *In vivo*, VTA orexin increases the basal firing rate of VTA dopamine neurons and augment their sensitivity to inputs from prefrontal cortex (Moorman and Aston-Jones, 2010). Thus, orexin-A may regulate the intrinsic excitability of VTA neurons thereby increasing their sensitivity to excitatory inputs. Indeed, intra-VTA orexin-A increases, while intra-VTA orexin receptor antagonism decreases the magnitude of phasic dopamine evoked by electrical stimulation of the VTA (España et al., 2010, 2011). Importantly, my data is the first evidence that orexin augments phasic dopamine release evoked by a motivationally salient event (i.e., the receipt of food reward) in awake and behaving animals. Moreover, I never observed a non-specific increase in dopamine release, suggesting that VTA orexin did not result in a general increase in dopamine neuron activity. Instead my data more closely resemble an increase in sensitivity to excitatory input, which is largely consistent

with orexin's effects on excitatory transmission onto VTA dopamine neurons. In sum, these data raise the possibility that orexin influences reward-directed behaviors, including reinstatement of food and drug seeking by modulating phasic dopamine release to rewards and their predictors. Additionally, it suggests that LH ghrelin could act on VTA projecting orexin neurons and thereby increasing NAc dopamine release evoked by food.

E. VTA orexin signaling is necessary for feeding induced by central ghrelin administration

Prior to my experiments, there was some evidence that food intake induced by central ghrelin was dependent on the orexin system. For example, ventricular or intra-LH administration of ghrelin induces Fos expression in LH orexin neurons (Olszewski et al., 2003b; Toshinai et al., 2003), while ghrelin administered directly into the LH promotes food intake (Olszewski et al., 2003b). Orexin activity appears to be critical for central ghrelin to augment feeding. Injecting antibodies against orexin-A and B attenuate food intake induced by central ghrelin and orexin knockout mice consume less food than wild type littermates following central ghrelin administration (Toshinai et al., 2003). However, globally interfering with the orexin system impairs sleep-wake regulation (Adamantidis and de Lecea, 2009). Thus, impairments in ghrelin-induced feeding could have resulted from differences in the amount time the animals were awake during the test session. My data further suggests that ghrelin augments feeding by acting on LH orexin neurons. Moreover, my results highlight VTA orexin receptors as key mediators of ghrelin's effects on food intake. Notably, the compound used in this study (SB 334,867) is 50 fold more selective for the Orexin-1 receptor compared to the Orexin-2 receptor. Thus, it is possible that Orexin-1 receptor signaling in the VTA is more critical than the Orexin-2 receptors.

Future studies will need to examine feeding related effects of VTA orexin receptor blockade more thoroughly as selective orexin receptor antagonists become available.

Importantly, these data suggest a potential mechanism underlying the ability of central ghrelin manipulations to augment phasic dopamine release. First, I observed increases in phasic dopamine release following both LV and LH ghrelin administration. Next, I recapitulated the results of LV and LH ghrelin with intra-VTA infusions of orexin-A. Here I show that VTA orexin receptors are required for LV ghrelin to promote feeding. Thus, ghrelin may be exciting LH orexin neurons, thereby increasing VTA orexin release to alter VTA dopamine neuron excitability. However, further experiments are required to determine the precise mechanisms underlying the ability of central ghrelin to augment phasic dopamine release.

F. Endogenous signaling related to physiological state augments phasic dopamine release evoked by food reward

My data indicate that ghrelin can influence phasic dopamine release. Notwithstanding, all of the effects I measured were in response to exogenous pharmacological challenges. Thus, it was unclear whether endogenous signaling related to physiological state would result in measurable differences in phasic dopamine release. I addressed this question by comparing the magnitude of phasic dopamine release evoked by food for all animals that were food-restricted (GHRP experiments) to all *ad libitum* fed animals (LV, LH, and VTA ghrelin experiments) for all trials that occurred before mid-session pharmacological manipulations. This analysis revealed that the magnitude of dopamine release evoked by food was significantly greater in food-restricted animals, thus establishing that natural fluctuations in physiological state are encoded by phasic dopamine release. Given that plasma ghrelin levels increase in response to food-

restriction (Tschöp et al., 2000; Cummings et al., 2004), these data further support the possibility that endogenous fluctuations in ghrelin interact with the mesolimbic system to augment phasic dopamine signaling evoked by food.

Prior to my experiments, microdialysis studies revealed that food intake increased dopamine levels in the NAc over many minutes, and the magnitude of the response was modulated by physiological state (Taber and Fibiger, 1997; Wilson et al., 1995; Ahn and Phillips, 1999; Bassareo and Di Chiara, 1999). More recently, food-restriction was shown to increase burst firing of dopamine neurons in anesthetized mice, suggesting that physiological state may alter phasic dopamine signaling (Branch et al., 2013). However, whether or not physiological state influenced burst firing of dopamine neurons in awake-behaving animals was unknown. These data demonstrate that in addition to altering long term changes in NAc dopamine evoked in response to feeding, endogenous signals related to physiological state also influence brief, phasic dopamine spikes evoked by food. Phasic dopamine spikes play a critical role in aspects of reinforcement, learning, and motivation. Therefore, regulation of food-evoked phasic dopamine release by physiological state may be one way in which hunger influences aspects of reward-directed behavior. Moreover, given that *ad libitum* fed rats exhibited smaller dopamine spikes than hungry rats, future studies should probe whether satiety signals also regulate phasic dopamine release.

G. Central ghrelin increases phasic mesolimbic signaling evoked by food-predictive cues

The ability of environmental cues to promote food intake is attenuated by interference with NAc dopamine receptors or inactivation of the NAc (Floresco et al., 2008; Lex and Hauber, 2008; Guy et al., 2011). In addition, cue-elicited food seeking is attenuated by satiety (Corbit et

al., 2007) as well as ghrelin receptor antagonism (Walker et al., 2012). Moreover, the BOLD response to food cues is diminished following satiety (Dimitropoulos et al., 2012), but the precise neural mechanisms involved were unknown. Similar results have been obtained in studies of drug self-administration. Food-restriction alters the ability of drug-paired cues to promote reinstatement (D'Cunha et al., 2013) and this effect requires NAc D1Rs (Tobin et al., 2013). Importantly, serum ghrelin levels are correlated with drug-seeking induced by cocaine cues (Tessari et al., 2007). Taken together, these data strongly suggest that physiological state 'gates' the ability of reward-paired cues to promote behavior through actions in the mesolimbic system. However, the mechanisms underlying the relationship between physiological state and cue-driven behavior remained unknown. My results strongly support the idea that by increasing ghrelin secretion, hunger potentiates the response of mesolimbic circuitry to motivationally salient cues thereby increasing the probability those cues can invigorate reward-seeking behaviors. These data have big implications for both food and drug seeking as it highlights a potential therapeutic target for preventing maladaptive behavior.

While I recorded from neurons in both the NAc core and shell, I did not compare effects across sub-regions. However, combining data across core and shell should not affect the interpretation of the results. Pharmacological evidence supports a role for NAc core in coordinating goal-directed behaviors, while the shell may suppress task-irrelevant behaviors (Floresco et al., 2008; Blaiss and Janak, 2009). Functional differences between NAc core and shell are supported by electrophysiological data. NAc core neurons respond in greater numbers and with larger increases in activity to a reward-paired cue, whereas more shell neurons respond to an unrewarded cue (Ambroggi et al., 2011). Inactivating vmPFC increases NAc shell activity evoked by an unrewarded cue and behavioral responding to the unrewarded stimulus

(Ghazizadeh et al., 2012). While there are differences in how the core and shell respond to rewarded versus unrewarded stimuli, the magnitude of NAc spiking evoked by a reward-paired cue has been directly linked to the subsequent intensity of approach behaviors (e.g., speed, latency). Critically, the link between approach behavior and NAc spiking evoked by the rewarded cue held when either the core or shell were considered separately (McGinty et al., 2013). Taken together, these studies suggest that increasing the NAc response to a CS+ in either the core or shell, without altering the NAc response to a neutral cue, should facilitate goal-directed behavior.

H. Implications for obesity

In natural foraging environments, regulation of cue and food evoked mesolimbic signals by physiological state would be highly adaptive. This would ensure that in times of caloric need, food consumption would be more effective at reinforcing the specific behaviors that led to the receipt of food. Modulation of cue-evoked mesolimbic signals could facilitate associations between food and stimuli that predict food availability, thus helping an organism more effectively find food in the future. In addition, by regulating cue-evoked signaling to previously learned cues, environmental cues would more effectively promote food seeking. In environments with scarce resources, these processes would help ensure survival by increasing an organism's ability to extract nutrients from the environment. However, in an obesogenic environment, where energy dense foods and associated stimuli are already capable of promoting intake in the absence of caloric need, the ability of hunger to increase the magnitude of mesolimbic signaling could lead to hypersensitivity and therefore promote over consumption.

Phasic dopamine signaling evoked in response to both food and food-predictive cues is sensitive to both caloric content, (Beeler et al., 2012; McCutcheon et al., 2012a) and the magnitude of reward (Tobler et al., 2005; Gan et al., 2010). Moreover, the magnitude of phasic dopamine release is correlated with the intensity of food-seeking actions (Wassum et al. 2012; Wassum et al. 2013). Thus, it is possible that densely caloric foods bias food choice behavior by evoking greater dopamine release than less caloric options. Importantly, youth at risk for obesity show greater striatal activation to receipt of rewarding food (Stice et al., 2011), while greater striatal responses to images of food are associated with future weight gain (Stice et al., 2010b). Thus, increased striatal responses to food as well as food cues are likely related to overconsumption. When considered in conjunction with the effects of ghrelin reported here, it is possible that increased responsivity to calorically dense foods could be further exacerbated when ghrelin levels are high and therefore promote overconsumption.

Overconsumption and the subsequent development of obesity compromise multiple aspects of striatal dopamine signaling. Data from both humans and rodents demonstrate that obesity results in the down-regulation of striatal D2R expression (Huang et al., 2006; Wang et al., 2009b; Johnson and Kenny, 2010). Importantly, the loss of D2Rs may contribute to the development of habitual-like feeding behavior (Johnson and Kenny, 2010). Obesity also impairs aspects of dopamine release. Rats bred for susceptibility to obesity and rats who have developed obesity exhibit widespread deficits in dopamine release in multiple dopamine terminal regions, including the NAc (Geiger et al., 2008, 2009). The dietary effects on dopamine release are probably due to reductions in dopamine synthesis as high fat diet consumption decreases TH expression (Li et al., 2009; Vucetic et al., 2012). Behaviorally, consumption of a high fat diet impairs the locomotor potentiating effects of amphetamine, which is dopamine dependent (Davis

et al., 2008; Speed et al., 2011). In addition to 'online' changes that occur during the onset and establishment of obesity, dietary insults during development can also have widespread and long lasting effects on aspects of dopamine signaling. Early life exposure to a high fat diet causes long-term alterations in biochemical markers of NAc dopamine signaling that last long after cessation of high fat diet consumption (Teegarden et al., 2009). Interestingly, offspring born to a mother who consumed a high fat diet exhibit alterations in TH and D2R expression that last after weaning (Vucetic et al., 2010). Thus, exposure to high fat diet throughout the life span can impair aspects of dopamine release, dopamine synthesis, and dopamine receptor expression. Given the role of the mesolimbic dopamine in motivation and reward-directed behaviors, these insults could contribute to excessive intake and reduced energy expenditure in obese individuals.

There is also considerable support for dietary effects on the DAT. Simply consuming a high fat diet can decrease DAT protein levels and DAT function in the ventral striatum, independent of the development of obesity (Cone et al., 2013). A similar deficit in reuptake and DAT trafficking has been observed following the development of an obese phenotype (Speed et al., 2011). This study found that obesity resulted in dysregulation of Akt signaling which impaired DAT trafficking into the plasma membrane (Speed et al., 2011). The deficits in DAT trafficking and Akt signaling reported are most likely due to insulin resistance specifically within dopamine neurons. Akt is phosphorylated following insulin receptor activation, and similar deficits in dopamine reuptake are observed following the development of peripheral insulin resistance after prolonged exposure to a high-fat diet (Morris et al., 2011). Insulin receptors are expressed in the VTA (Figlewicz et al., 2003) and bath application of insulin onto VTA slices augments DAT trafficking and dopamine reuptake (Mebel et al., 2012). Critically, restoring Akt signaling by virally increasing VTA insulin receptor expression rescues DAT impairments and

reduces hyperphagia (Speed et al., 2011). Thus, once an obese phenotype is established, insensitivity to peripheral hormones and their ability to regulate food intake may engage a feed-forward process that perpetuates further intake.

There is also evidence that obesity effects how the mesolimbic system responds specifically to food and food related cues. Satiety normally reduces the response to food cues in the human caudate, but this effect is absent in obese individuals (Dimitropoulos et al., 2012). Thus, the obese may be incapable of regulating their sensitivity to food cues, which could promote further intake. Moreover, weight gain over a 6-month period is associated with an attenuated striatal response to the receipt of palatable food compared to weight stable individuals (Stice et al., 2010a), suggesting that body weight status modulates food-evoked mesolimbic signaling. Taken together, at a broad level (e.g., fMRI), the mesolimbic response to food and related cues and their sensitivity to physiological state appear to be compromised in obesity. Discovering how these insults occur, and how they contribute to the maintenance of obesity should be the topic of future studies.

Overall, the data from human and rodent subjects supports the idea that the development of obesity correlates with the development of dopamine hypofunction. Suppressing striatal dopamine synthesis or blocking NAc dopamine receptors both impair motivation for food (Szczypka et al., 2001; Salamone and Correa 2012), while elevating striatal dopamine increases motivation (Beeler et al., 2010). These results, along with many others, have led to the hypothesis that modulating dopamine levels may differentiate between exploit versus exploratory foraging strategies (Beeler, 2012). That is, low levels of dopamine favor reduced effort and consumption of low-cost options whereas higher levels of dopamine facilitate high-effort exploratory options. In an obesogenic society, minimal effort is required to obtain and consume

unhealthy food. Notably, the perceived, but not actual, distance between one's home and a supermarket is closely associated with reduced intake of healthy food (Caspi et al., 2012). That is, those who believe that more effort is required to consume healthy food are less likely to eat healthy. Moreover, living near grocery stores lowers obesity risk (Morland et al., 2006). I hypothesize that this relationship stems from the effort needed to travel to access healthy food. Otherwise, the perception of high cost (increased travel and preparation time) can bias food choices towards low-cost unhealthy options. Given the role of dopamine in effort related responding for food, obesity-related insults to the dopamine system may further bias food choices toward low-effort, high calorie options thereby perpetuating the prevalence of the disorder.

CITATIONS

Abbott CR, Monteiro M, Small CJ, Sajedi A, Smith KL, Parkinson JRC, Ghatei MA, Bloom SR. The inhibitory effects of peripheral administration of peptide YY 3-36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res* 1044: 127–131, 2005.

Aberman JE, Ward SJ, Salamone JD. Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. *Pharmacol Biochem Behav* 61: 341–348, 1998.

Abizaid A, Liu Z-WW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschöp MH, Gao XB, Horvath TL. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 116: 3229–3239, 2006.

Adamantidis A, de Lecea L. The hypocretins as sensors for metabolism and arousal. *J Physiol* 587: 33–40, 2009.

Adamantidis A, de Lecea L. Hypocretin/Orexin and MCH and Receptors. In: *Encyclopedia of Neuroscience*. 2010, p. 51–56.

Adamantidis AR, Tsai H-C, Boutrel B, Zhang F, Stuber GD, Budygin EA, Touriño C, Bonci A, Deisseroth K, de Lecea L. Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. *J Neurosci* 31: 10829–10835, 2011.

Ahn S, Phillips AG. Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. *J Neurosci* 19: RC29, 1999.

Alhadeff AL, Rupprecht LE, Hayes MR. GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology* 153: 647–58, 2012.

Allas S, Abribat T. Clinical perspectives for ghrelin-derived therapeutic products. *Endocr. Dev.* 25: 157–166, 2013.

Alvarez-Crespo M, Skibicka KP, Farkas I, Molnár CS, Egecioglu E, Hrabovszky E, Liposits Z, Dickson SL. The Amygdala as a Neurobiological Target for Ghrelin in Rats: Neuroanatomical, Electrophysiological and Behavioral Evidence. *PLoS One* 7(10): e46321, 2012.

Ambroggi F, Ishikawa A, Fields HL, Nicola SM. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron* 59: 648–661, 2008.
Ambroggi F, Ghazizadeh A, Nicola SM, Fields HL. Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. *J Neurosci* 31: 6820–6830, 2011.

Andrews ZB. Central mechanisms involved in the orexigenic actions of ghrelin. *Peptides* 2011.

Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nat Neurosci* 14: 351–355, 2011.

De Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MAL, Simon SA. Food Reward in the Absence of Taste Receptor Signaling. *Neuron* 57: 930–941, 2008.

Aston-Jones G, Smith RJ, Sartor GC, Moorman DE, Massi L, Tahsili-Fahadan P, Richardson KA. Lateral hypothalamic orexin/hypocretin neurons: A role in reward-seeking and addiction. *Brain Res* 1314: 74–90, 2010.

Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. *Nature* 488: 172–177, 2012.

Ayyad C, Andersen T. Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999. *Obes Rev* 1: 113–119, 2000.

Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. *Gastroenterology* 132: 2131–2157, 2007.

Bakshi VP, Kelley AE. Feeding induced by opioid stimulation of the ventral striatum: role of opiate receptor subtypes. *J Pharmacol Exp Ther* 265: 1253–1260, 1993.

Baldo BA, Kelley AE. Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. *Am J Physiol Regul Integr Comp Physiol* 281: R1232–R1242, 2001.

Baldo BA, Kelley AE. Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. *Psychopharmacology (Berl)* 191: 439–459, 2007.

Baldo BA, Sadeghian K, Basso AM, Kelley AE, Maria A. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behav Brain Res* 137: 165–177, 2002.

Balla T. Phosphoinositide-derived messengers in endocrine signaling. *J. Endocrinol.* 188: 135–153, 2006.

Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, Schmauss C, Zakharenko SS, Zablow L, Sulzer D. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron* 42: 653–663, 2004.

Banks WA, Coon AB, Robinson SM, Moinuddin A, Shultz JM, Nakaoke R, Morley JE. Triglycerides Induce Leptin Resistance at the Blood-Brain Barrier. *Diabetes* 53: 1253–1260, 2004.

Banks WA, Tschöp M, Robinson SM, Heiman ML, Op MT. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* 302: 822–827, 2002.

Bass CE, Grinevich VP, Gioia D, Day-Brown JD, Bonin KD, Stuber GD, Weiner JL, Budygin E a. Optogenetic stimulation of VTA dopamine neurons reveals that tonic but not phasic patterns of dopamine transmission reduce ethanol self-administration. *Front Behav Neurosci* 7: 173, 2013.

Bassareo V, Di Chiara G. Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neuroscience* 89: 637–641, 1999.

Beeler JA. Thorndike's law 2.0: Dopamine and the regulation of thrift. *Front. Neurosci.* 2012.

Beeler JA, Daw N, Frazier CRM, Zhuang X. Tonic dopamine modulates exploitation of reward learning. *Front Behav Neurosci* 4: 170, 2010.

Beeler JA, McCutcheon JE, Cao ZFH, Murakami M, Alexander E, Roitman MF, Zhuang X. Taste uncoupled from nutrition fails to sustain the reinforcing properties of food. *Eur J Neurosci* 36: 2533–46, 2012.

Begg DP, Woods SC. The central insulin system and energy balance. *Handb Exp Pharmacol* 209: 111–129, 2012.

Benoit-Marand M, Borrelli E, Gonon F. Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics in vivo. *J Neurosci* 21: 9134–9141, 2001.

Berendse HW, Groenewegen HJ. Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J Comp Neurol* 299: 187–228, 1990.

Berridge KC, Robinson TE. What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* 28: 309–369, 1998.

Berthoud H-R, Münzberg H. The lateral hypothalamus as integrator of metabolic and environmental needs: from electrical self-stimulation to opto-genetics. *Physiol Behav* 104: 29–39, 2011.

Betley JN, Cao ZFH, Ritola KD, Sternson SM. Parallel, redundant circuit organization for homeostatic control of feeding behavior. *Cell* 155: 1337–1350, 2013.

Blaiss CA, Janak PH. The nucleus accumbens core and shell are critical for the expression, but not the consolidation, of Pavlovian conditioned approach. *Behav Brain Res* 200: 22–32, 2009.

Blank T, Nijholt I, Teichert U, Kügler H, Behrsing H, Fienberg A, Greengard P, Spiess J. The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal N-methyl-D-aspartate responses. *Proc Natl Acad Sci U S A* 94: 14859–14864, 1997.

Borgland SL, Storm E, Bonci A. Orexin B/hypocretin 2 increases glutamatergic transmission to ventral tegmental area neurons. *Eur J Neurosci* 28: 1545–56, 2008.

Borgland SL, Taha SA, Sarti F, Fields HL, Bonci A. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* 49: 589–601, 2006.

Borgland SL, Ungless MA, Bonci A. Convergent actions of orexin/hypocretin and CRF on dopamine neurons: Emerging players in addiction. *Brain Res* 1314: 139–144, 2010.

Branch SY, Goertz RB, Sharpe AL, Pierce J, Roy S, Ko D, Paladini C a, Beckstead MJ. Food restriction increases glutamate receptor-mediated burst firing of dopamine neurons. *J Neurosci* 33: 13861–72, 2013.

Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology* 151: 4745–4755, 2010.

Briggs DI, Lockie SH, Wu Q, Lemus MB, Stark R, Andrews ZB. Calorie-restricted weight loss reverses high-fat diet-induced ghrelin resistance, which contributes to rebound weight gain in a ghrelin-dependent manner. *Endocrinology* 154: 709–717, 2013.

Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A. Synaptic and Behavioral Profile of Multiple Glutamatergic Inputs to the Nucleus Accumbens. *Neuron* 76: 790–803, 2012.

Broberger C, Visser TJ, Kuhar MJ, Hökfelt T. Neuropeptide Y innervation and neuropeptide-Y-Y1-receptor-expressing neurons in the paraventricular hypothalamic nucleus of the mouse. *Neuroendocrinology* 70: 295–305, 1999.

Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol* 338: 255–278, 1993.

Bromberg-Martin ES, Matsumoto M, Hikosaka O. Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron* 68: 815–834, 2010.

Brown HD, McCutcheon JE, Cone JJ, Ragozzino ME, Roitman MF. Primary food reward and reward-predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum. *Eur J Neurosci* 34: 1997–2006, 2011.

Burdakov D, Luckman SM, Verkhratsky A. Glucose-sensing neurons of the hypothalamus. *Philos Trans R Soc Lond B Biol Sci* 360: 2227–2235, 2005.

Cabral A, Fernandez G, Perello M. Analysis of brain nuclei accessible to ghrelin present in the cerebrospinal fluid. *Neuroscience* 253: 406–15, 2013.

Cacciapaglia F, Wightman RM, Carelli RM. Rapid Dopamine Signaling Differentially Modulates Distinct Microcircuits within the Nucleus Accumbens during Sucrose-Directed Behavior. *J. Neurosci.* 31: 13860–13869, 2011.

Cachope R, Mateo Y, Mathur BN, Irving J, Wang HL, Morales M, Lovinger DM, Cheer JF. Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: Setting the tone for reward processing. *Cell Rep* 2: 33–41, 2012.

Cador M, Kelley AE, Le Moal M, Stinus L. Ventral tegmental area infusion of substance P, neurotensin and enkephalin: differential effects on feeding behavior. *Neuroscience* 18: 659–669, 1986.

Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC, Weigle DS. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J Clin Endocrinol Metab* 89: 1319–1324, 2004.

Camiña JP, Carreira MC, Messari SE, Llorens-Cortes C, Smith RG, Casanueva FF. Desensitization and Endocytosis Mechanisms of Ghrelin-Activated Growth Hormone Secretagogue Receptor 1a. *Endocrinology* 145: 930–940, 2004.

Carr DB, Sesack SR. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci* 20: 3864–3873, 2000.

Carr KD, Kim GY, Cabeza de Vaca S. Rewarding and locomotor-activating effects of direct dopamine receptor agonists are augmented by chronic food restriction in rats. *Psychopharmacology (Berl)* 154: 420–428, 2001.

Carr KD. Feeding, drug abuse, and the sensitization of reward by metabolic need. *Neurochem Res* 21: 1455–1467, 1996.

Carr KD. Chronic food restriction: Enhancing effects on drug reward and striatal cell signaling. *Physiol Behav* 91: 459–472, 2007.

Cason AM, Smith RJ, Tahsili-Fahadan P, Moorman DE, Sartor GC, Aston-Jones G. Role of orexin/hypocretin in reward-seeking and addiction: Implications for obesity. *Physiol Behav* 100: 419–428, 2010.

Caspi CE, Kawachi I, Subramanian S V., Adamkiewicz G, Sorensen G. The relationship between diet and perceived and objective access to supermarkets among low-income housing residents. *Soc Sci Med* 75: 1254–1262, 2012.

Chao SZ, Ariano MA, Peterson DA, Wolf ME. D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons. *J Neurochem* 83: 704–712, 2002.

Cheer JF, Aragona BJ, Heien MLAV, Seipel AT, Carelli RM, Wightman RM. Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron* 54: 237–44, 2007.

Chen L, Xing T, Wang M, Miao Y, Tang M, Chen J, Li G, Ruan DY. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. *Eur J Neurosci* 33: 266–275, 2011.

Choi B, Schnall PL, Yang H, Dobson M, Landsbergis P, Israel L, Karasek R, Baker D. Sedentary work, low physical job demand, and obesity in US workers. *Am J Ind Med* 53: 1088–1101, 2010.

Chen L, Lodge DJ. The lateral mesopontine tegmentum regulates both tonic and phasic activity of VTA dopamine neurons. *J Neurophysiol* 110: 2287–94, 2013.

Choi DL, Davis JF, Fitzgerald ME, Benoit SC. The role of orexin-A in food motivation, reward-based feeding behavior and food-induced neuronal activation in rats. *Neuroscience* 167: 11–20, 2010.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *J Neurosci* 21: 9471–9477, 2001.

Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI. The dopamine transporter: immunochemical characterization and localization in brain [Online]. *J Neurosci* 15: 1714–1723, 1995.

Ciofi P. The arcuate nucleus as a circumventricular organ in the mouse. *Neurosci Lett* 487: 187–190, 2011.

Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature* 482: 85–88, 2012.

Cone JJ, Chartoff EH, Potter DN, Ebner SR, Roitman MF. Prolonged high fat diet reduces dopamine reuptake without altering DAT gene expression. *PLoS One* 8: e58251, 2013.

Cone JJ, McCutcheon JE, Roitman MF. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *J Neurosci* 34: 4905–13, 2014.

Corbit LH, Balleine BW. The general and outcome-specific forms of pavlovian-instrumental transfer are differentially mediated by the nucleus accumbens core and shell. *J Neurosci* 31: 11786–11794, 2011.

Corbit LH, Janak PH, Balleine BW. General and outcome-specific forms of Pavlovian-instrumental transfer: The effect of shifts in motivational state and inactivation of the ventral tegmental area. *Eur J Neurosci* 26: 3141–3149, 2007.

Corbit LH, Muir JL, Balleine BW. The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. *J Neurosci* 21: 3251–3260, 2001.

Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, Cone RD. Integration of npy, agrp, and melanocortin signals in the hypothalamic paraventricular nucleus: Evidence of a cellular basis for the adipostat. *Neuron* 24: 155–163, 1999.

Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37: 649–661, 2003.

Cragg SJ, Rice ME. DAncing past the DAT at a DA synapse. *Trends Neurosci.* 27: 270–277, 2004.

Cruz CRY, Smith RG, Russell C, Cruz Y. The Growth Hormone Secretagogue Receptor. *Mol Med* 77: 47–88, 2008.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50: 1714–1719, 2001.

Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 287: E297–304, 2004.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 346: 1623–1630, 2002.

Currie J, DellaVigna S, Moretti E, Pathania V. The Effect of Fast Food Restaurants on Obesity and Weight Gain. *Am. Econ. J. Econ. Policy* 2: 32–63, 2010.

D'Agostino AE, Small DM. Neuroimaging the interaction of mind and metabolism in humans. *Mol. Metab.* 1: 10–20, 2012.

D'Cunha TM, Sedki F, MacRi J, Casola C, Shalev U. The effects of chronic food restriction on cue-induced heroin seeking in abstinent male rats. *Psychopharmacology (Berl)* 225: 241–50, 2013.

Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in Ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123: 1120–1128, 2002.

Davis JF, Tracy AL, Schurdak JD, Tschöp MH, Lipton JW, Clegg DJ, Benoit SC. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behav Neurosci* 122: 1257–63, 2008.

Day JJ, Jones JL, Wightman RM, Carelli RM. Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. *Biol Psychiatry* 68: 306–309, 2010.

Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat Neurosci* 10: 1020–8, 2007.

Day JJ, Wheeler RA, Roitman MF, Carelli RM. Nucleus accumbens neurons encode Pavlovian approach behaviors: evidence from an autoshaping paradigm. *Eur J Neurosci* 23: 1341–1351, 2006.

Dayan P, Balleine BW. Reward, motivation, and reinforcement learning. *Neuron* 36: 285–298, 2002.

Delmas P, Coste B, Gamper N, Shapiro MS. Phosphoinositide lipid second messengers: New paradigms for calcium channel modulation. *Neuron* 47: 179–182, 2005.

Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB, Banks WA, Morley JE, Pinto S, Sherwin RS, Xu L, Yamada KA, Sleeman MW, Tschöp MH, Horvath TL. Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* 9: 381–388, 2006.

Dimitropoulos A, Tkach J, Ho A, Kennedy J. Greater corticolimbic activation to high-calorie food cues after eating in obese vs. normal-weight adults. *Appetite* 58: 303–312, 2012.

Domesick VB. Neuroanatomical Organization of Dopamine Neurons in the Ventral Tegmental Area. *Ann NY Acad Life Sci* 537:10-26, 1988.

Domingos AI, Vaynshteyn J, Voss HU, Ren X, Gradinaru V, Zang F, Deisseroth K, de Araujo IE, Friedman J. Leptin regulates the reward value of nutrient. *Nat Neurosci* 14: 1562–8, 2011.

Domingos AI, Sordillo A, Dietrich MO, Liu ZW, Tellez LA, Vaynshteyn J, Ferreira JG, Ekstrand MI, Horvath TL, de Araujo IE, Friedman JM. Hypothalamic melanin concentrating hormone neurons communicate the nutrient value of sugar. *Elife* 2013, 2013.

Dossat AM, Lilly N, Kay K, Williams DL. Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake. *J Neurosci* 31: 14453–7, 2011.

Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JEW, Overton PG, Redgrave P. How visual stimuli activate dopaminergic neurons at short latency. *Science* 307: 1476–1479, 2005.

Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC. Effects of a fixed meal pattern on ghrelin secretion: Evidence for a learned response independent of nutrient status. *Endocrinology* 147: 23–30, 2006.

Drewnowski A, Specter SE. Poverty and obesity: the role of energy density and energy costs. *Am J Clin Nutr* 79: 6–16, 2004.

Dreyer JK, Herrik KF, Berg RW, Hounsgaard JD. Influence of phasic and tonic dopamine release on receptor activation. *J Neurosci* 30: 14273–14283, 2010.

Dumartin B, Doudnikoff E, Gonon F, Bloch B. Differences in ultrastructural localization of dopaminergic D1 receptors between dorsal striatum and nucleus accumbens in the rat. *Neurosci Lett* 419: 273–277, 2007.

Dunah AW, Standaert DG. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *J Neurosci* 21: 5546–5558, 2001.

English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH. Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab* 87: 2984–2987, 2002.

España RA, Oleson EB, Locke JL, Brookshire BR, Roberts DCS, Jones SR. The hypocretin-orexin system regulates cocaine self-administration via actions on the mesolimbic dopamine system. *Eur J Neurosci* 31: 336–48, 2010.

España RA, Melchior JR, Roberts DCS, Jones SR. Hypocretin 1/orexin A in the ventral tegmental area enhances dopamine responses to cocaine and promotes cocaine self-administration. *Psychopharmacology (Berl)* 214: 415–426, 2011.

Fadel J, Deutch AY. Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* 111: 379–387, 2002.

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165–168, 1997.

Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. 2007.

Ferriday D, Brunstrom JM. How does food-cue exposure lead to larger meal sizes? *Br J Nutr* 100: 1325–1332, 2008.

Ferrini F, Salio C, Lossi L, Merighi A. Ghrelin in central neurons. *Curr Neuropharmacol* 7: 37–49, 2009.

Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res* 964: 107–15, 2003.

Finger BC, Dinan TG, Cryan JF. Diet-induced obesity blunts the behavioural effects of ghrelin: Studies in a mouse-progressive ratio task. *Psychopharmacology (Berl)* 220: 173–181, 2012.

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers C a, Clinton SM, Phillips PEM, Akil H. A selective role for dopamine in stimulus-reward learning. *Nature* 469: 53–7, 2011.

Floresco SB, McLaughlin RJ, Haluk DM. Opposing roles for the nucleus accumbens core and shell in cue-induced reinstatement of food-seeking behavior. *Neuroscience* 154: 877–884, 2008.

Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, Gaylinn BD, Thorner MO, Cummings DE. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab* 93: 1971–1979, 2008.

Frank LD, Saelens BE, Powell KE, Chapman JE. Stepping towards causation: Do built environments or neighborhood and travel preferences explain physical activity, driving, and obesity? *Soc Sci Med* 65: 1898–1914, 2007.

Fulton S, Pissios P, Manchon RPP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51: 811–822, 2006.

Futami T, Takakusaki K, Kitai ST. Glutamatergic and cholinergic inputs from the pedunculopontine tegmental nucleus to dopamine neurons in the substantia nigra pars compacta. *Neurosci Res* 21: 331–342, 1995.

Gan JO, Walton ME, Phillips PEM. Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. *Nat Neurosci* 13: 25–27, 2010.

Garris PA, Ciolkowski EL, Pastore P, Wightman RM. Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. *J Neurosci* 14: 6084–6093, 1994.

Gautier JF, Chen K, Salbe AD, Bandy D, Pratley RE, Heiman M, Ravussin E, Reiman EM, Tataranni PA. Differential brain responses to satiation in obese and lean men. *Diabetes* 49: 838–846, 2000.

Gehlert DR. Role of hypothalamic neuropeptide Y in feeding and obesity. *Neuropeptides* 33: 329–338, 1999.

Geiger BM, Behr GG, Frank LE, Caldera-Siu AD, Beinfeld MC, Kokkotou EG, Pothos EN. Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. *FASEB J* 22: 2740–6, 2008.

Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience* 159: 1193–9, 2009.

Geisler S, Zahm DS. Neurotensin afferents of the ventral tegmental area in the rat: [1] Re-examination of their origins and [2] responses to acute psychostimulant and antipsychotic drug administration. *Eur J Neurosci* 24: 116–134, 2006.

Geisler S, Derst C, Veh RW, Zahm DS. Glutamatergic afferents of the ventral tegmental area in the rat. *J Neurosci* 27: 5730–5743, 2007.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Sibley DR. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250: 1429–1432, 1990.

Ghazizadeh A, Ambroggi F, Odean N, Fields HL. Prefrontal Cortex Mediates Extinction of Responding by Two Distinct Neural Mechanisms in Accumbens Shell. *J. Neurosci.* 32: 726–737, 2012.

Gibbs J, Fauser DJ, Rowe EA, Rolls BJ, Rolls ET, Maddison SP. Bombesin suppresses feeding in rats. *Nature* 282: 208–210, 1979.

Gibbs J, Smith GP. Satiety: the roles of peptides from the stomach and the intestine. *Fed Proc* 45: 1391–1395, 1986.

Glimcher PW, Giovino AA, Hoebel BG. Neurotensin self-injection in the ventral tegmental area. *Brain Res* 403: 147–150, 1987.

Gore BB, Zweifel LS. Genetic reconstruction of dopamine d1 receptor signaling in the nucleus accumbens facilitates natural and drug reward responses. *J Neurosci* 33: 8640–9, 2013.

Goto Y, O'Donnell P. Network synchrony in the nucleus accumbens in vivo. *J Neurosci* 21: 4498–4504, 2001.

Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: single spike firing. *J Neurosci* 4: 2866–2876, 1984.

Grace AA, Floresco SB, Goto Y, Lodge DJ. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci.* 30: 220–227, 2007.

Green DM, Swets JA. Signal detection theory and psychophysics. *New York: Wiley* 1: 521, 1966.

Greenman Y, Kuperman Y, Drori Y, Asa SL, Navon I, Forkosh O, Gil S, Stern N, Chen A. Postnatal ablation of POMC neurons induces an obese phenotype characterized by decreased food intake and enhanced anxiety-like behavior. *Mol Endocrinol* 27: 1091–102, 2013.

Groenewegen HJ, Berendse HW, Meredith GE, Haber SN, Voorn P, Wolters JG, Lohman AHM. Functional anatomy of the ventral, limbic system-innervated striatum. In: *The mesolimbic dopamine system: from motivation to action*. 1991, p. 19–59.

Groenewegen HJ, Wright CI, Beijer AVJ, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. In: *Annals of the New York Academy of Sciences*. 1999, p. 49–63.

Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, Brüning JC. Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci* 8: 1289–1291, 2005.

Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, Witcher DR, Luo S, Onyia JE, Hale JE. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A* 105: 6320–6325, 2008.

Guy EG, Choi E, Pratt WE. Nucleus accumbens dopamine and mu-opioid receptors modulate the reinstatement of food-seeking behavior by food-associated cues. *Behav Brain Res* 219: 265–272, 2011.

Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci U S A* 96: 10911–10916, 1999.

Hajnal A, Smith GP, Norgren R. Oral sucrose stimulation increases accumbens dopamine in the rat. *Am J Physiol Regul Integr Comp Physiol* 286: R31–R37, 2004.

Hallett PJ, Spoelgen R, Hyman BT, Standaert DG, Dunah AW. Dopamine D1 activation potentiates striatal NMDA receptors by tyrosine phosphorylation-dependent subunit trafficking. *J Neurosci* 26: 4690–4700, 2006.

Hammond RA, Levine R. The economic impact of obesity in the United States. *Diabetes Metab Syndr Obes* 3: 285–295, 2010.

Han XF, Zhu YL, Hernandez M, Keating DJ, Chen C. Ghrelin reduces voltage-gated potassium currents in GH3 cells via cyclic GMP pathways. *Endocrine* 28: 217–224, 2005.

Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437: 556–559, 2005.

Harris JL, Bargh JA, Brownell KD. Priming effects of television food advertising on eating behavior. *Health Psychol* 28: 404–413, 2009.

Heien MLAV, Johnson MA, Wightman RM. Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Anal Chem* 76: 5697–704, 2004.

Hernandez L, Hoebel BG. Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. *Physiol Behav* 44: 599–606, 1988.

Hernández-López S, Bargas J, Surmeier DJ, Reyes A, Galarraga E. D1 receptor activation enhances evoked discharge in neostriatal medium spiny neurons by modulating an L-type Ca2+ conductance. *J Neurosci* 17: 3334–3342, 1997.

Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H, Surmeier DJ. D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin- signaling cascade [Online]. *J Neurosci* 20: 8987–8995, 2000. 11124974.

Herrington J, Hille B. Growth hormone-releasing hexapeptide elevates intracellular calcium in rat somatotropes by two mechanisms. *Endocrinology* 135: 1100–1108, 1994.

Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI. Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. *J Neurosci* 15: 5222–5237, 1995.

Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI. Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J Comp Neurol* 388: 211–227, 1997.

Hewson AK, Dickson SL. Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J Neuroendocrinol* 12: 1047–1049, 2000.

Hidekitamura JK, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 141: 4797–4800, 2000.

Higley MJ, Sabatini BL. Competitive regulation of synaptic Ca2+ influx by D2 dopamine and A2A adenosine receptors. *Nat Neurosci* 13: 958–966, 2010.

Holland PC, Petrovich GD, Gallagher M. The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats. *Physiol Behav* 76: 117–129, 2002.

Holst B, Holliday ND, Bach A, Elling CE, Cox HM, Schwartz TW. Common structural basis for constitutive activity of the ghrelin receptor family. *J Biol Chem* 279: 53806–53817, 2004.

Hommel JD, Trinko R, Sears RM, Georgescu D, Liu Z-WW, Gao X-BB, Thurmon JJ, Marinelli M, DiLeone RJ. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51: 801–810, 2006.

Hopf FW, Cascini MG, Gordon AS, Diamond I, Bonci A. Cooperative activation of dopamine D1 and D2 receptors increases spike firing of nucleus accumbens neurons via G-protein betagamma subunits. *J Neurosci* 23: 5079–5087, 2003.

Hosp JA, Pekanovic A, Rioult-Pedotti MS, Luft AR. Dopaminergic projections from midbrain to primary motor cortex mediate motor skill learning. *J Neurosci* 31: 2481–2487, 2011.

Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273: 974–977, 1996.

Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 289: 1785–1791, 2003.

Huo L, Gamber K, Greeley S, Silva J, Huntoon N, Leng XH, Bjørbæk C. Leptin-Dependent Control of Glucose Balance and Locomotor Activity by POMC Neurons. *Cell Metab* 9: 537–547, 2009.

Huang X-FFX, Zavitsanou K, Yu Y, Wang H, Chen F, Lawrence AJ, Deng C. Dopamine transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-induced obesity. *Behav Brain Res* 175: 415–419, 2006.

Hyland BI, Reynolds JNJ, Hay J, Perk CG, Miller R. Firing modes of midbrain dopamine cells in the freely moving rat. *Neuroscience* 114: 475–492, 2002.

Ikemoto S, Glazier BS, Murphy JM, McBride WJ. Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *J Neurosci* 17: 8580–8587, 1997.

Ikemoto S. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* 56: 27–78, 2007.

Ikemoto S. Brain reward circuitry beyond the mesolimbic dopamine system: A neurobiological theory. *Neurosci Biobehav Rev* 35: 129–150, 2010.

Ilango A, Kesner AJ, Broker CJ, Wang D V, Ikemoto S. Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: parametric and reinforcement-schedule analyses. *Front Behav Neurosci* 8: 155, 2014.

Imeryüz N, Yeğen BC, Bozkurt A, Coşkun T, Villanueva-Peñacarrillo ML, Ulusoy NB. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol* 273: G920–G927, 1997.

James MH, Charnley JL, Levi EM, Jones E, Yeoh JW, Smith DW, Dayas C V. Orexin-1 receptor signalling within the ventral tegmental area, but not the paraventricular thalamus, is critical to regulating cue-induced reinstatement of cocaine-seeking. *Int J Neuropsychopharmacol* 14: 684–690, 2011.

Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice : implications for its involvement in brain reward. *Addict Biol* 11: 45–54, 2006.

Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JAJA. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol* 12: 6–16, 2007.

Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict Biol* 13: 358–363, 2008.

Jerlhag E, Janson AC, Waters S, Engel JA. Concomitant Release of Ventral Tegmental Acetylcholine and Accumbal Dopamine by Ghrelin in Rats. *PLoS One* 7, 2012.

Johnson AW, Gallagher M, Holland PC. The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. *J Neurosci* 29: 696–704, 2009.

Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci* 13: 635–41, 2010.

Jones JL, Day JJ, Aragona BJ, Wheeler RA, Wightman RM, Carelli RM. Basolateral Amygdala Modulates Terminal Dopamine Release in the Nucleus Accumbens and Conditioned Responding. *Biol Psychiatry* 67: 737–744, 2010a.

Jones JL, Day JJ, Wheeler RA, Carelli RM. The basolateral amygdala differentially regulates conditioned neural responses within the nucleus accumbens core and shell. *Neuroscience* 169: 1186–1198, 2010b.

Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinck JW, Taborsky GJ, Porte D. Evidence of cosecretion of islet amyloid polypeptide and insulin bv ??-cells. *Diabetes* 39: 634–638, 1990.

Kanoski SE, Fortin SM, Ricks KM, Grill HJ. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol Psychiatry* 73: 915–923, 2012.

Katsuki A, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K, Yano Y, Adachi Y, Sumida Y. Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 151: 573–577, 2004.

Kelley AE, Domesick VB, Nauta WJH. The amygdalostriatal projection in the rat. An anatomical study by anterograde and retrograde tracing methods. *Neuroscience* 7: 615–630, 1982.

Kelley AE, Swanson CJ. Feeding induced by blockade of AMPA and kainate receptors within the ventral striatum: A microinfusion mapping study. *Behav Brain Res* 89: 107–113, 1997.

Kelley AE. Ventral striatal control of appetitive motivation: Role in ingestive behavior and reward-related learning. In: *Neuroscience and Biobehavioral Reviews*. 2004, p. 765–776.

Kempadoo KA, Tourino C, Cho SL, Magnani F, Leinninger G-M, Stuber GD, Zhang F, Myers MG, Deisseroth K, de Lecea L, Bonci A. Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. *J Neurosci* 33: 7618–26, 2013.

Kennedy RT, Jones SR, Wightman RM. Dynamic observation of dopamine autoreceptor effects in rat striatal slices. *J Neurochem* 59: 449–455, 1992.

Kenny PJ. Reward Mechanisms in Obesity: New Insights and Future Directions. *Neuron* 69: 664–679, 2011.

Kim J, Nakajima K, Oomura Y, Wayner MJ, Sasaki K. Electrophysiological effects of ghrelin on pedunculopontine tegmental neurons in rats: An in vitro study. *Peptides* 30: 745–757, 2009.

King SJ, Isaacs AM, O'Farrell E, Abizaid A, O'Farrell E. Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. *Horm Behav* 60: 572–80, 2011.

Koch M, Schmid A, Schnitzler H-U. Role of nucleus accumbens dopamine D 1 and D 2 receptors in instrumental and Pavlovian paradigms of conditioned reward. *Psychopharmacology (Berl).* 152: 67–73, 2000.

Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 52: 948–956, 2003.

Kohno D, Yada T. Arcuate NPY neurons sense and integrate peripheral metabolic signals to control feeding. *Neuropeptides* 46: 315–319, 2012.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660, 1999.

Kolotkin RL, Meter K, Williams GR. Quality of life and obesity. *Obes Rev* 2: 219–229, 2001.

Koob GF, Riley SJ, Smith SC, Robbins TW. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *J Comp Physiol Psychol* 92: 917–927, 1978.

Koordeman R, Anschutz DJ, van Baaren RB, Engels RCME. Exposure to soda commercials affects sugar-sweetened soda consumption in young women. An observational experimental study. *Appetite* 54: 619–622, 2010.

Kopelman P. Health risks associated with overweight and obesity. *Obes Rev* 8 Suppl 1: 13–17, 2007.

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J Neurosci* 23: 7–11, 2003.

Krashes MJ, Shah BP, Madara JC, Olson DP, Strochlic DE, Garfield AS, Vong L, Pei H, Watabe-Uchida M, Uchida N, Liberles SD, Lowell BB. An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. *Nature* 507: 238–42, 2014.

Krause M, German PW, Taha SA, Fields HL. A pause in nucleus accumbens neuron firing is required to initiate and maintain feeding. *J Neurosci* 30: 4746–4756, 2010.

Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci* 15: 4–7, 2012.

Kroemer NB, Krebs L, Kobiella A, Grimm O, Pilhatsch M, Bidlingmaier M, Zimmermann US, Smolka MN. Fasting levels of ghrelin covary with the brain response to food pictures. *Addict Biol* 18: 855–862, 2013.

Labouèbe G, Liu S, Dias C, Zou H, Wong JCY, Karunakaran S, Clee SM, Phillips AG, Boutrel B, Borgland SL. Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. *Nat Neurosci* 16: 300–8, 2013.

Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J. Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* 57: 760–773, 2008.

Lammel S, Ion DI, Roeper J, Malenka RC. Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. *Neuron* 70: 855–862, 2011.

Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC. Input-specific control of reward and aversion in the ventral tegmental area. *Nature* 2012.

Lawrence CB, Snape AC, Baudoin FM-HH, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* 143: 155–62, 2002.

de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG, Ecea LDEL, Ilduff TSK, Eyron CP, Ao XG, Oye PEF, Anielson PED, Ukuhara CF. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 95: 322–327, 1998.

Legradi G, Lechan RM, Légrádi G. The arcuate nucleus is the major source for neuropeptide Y-innervation of thyrotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. [Online]. *Endocrinology* 139: 3262–70, 1998.

Leibowitz SF, Hammer NJ, Chang K. Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. *Physiol Behav* 27: 1031–1040, 1981.

Leidy HJ, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL, Williams NI. Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. In: *Journal of Clinical Endocrinology and Metabolism*. 2004, p. 2659–2664.

Lenoir M, Serre F, Cantin L, Ahmed SH. Intense sweetness surpasses cocaine reward. *PLoS One* 2(8): e698, 2007.

Lesser LI, Zimmerman FJ, Cohen D a. Outdoor advertising, obesity, and soda consumption: a cross-sectional study. *BMC Public Health* 13: 20, 2013.

Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, Price DL, Maggio R, Brann MR, Ciliax BJ. Localization of D1and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc Natl Acad Sci USA* 90: 8861–8865, 1993.

Levin BE, Dunn-Meynell AA, Routh VH. Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *Am J Physiol* 276: R1223–R1231, 1999.

Levin BE, Kang L, Sanders NM, Dunn-Meynell AA. Role of Neuronal Glucosensing in the Regulation of Energy Homeostasis. *Diabetes* 55: S122–S130, 2006.

Lex A, Hauber W. Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. *Learn Mem* 15: 483–491, 2008.

Liang N-C, Hajnal A, Norgren R. Sham feeding corn oil increases accumbens dopamine in the rat. *Am J Physiol Regul Integr Comp Physiol* 291: R1236–R1239, 2006.

Li Y, South T, Han M, Chen J, Wang R, Huang X-FF. High-fat diet decreases tyrosine hydroxylase mRNA expression irrespective of obesity susceptibility in mice. *Brain Res* 1268: 181–9, 2009.

Lindgren N, Xu ZQ, Herrera-Marschitz M, Haycock J, Hökfelt T, Fisone G. Dopamine D(2) receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. *Eur J Neurosci* 13: 773–780, 2001.

Lodge DJ, Grace AA. The hippocampus modulates dopamine neuron responsivity by regulating the intensity of phasic neuron activation. *Neuropsychopharmacology* 31: 1356–1361, 2006a.

Lodge DJ, Grace AA. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *Proc Natl Acad Sci U S A* 103: 5167–5172, 2006b.

Lovibond PF. Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. *J Exp Psychol Anim Behav Process* 9: 225–247, 1983.

Lu XY, Ghasemzadeh MB, Kalivas PW. Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* 82: 767–780, 1997.

Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310: 683–685, 2005.

Lutz TA. Control of energy homeostasis by amylin. *Cell Mol Life Sci* 69: 1947–65, 2012.

Madden CJ, Morrison SF. Neurons in the paraventricular nucleus of the hypothalamus inhibit sympathetic outflow to brown adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 296: R831–R843, 2009.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155–1161, 1995.

Mahler S V., Aston-Jones GS. Fos Activation of Selective Afferents to Ventral Tegmental Area during Cue-Induced Reinstatement of Cocaine Seeking in Rats. *J Neurosci* 32: 13309–13325, 2012.

Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. *Cell Metab* 7: 400–409, 2008.

Mangiavacchi S, Wolf ME. D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. *J Neurochem* 88: 1261–1271, 2004.

Margules DL, Olds J. Identical “feeding” and “rewarding” systems in the lateral hypothalamus of rats. *Science (80-)* 135: 374–375, 1962.

Matheny M, Strehler KYE, King M, Tümer N, Scarpace PJ. Targeted leptin receptor blockade: role of ventral tegmental area and nucleus of the solitary tract leptin receptors in body weight homeostasis. *J Endocrinol* 222: 27–41, 2014.

Matsuda W, Furuta T, Nakamura KC, Hioki H, Fujiyama F, Arai R, Kaneko T. Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *J Neurosci* 29: 444–453, 2009.

Matsumoto M, Hikosaka O. Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* 459: 837–841, 2009.

McCann B, Frank L. Driving, Walking, and Where You Live: Links to Obesity. *TDM Rev* 13: p 16, 2005.

McCutcheon JE, Beeler JA, Roitman MF. Sucrose-predictive cues evoke greater phasic dopamine release than saccharin-predictive cues. *Synapse* 66: 346–51, 2012a.

McCutcheon JE, Conrad KL, Carr SB, Ford KA, McGehee DS, Marinelli M. Dopamine neurons in the ventral tegmental area fire faster in adolescent rats than in adults. *J. Neurophysiol.* (2012b).

McGinty VB, Lardeux S, Taha SA, Kim JJ, Nicola SM. Invigoration of reward seeking by cue and proximity encoding in the nucleus accumbens. *Neuron* 78: 910–922, 2013.

Mebel DM, Wong JCY, Dong YJ, Borgland SL. Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. *Eur J Neurosci* 36: 2336–2346, 2012.

Melis MR, Mascia MS, Succu S, Torsello A, Muller EE, Deghenghi R, Argiolas A. Ghrelin injected into the paraventricular nucleus of the hypothalamus of male rats induces feeding but not penile erection. *Neurosci. Lett.* 329: 339–343, 2002.

Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol* 403: 261–80, 1999.

Meredith GE, Agolia R, Arts MPM, Groenewegen HJ, Zahm DS. Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. *Neuroscience* 50: 149–162, 1992.

Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE. The structural basis for mapping behavior onto the ventral striatum and its subdivisions. *Brain Struct Funct* 213: 17–27, 2008.

Merkestein M, Brans MAD, Luijendijk MCM, de Jong JW, Egecioglu E, Dickson SL, Adan RAH. Ghrelin Mediates Anticipation to a Palatable Meal in Rats. *Obesity* 20: 963–971, 2012.

Mesaros A, Koralov SB, Rother E, Wunderlich FT, Ernst MB, Barsh GS, Rajewsky K, Brüning JC. Activation of Stat3 Signaling in AgRP Neurons Promotes Locomotor Activity. *Cell Metab* 7: 236–248, 2008.

Mietlicki-Baase EG, Reiner DJ, Cone JJ, Olivos DR, McGrath LE, Zimmer DJ, Roitman MF, Hayes MR. Amylin Modulates the Mesolimbic Dopamine System to Control Energy Balance. *Neuropsychopharmacology* (2014).

Mietlicki-Baase EG, Rupprecht LE, Olivos DR, Zimmer DJ, Alter MD, Pierce RC, Schmidt HD, Hayes MR. Amylin receptor signaling in the ventral tegmental area is physiologically relevant for the control of food intake. *Neuropsychopharmacology* 38: 1685–97, 2013.

Mink M, Evans A, Moore CG, Calderon KS, Deger S. Nutritional imbalance endorsed by televised food advertisements. *J Am Diet Assoc* 110: 904–910, 2010.

Mirenowicz J, Schultz W. Importance of unpredictability for reward responses in primate dopamine neurons. *J Neurophysiol* 72: 1024–1027, 1994.

Mirenowicz J, Schultz W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379: 449–451, 1996.

Mizuno T, Schmauss C, Rayport S. Distinct roles of presynaptic dopamine receptors in the differential modulation of the intrinsic synapses of medium-spiny neurons in the nucleus accumbens. *BMC Neurosci* 8: 8, 2007.

Mogenson GJ, Jones DL, Yim CY. From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* 14: 69–97, 1980.

Morland K, Diez Roux A V., Wing S. Supermarkets, other food stores, and obesity: The Atherosclerosis Risk in Communities Study. *Am J Prev Med* 30: 333–339, 2006.

Moorman DE, Aston-Jones G. Orexin/hypocretin modulates response of ventral tegmental dopamine neurons to prefrontal activation: diurnal influences. *J Neurosci* 30: 15585–15599, 2010.

Morris JK, Bomhoff GL, Gorres BK, Davis VA, Kim J, Lee P-PP, Brooks WM, Gerhardt GA, Geiger PC, Stanford JA. Insulin resistance impairs nigrostriatal dopamine function. *Exp Neurol* 231: 171–80, 2011.

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature* 443: 289–95, 2006.

Morton GJ, Schwartz MW. The NPY/AgRP neuron and energy homeostasis. *Int J Obes Relat Metab Disord* 25 Suppl 5: S56–S62, 2001.

Münzberg H, Björnholm M, Bates SH, Myers MG. Leptin receptor action and mechanisms of leptin resistance. *Cell. Mol. Life Sci.* 62: 642–652, 2005.

Muschamp JW, Hollander J a, Thompson JL, Voren G, Hassinger LC, Onvani S, Kamenecka TM, Borgland SL, Kenny PJ, Carlezon W a. Hypocretin (orexin) facilitates reward by attenuating the antireward effects of its cotransmitter dynorphin in ventral tegmental area. *Proc Natl Acad Sci U S A* 111: E1648–55, 2014.

Myers MG, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 70: 537–556, 2008.

Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* 873: 181–187, 2000.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 409: 194–198, 2001.

Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 26: 2274–2279, 2005.

Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. *J Recept Signal Transduct Res* 24: 165–205, 2004.

Nicola SM, Yun IA, Wakabayashi KT, Fields HL. Cue-evoked firing of nucleus accumbens neurons encodes motivational significance during a discriminative stimulus task. *J Neurophysiol* 91: 1840–1865, 2004.

Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 311: 806–814, 2014.

Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278: 135–138, 1997.

Olszewski PK, Grace MK, Billington CJ, Levine AS. Hypothalamic paraventricular injections of ghrelin: Effect on feeding and c-Fos immunoreactivity. *Peptides* 24: 919–923, 2003a.

Olszewski PK, Li D, Grace MK, Billington CJ, Kotz CM, Levine AS. Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* 24: 597–602, 2003b.

Ott V, Friedrich M, Zemlin J, Lehnert H, Schultes B, Born J, Hallschmid M. Meal anticipation potentiates postprandial ghrelin suppression in humans. *Psychoneuroendocrinology* 37: 1096–1100, 2012.

Owen N, Sparling PB, Healy GN, Dunstan DW, Matthews CE. Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin Proc* 85: 1138–41, 2010.

Owesson-White CA, Roitman MF, Sombers LA, Belle AM, Keithley RB, Peele JL, Carelli RM, Wightman RM. Sources contributing to the average extracellular concentration of dopamine in the nucleus accumbens. *J Neurochem* 121: 252–262, 2012.

Paeratakul S, Ferdinand DP, Champagne CM, Ryan DH, Bray GA. Fast-food consumption among US adults and children: Dietary and nutrient intake profile. *J Am Diet Assoc* 103: 1332–1338, 2003.

Palyha OC, Feighner SD, Tan CP, McKee KK, Hreniuk DL, Gao YD, Schleim KD, Yang L, Morriello GJ, Nargund R, Patchett AA, Howard AD, Smith RG. Ligand activation domain of human orphan growth hormone (GH) secretagogue receptor (GHS-R) conserved from Pufferfish to humans. *Mol Endocrinol* 14: 160–169, 2000.

Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, Morisset S, Nivot S, Vie-Luton M-P, Grouselle D, de Kerdanet M, Kadiri A, Epelbaum J, Le Bouc Y, Amselem S. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest* 116: 760–768, 2006.

Parton LE, Ye CP, Coppari R, Enriori PJ, Choi B, Zhang C-Y, Xu C, Vianna CR, Balthasar N, Lee CE, Elmquist JK, Cowley MA, Lowell BB. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature* 449: 228–232, 2007.

Paxinos G, Watson C *The rat brain in stereotaxic coordinates.* London: Elsevier Academic, 2007.

Peciña S, Berridge KC. Opioid site in nucleus accumbens shell mediates eating and hedonic "liking" for food: Map based on microinjection Fos plumes. *Brain Res.* 863: 71–86, 2000.

Peciña S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J Neurosci* 25: 11777–11786, 2005.

Pei H, Sutton AK, Burnett KH, Fuller PM, Olson DP. AVP neurons in the paraventricular nucleus of the hypothalamus regulate feeding. *Mol Metab* 3: 209–215, 2014.

Perello M, Sakata I, Birnbaum S, Chuang J-CC, Osborne-Lawrence S, Rovinsky SA, Woloszyn J, Yanagisawa M, Lutter M, Zigman JM. Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol Psychiatry* 67: 880–6, 2010.

Perez MF, White FJ, Hu X-TT. Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *J Neurophysiol* 96: 2217–2228, 2006.

Perreault M, Istrate N, Wang L, Nichols AJ, Tozzo E, Stricker-Krongrad A. Resistance to the orexigenic effect of ghrelin in dietary-induced obesity in mice: reversal upon weight loss. *Int J Obes Relat Metab Disord* 28: 879–885, 2004.

Perry ML, Leinninger GM, Chen R, Luderman KD, Yang H, Gnegy ME, Myers MG, Kennedy RT. Leptin promotes dopamine transporter and tyrosine hydroxylase activity in the nucleus accumbens of Sprague-Dawley rats. *J Neurochem* 114: 666–674, 2010.

Petrovich GD. Forebrain networks and the control of feeding by environmental learned cues. *Physiol. Behav.* 121: 10–18, 2013.

Peyron C, Tighe DK, van den Pol a N, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. [Online]. *J Neurosci* 18: 9996–10015, 1998.

Phillips, Paul EM, Stuber GD, Phillips PEMP, Stuber GDG, Heien MLAVM, Wightman RM, Carelli RM. Subsecond dopamine release promotes cocaine seeking. *Nature* 422: 614–618, 2003.

Piernas C, Popkin BM. Trends in snacking among U.S. children. *Health Aff* 29: 398–404, 2010a.

Piernas C, Popkin BM. Snacking increased among U.S. adults between 1977 and 2006. *J Nutr* 140: 325–332, 2010b.

Podda M V., Riccardi E, D'Ascenzo M, Azzena GB, Grassi C. Dopamine D1-like receptor activation depolarizes medium spiny neurons of the mouse nucleus accumbens by inhibiting inwardly rectifying K+ currents through a cAMP-dependent protein kinase A-independent mechanism. *Neuroscience* 167: 678–690, 2010.

Popkin BM, Nielsen SJ. The sweetening of the world's diet. *Obes Res* 11: 1325–1332, 2003.

Porter-Stransky KA, Seiler JL, Day JJ, Aragona BJ. Development of behavioral preferences for the optimal choice following unexpected reward omission is mediated by a reduction of D2-like receptor tone in the nucleus accumbens. *Eur J Neurosci* 38: 2572–2588, 2013.

Pothos EN, Przedborski S, Davila V, Schmitz Y, Sulzer D. D2-Like dopamine autoreceptor activation reduces quantal size in PC12 cells. *J Neurosci* 18: 5575–5585, 1998.

Pöykkö SM, Kellokoski E, Hörkkö S, Kauma H, Kesäniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. *Diabetes* 52: 2546–2553, 2003.

Prevention CDC. Trends in intake of energy and macronutrients - United States, 1971-2000. *Morb Mortal Wkly Rep* 53: 80–82, 2004.

Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, Guan X, Yu H, Shen Z, Feng Y, Frazier E, Chen A, Camacho RE, Shearman LP, Gopal-Truter S, MacNeil DJ, Van der Ploeg LHT, Marsh DJ. Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol Cell Biol* 22: 5027–5035, 2002.

Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 380: 243–247, 1996.

Quarta D, Di Francesco C, Melotto S, Mangiarini L, Heidbreder C, Hedou G. Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. *Neurochem Int* 54: 89–94, 2009.

Ragsdale CW, Graybiel AM. Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat. *J Comp Neurol* 269: 506–522, 1988.

Ren X, Ferreira JG, Zhou L, Shammah-Lagnado SJ, Yeckel CW, de Araujo IE. Nutrient selection in the absence of taste receptor signaling. *J Neurosci* 30: 8012–23, 2010.

Rice ME. Distinct regional differences in dopamine-mediated volume transmission. In: *Progress in Brain Research*. 2000, p. 277–290.

Richard JM, Castro DC, DiFeliceantonio AG, Robinson MJF, Berridge KC. Mapping brain circuits of reward and motivation: In the footsteps of Ann Kelley. *Neurosci. Biobehav. Rev.* 37: 1919–1931, 2013.

Ritter S, Li AJ, Wang Q, Dinh TT. Minireview: The value of looking backward: The essential role of the hindbrain in counterregulatory responses to glucose deficit. *Endocrinology* 152: 4019–4032, 2011.

Roesch MR, Calu DJ, Schoenbaum G. Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. *Nat Neurosci* 10: 1615–1624, 2007.

Roitman JD, Loriaux AL. Nucleus accumbens responses differentiate execution and restraint in reward-directed behavior. *J Neurophysiol* 111: 350–60, 2014.

Roitman MF, Stuber GD, Phillips PEM, Wightman RM, Carelli RM. Dopamine operates as a subsecond modulator of food seeking. *J Neurosci* 24: 1265–71, 2004.

Roitman MF, Wheeler RA, Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron* 45: 587–97, 2005.

Roitman MF, Van Dijk G, Thiele TE, Bernstein IL. Dopamine mediation of the feeding response to violations of spatial and temporal expectancies. *Behav Brain Res* 122: 193–199, 2001.

Rolls BJ, Roe LS, Meengs JS. Larger portion sizes lead to a sustained increase in energy intake over 2 days. *J Am Diet Assoc* 106: 543–549, 2006.

Rolls BJ, Roe LS, Meengs JS. The effect of large portion sizes on energy intake is sustained for 11 days. *Obesity (Silver Spring)* 15: 1535–1543, 2007.

Rolls ET, Murzi E, Yaxley S, Thorpe SJ, Simpson SJ. Sensory-specific satiety: food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. *Brain Res* 368: 79–86, 1986.

Rorsman P, Braun M. Regulation of insulin secretion in human pancreatic islets. *Annu Rev Physiol* 75: 155–79, 2013.

Le Roux CW, Neary NM, Halsey TJ, Small CJ, Martinez-Isla AM, Ghatei MA, Theodorou NA, Bloom SR. Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. 2005a.

Le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. *J Clin Endocrinol Metab* 90: 1068–1071, 2005b.

Rüter J, Kobelt P, Tebbe JJ, Avsar Y, Veh R, Wang L, Klapp BF, Wiedenmann B, Taché Y, Mönnikes H. Intraperitoneal injection of ghrelin induces Fos expression in the paraventricular nucleus of the hypothalamus in rats. *Brain Res* 991: 26–33, 2003.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 573–85, 1998.

Salamone JD, Correa MM. The Mysterious Motivational Functions of Mesolimbic Dopamine. *Neuron* 76: 470–485, 2012.

Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D, Mahan K. Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology (Berl)* 104: 515–521, 1991.

Sánchez J, Oliver P, Palou A, Picó C. The inhibition of gastric ghrelin production by food intake in rats is dependent on the type of macronutrient. *Endocrinology* 145: 5049–5055, 2004.

Saunders BT, Robinson TE. The role of dopamine in the accumbens core in the expression of pavlovian-conditioned responses. *Eur J Neurosci* 36: 2521–2532, 2012.

Scherbaum WA. The role of amylin in the physiology of glycemic control. *Exp Clin Endocrinol Diabetes* 106: 97–102, 1998.

Schott BH, Minuzzi L, Krebs RM, Elmenhorst D, Lang M, Winz OH, Seidenbecher CI, Coenen HH, Heinze H-J, Zilles K, Düzel E, Bauer A. Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release. *J Neurosci* 28: 14311–14319, 2008.

Schultz W. Predictive reward signal of dopamine neurons. *J Neurophysiol* 80: 1–27, 1998.

Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science* 275: 1593–1599, 1997.

Schultz W, Dickinson A. Neuronal coding of prediction errors. *Annu Rev Neurosci* 23: 473–500, 2000.

Schüssler P, Kluge M, Yassouridis A, Dresler M, Uhr M, Steiger A. Ghrelin Levels Increase After Pictures Showing Food. *Obesity* 20: 1212–1217, 2012.

Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2: 589–593, 1996.

Sclafani A, Glendinning JI. Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. *Am J Physiol Regul Integr Comp Physiol* 289: R712–R720, 2005.

Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* 320: 145–160, 1992.

Shi L, Bian X, Qu Z, Ma Z, Zhou Y, Wang K, Jiang H, Xie J. Peptide hormone ghrelin enhances neuronal excitability by inhibition of Kv7/KCNQ channels. *Nat Commun* 4: 1435, 2013.

Shimizu I, Hirota M, Ohboshi C, Shima K. Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* 121: 1076–1082, 1987.

Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K. Rapid publication ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 50: 227–232, 2001.

Shrestha YB, Wickwire K, Giraudo S. Effect of reducing hypothalamic ghrelin receptor gene expression on energy balance. *Peptides* 30: 1336–1341, 2009.

Sinkala E, McCutcheon JE, Schuck MJ, Schmidt E, Roitman MF, Eddington DT. Electrode calibration with a microfluidic flow cell for fast-scan cyclic voltammetry. *Lab Chip* 12: 2403–2408, 2012.

Skibicka KP, Dickson SL. Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 32: 2265–73, 2011.

Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180: 129–37, 2011.

Skibicka KP, Shirazi RH, Rabasa-Papio C, Alvarez-Crespo M, Neuber C, Vogel H, Dickson SL. Divergent circuitry underlying food reward and intake effects of ghrelin: Dopaminergic VTA-accumbens projection mediates ghrelin's effect on food reward but not food intake. *Neuropharmacology* 73C: 274–283, 2013.

Small DM, Jones-Gotman M, Dagher A. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage* 19: 1709–1715, 2003.

Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M. Changes in brain activity related to eating chocolate: from pleasure to aversion. *Brain* 124: 1720–1733, 2001.

Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 213: 1036–1037, 1981.

Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J Neurosci* 20: 7737–7742, 2000.

Snyder GL, Allen PB, Fienberg AA, Valle CG, Huganir RL, Nairn AC, Greengard P. Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. *J Neurosci* 20: 4480–4488, 2000.

Sombers LA, Beyene M, Carelli RM, Wightman RM. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. *J Neurosci* 29: 1735–42, 2009.

Song L, Zhu Q, Liu T, Yu M, Xiao K, Kong Q, Zhao R, Li GD, Zhou Y. Ghrelin Modulates Lateral Amygdala Neuronal Firing and Blocks Acquisition for Conditioned Taste Aversion. *PLoS One* 8(6): e65422, 2013.

Speed N, Saunders C, Davis AR, Owens WA, Matthies HJG, Saadat S, Kennedy JP, Vaughan RA, Neve RL, Lindsley CW, Russo SJ, Daws LC, Niswender KD, Galli A. Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. *PLoS One* 6: e25169, 2011.

Srinivasan S, Simms JA, Nielsen CK, Lieske SP, Bito-Onon JJ, Yi H, Hopf FW, Bonci A, Bartlett SE. The Dual Orexin/Hypocretin Receptor Antagonist, Almorexant, in the Ventral Tegmental Area Attenuates Ethanol Self-Administration. *PLoS One* 7, 2012.

Steinberg EE, Boivin JR, Saunders BT, Witten IB, Deisseroth K, Janak PH. Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. *PLoS One* 9: e94771, 2014.

Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH. A causal link between prediction errors, dopamine neurons and learning. *Nat Neurosci* 16: 966–73, 2013.

Stice E, Yokum S, Blum K, Bohon C. Weight gain is associated with reduced striatal response to palatable food. *J Neurosci* 30: 13105–13109, 2010a.

Stice E, Yokum S, Bohon C, Marti N, Smolen A. Reward circuitry responsivity to food predicts future increases in body mass: Moderating effects of DRD2 and DRD4. *Neuroimage* 50: 1618–1625, 2010b.

Stice E, Yokum S, Burger KS, Epstein LH, Small DM. Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. *J Neurosci* 31: 4360–4366, 2011.

Stice E, Burger K, Yokum S. Relative ability of fat and sugar tastes to activate reward, gustatory, and somatosensory regions. *Am. J. Clin. Nutr.* 98: 1377-84 (2013a).

Stice E, Burger K, Yokum S. Caloric deprivation increases responsivity of attention and reward brain regions to intake, anticipated intake, and images of palatable foods. *Neuroimage* 67: 322–330, 2013b.

Stice E, Figlewicz DP, Gosnell BA, Levine AS, Pratt WE. The contribution of brain reward circuits to the obesity epidemic. *Neurosci. Biobehav. Rev.* 37: 2047–2058, 2013c.

Stopper CM, Khayambashi S, Floresco SB. Receptor-specific modulation of risk-based decision making by nucleus accumbens dopamine. *Neuropsychopharmacology* 38: 715–28, 2013.

Stratford TR, Kelley a E. GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. [Online]. *J Neurosci* 17: 4434–40, 1997.

Stratford TR, Kelley AE. Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. *J Neurosci* 19: 11040–11048, 1999.

Strine TW, Mokdad AH, Dube SR, Balluz LS, Gonzalez O, Berry JT, Manderscheid R, Kroenke K. The association of depression and anxiety with obesity and unhealthy behaviors among community-dwelling US adults. *Gen Hosp Psychiatry* 30: 127–137, 2008.

Stuber GD, Klanker M, de Ridder B, Bowers MS, Joosten RN, Feenstra MG, Bonci A. Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science* 321: 1690–2, 2008.

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, Bonci A. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* 475: 377–380, 2011.

Sturm R, Wells KB. Does obesity contribute as much to morbidity as poverty or smoking? *Public Health* 115: 229–235, 2001.

Sturm R. The effects of obesity, smoking, and drinking on medical problems and costs. Obesity outranks both smoking and drinking in its deleterious effects on health and health costs [Online]. *Health Aff* 21: 245–253, 2002.

Sugam JA, Day JJ, Wightman RM, Carelli RM. Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior. *Biol Psychiatry* 71: 199–205, 2012.

Sulzer D. How Addictive Drugs Disrupt Presynaptic Dopamine Neurotransmission. *Neuron* 69: 628–649, 2011.

Sun X, Milovanovic M, Zhao Y, Wolf ME. Acute and chronic dopamine receptor stimulation modulates AMPA receptor trafficking in nucleus accumbens neurons cocultured with prefrontal cortex neurons. *J Neurosci* 28: 4216–4230, 2008.

Sun X, Veldhuizen MG, Wray AE, de Araujo IE, Sherwin RS, Sinha R, Small DM. The neural signature of satiation is associated with ghrelin response and triglyceride metabolism. *Physiol Behav* In press, 2014.

Surmeier DJ, Bargas J, Hemmings HC, Nairn AC, Greengard P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14: 385–397, 1995.

Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci.* 30: 228–235, 2007.

Swanson LW, Kuypers HG. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J Comp Neurol* 194: 555–570, 1980.

Swinburn B, Egger G, Raza F. Dissecting obesogenic environments: the development and application of a framework for identifying and prioritizing environmental interventions for obesity. *Prev Med (Baltim)* 29: 563–570, 1999.

Szczypka MS, Kwok K, Brot MD, Marck BT, Matsumoto AM, Donahue BA, Palmiter RD. Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. *Neuron* 30: 819–828, 2001.

Szczypka MS, Rainey M a, Kim DS, Alaynick W a, Marck BT, Matsumoto a M, Palmiter RD. Feeding behavior in dopamine-deficient mice. [Online]. *Proc Natl Acad Sci U S A* 96: 12138–43, 1999.

Szentirmai E, Kapás L, Krueger JM. Ghrelin microinjection into forebrain sites induces wakefulness and feeding in rats. *Am J Physiol Regul Integr Comp Physiol* 292: R575–R585, 2007.

Taber MT, Fibiger HC. Feeding-evoked dopamine release in the nucleus, accumbens: regulation by glutamatergic mechanisms. *Neuroscience* 76: 1105–1112, 1997.

Taha SA, Fields HL. Encoding of palatability and appetitive behaviors by distinct neuronal populations in the nucleus accumbens. *J Neurosci* 25: 1193–1202, 2005.

Taha SA, Nicola SM, Fields HL. Cue-evoked encoding of movement planning and execution in the rat nucleus accumbens. *J Physiol* 584: 801–818, 2007.

Takano S, Kim J, Ikari Y, Ogaya M, Nakajima K, Oomura Y, Wayner MJ, Sasaki K. Electrophysiological effects of ghrelin on laterodorsal tegmental neurons in rats: An in vitro study. *Peptides* 30: 1901–1908, 2009.

Tamura H, Kamegai J, Shimizu T, Ishii S, Sugihara H, Oikawa S. Ghrelin stimulates GH but not food intake in arcuate nucleus ablated rats. *Endocrinology* 143: 3268–3275, 2002.

Tang DW, Fellows LK, Small DM, Dagher a. Food and drug cues activate similar brain regions: a meta-analysis of functional MRI studies. *Physiol Behav* 106: 317–24, 2012.

Taslimi Z, Arezoomandan R, Omranifard A, Ghalandari-Shamami M, Riahi E, Vafaei AA, Rashidy-Pour A, Haghparast A. Orexin A in the ventral tegmental area induces conditioned place preference in a dose-dependent manner: Involvement of D1/D2 receptors in the nucleus accumbens. *Peptides* 37: 225–232, 2012.

Teegarden SL, Scott a N, Bale TL. Early life exposure to a high fat diet promotes long-term changes in dietary preferences and central reward signaling. *Neuroscience* 162: 924–32, 2009.

Teitelbaum P, Epstein AN. The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol Rev* 69: 74–90, 1962.

Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA, Heidbreder CA, Melotto S. Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. *Addict Biol* 12: 22–9, 2007.

Thompson JL, Borgland SL. Presynaptic leptin action suppresses excitatory synaptic transmission onto ventral tegmental area dopamine neurons. *Biol Psychiatry* 73: 860–868, 2013.

Thorpe AJ, Cleary JP, Levine AS, Kotz CM. Centrally administered orexin A increases motivation for sweet pellets in rats. *Psychopharmacology (Berl)* 182: 75–83, 2005.

Thorpe KE. The rise in health care spending and what to do about it. *Health Aff (Millwood)* 24: 1436–45, 2005.

Thorpe KE. Factors accounting for the rise in health-care spending in the United States: The role of rising disease prevalence and treatment intensity. *Public Health* 120: 1002–1007, 2006.

Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ. Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* 75: 58–64, 2012.

Tobin S, Sedki F, Abbas Z, Shalev U. Antagonism of the dopamine D1-like receptor in mesocorticolimbic nuclei attenuates acute food deprivation-induced reinstatement of heroin seeking in rats. *Eur J Neurosci* 37: 972–981, 2013.

Tobler PN, Dickinson A, Schultz W. Coding of predicted reward omission by dopamine neurons in a conditioned inhibition paradigm. *J Neurosci* 23: 10402–10410, 2003.

Tobler PN, Fiorillo CD, Schultz W. Adaptive coding of reward value by dopamine neurons. *Science* 307: 1642–1645, 2005.

van den Top M, Lee K, Whyment AD, Blanks AM, Spanswick D. Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci* 7: 493–494, 2004.

Toshinai K et al., Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan J-L, Wang Q-P, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M. Ghrelin-Induced Food Intake Is Mediated via the Orexin Pathway. *Endocrinology* 144: 1506–1512, 2003.

Tsai H-C, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* 324: 1080–1084, 2009.

Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 407: 908–913, 2000.

Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 50: 707–709, 2001.

Tups A, Helwig M, Khorooshi RMH, Archer ZA, Klingensport M, Mercer JG. Circulating ghrelin levels and central ghrelin receptor expression are elevated in response to food deprivation in a seasonal mammal (Phodopus sungorus). *J Neuroendocrinol* 16: 922–928, 2004.

Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379: 69–72, 1996.

Tye KM, Janak PH. Amygdala neurons differentially encode motivation and reinforcement. *J Neurosci* 27: 3937–3945, 2007.

U.S. Department of Health adn Human Services. *Health, United States, 2012 (05/30/2013).* 2012.

Volkow ND, Wang G-JJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci* 15: 37–46, 2011.

Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: Neurobiological overlaps. *Obes. Rev.* 14: 2–18, 2013.

Vucetic Z, Carlin JL, Totoki K, Reyes TM. Epigenetic dysregulation of the dopamine system in diet-induced obesity. *J Neurochem* 120: 891–8, 2012.

Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology* 151: 4756–4764, 2010.

Voorn P, Vanderschuren LJMJ, Groenewegen HJ, Robbins TW, Pennartz CMA. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci.* 27: 468–474, 2004.

Waelti P, Dickinson A, Schultz W. Dopamine responses comply with basic assumptions of formal learning theory. *Nature* 412: 43–48, 2001.

Walker AK, Ibia IE, Zigman JM. Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. *Physiol. Behav.* 2012.

Wan X, Peoples LL. Firing patterns of accumbal neurons during a pavlovian-conditioned approach task. *J Neurophysiol* 96: 652–660, 2006.

Wanat MJ, Kuhnen CM, Phillips PEM. Delays conferred by escalating costs modulate dopamine release to rewards but not their predictors. *J Neurosci* 30: 12020–12027, 2010.

Wang L, Saint-Pierre DH, Taché Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y - synthesizing neurons in mouse hypothalamic arcuate nucleus. *Neurosci Lett* 325: 47–51, 2002.

Wang B, You ZB, Wise RA. Reinstatement of Cocaine Seeking by Hypocretin (Orexin) in the Ventral Tegmental Area: Independence from the Local Corticotropin-Releasing Factor Network. *Biol Psychiatry* 65: 857–862, 2009.

Wang G-J, Volkow ND, Thanos PK, Fowler JS. Imaging of brain dopamine pathways: implications for understanding obesity. *J Addict Med* 3: 8–18, 2009b.

Wassum KM, Ostlund SB, Loewinger GC, Maidment NT. Phasic mesolimbic dopamine release tracks reward seeking during expression of pavlovian-to-instrumental transfer. *Biol Psychiatry* 73: 747–55, 2013.

Wassum KM, Ostlund SB, Maidment NT. Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. *Biol Psychiatry* 71: 846–854, 2012.

Watabe-Uchida M, Zhu L, Ogawa SKK, Vamanrao A, Uchida N. Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* 74: 858–873, 2012.

Weinberg ZY, Nicholson ML, Currie PJ. 6-Hydroxydopamine lesions of the ventral tegmental area suppress ghrelin's ability to elicit food-reinforced behavior. *Neurosci Lett* 499: 70–73, 2011.

Weingarten HP, Chang PK, McDonald TJ. Comparison of the metabolic and behavioral disturbances following paraventricular- and ventromedial-hypothalamic lesions. *Brain Res Bull* 14: 551–559, 1985.

Weingarten HP. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science* 220: 431–433, 1983.

West DB, Fey D, Woods SC. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol* 246: R776–R787, 1984.

WHO. WHO | Obesity and overweight [Online]. *World Heal. Organ. Media Cent. Fact Sheet No. 311* 2012..

Wickham RJ, Solecki W, Rathbun LR, Neugebauer NM, Wightman RM, Addy N a. Advances in studying phasic dopamine signaling in brain reward mechanisms. *Front Biosci (Elite Ed)* 5: 982–99, 2013.

Williams DL, Grill HJ, Cummings DE, Kaplan JM. Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* 144: 5184–5187, 2003.

Wilson C, Nomikos GG, Collu M, Fibiger HC. Dopaminergic correlates of motivated behavior: importance of drive. *J Neurosci* 15: 5169–5178, 1995.

Winston C. On the Performance of the U.S. Transportation System: Caution Ahead. *J Econ Lit* 51: 773–824, 2013.

Witten IB, Steinberg EE, Lee SY, Davidson TJ, Zalocusky KA, Brodsky M, Yizhar O, Cho SL, Gong S, Ramakrishnan C, Stuber GD, Tye KM, Janak PH, Deisseroth K. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron* 72: 721–33, 2011.

Wong ML, Licinio J, Yildiz BO, Mantzoros CS, Prolo P, Kling M, Gold PW. Simultaneous and Continuous 24-Hour Plasma and Cerebrospinal Fluid Leptin Measurements: Dissociation of Concentrations in Central and Peripheral Compartments. *J Clin Endocrinol Metab* 89: 258–265, 2004.

Woods SC, Ramsay DS. Pavlovian influences over food and drug intake. In: *Behavioural Brain Research*. 2000, p. 175–182.

Woods SC. The eating paradox: how we tolerate food. *Psychol Rev* 98: 488–505, 1991.

Wortley KE, Del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, Sleeman MW. Absence of ghrelin protects against early-onset obesity. *J Clin Invest* 115: 3573–3578, 2005.

Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. 2001.
Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141: 4325–4328, 2000.

Wright CI, Beijer A V, Groenewegen HJ. Basal amygdaloid complex afferents to the rat nucleus accumbens are compartmentally organized. *J Neurosci* 16: 1877–1893, 1996.

Wright CI, Groenewegen HJ. Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. *Neuroscience* 73: 359–373, 1996.

Wu Q, Boyle MP, Palmiter RD. Loss of GABAergic Signaling by AgRP Neurons to the Parabrachial Nucleus Leads to Starvation. *Cell* 137: 1225–1234, 2009.

Wyvell CL, Berridge KC. Incentive sensitization by previous amphetamine exposure: increased cue-triggered “wanting” for sucrose reward. *J Neurosci* 21: 7831–7840, 2001.

Xi D, Gandhi N, Lai M, Kublaoui BM. Ablation of Sim1 neurons causes obesity through hyperphagia and reduced energy expenditure. *PLoS One* 7, 2012.

Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami KI, Sugiyama F, Goto K, Yanagisawa M, Sakurai T. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38: 701–713, 2003.

Yamazaki M, Kobayashi H, Tanaka T, Kangawa K, Inoue K, Sakai T. Ghrelin-induced growth hormone release from isolated rat anterior pituitary cells depends on intracellullar and extracellular Ca2+ sources. *J Neuroendocrinol* 16: 825–831, 2004.

Yang J, Brown MS, Liang G, Grishin N V., Goldstein JL. Identification of the Acyltransferase that Octanoylates Ghrelin, an Appetite-Stimulating Peptide Hormone. *Cell* 132: 387–396, 2008.

Young LR, Nestle M. The contribution of expanding portion sizes to the US obesity epidemic. *Am J Public Health* 92: 246–249, 2002.

Yun IA, Nicola SM, Fields HL. Contrasting effects of dopamine and glutamate receptor antagonist injection in the nucleus accumbens suggest a neural mechanism underlying cue-evoked goal-directed behavior. *Eur J Neurosci* 20: 249–263, 2004.

Yung KKL, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI. Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience* 65: 709–730, 1995.

Van Zessen R, Phillips JL, Budygin EA, Stuber GD. Activation of VTA GABA Neurons Disrupts Reward Consumption. *Neuron* 73: 1184–1194, 2012.

Zheng H, Patterson LM, Berthoud H-R. Orexin signaling in the ventral tegmental area is required for high-fat appetite induced by opioid stimulation of the nucleus accumbens. *J Neurosci* 27: 11075–11082, 2007.

Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* 494: 528–48, 2006.

Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK. Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J Clin Invest* 115: 3564–3572, 2005.

Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, Darvas M, Kim MJ, Mizumori SJY, Paladini C a, Phillips PEM, Palmiter RD. Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc Natl Acad Sci U S A* 106: 7281–7288, 2009.

COPYRIGHT STATEMENT

Author's note: The second chapter of this thesis has been published in The Journal of Neuroscience. The article is titled "Ghrelin as interface between physiological state and phasic dopamine signaling" and is cited in the References section (PMID# 24695709). Below is the current copyright information from Journal of Neuroscience that states I do not need permission to reprint it here.

CURRICULUM VITAE

Jackson J. Cone

Email: jcone2@uic.edu

Education

2009- Present University of Illinois, Chicago
Ph.D. Candidate in Neuroscience

2008 University of California, Berkeley
B.A. in Molecular and Cell Biology (Neurobiology track)
Minor in Public Policy

Research Experience

2009-Present *Graduate Student*
Laboratory of Mitchell Roitman, Ph.D.
University of Illinois, Chicago

2006-2009 *Research Student* (2006-07), *Staff Research Associate* (2007-09)
Laboratory of Patricia Janak, Ph.D.
University of California, San Francisco

2004-2006 *Lab Manager and Research Assistant*
Laboratory of Jack Glaser, Ph.D.
Goldman School of Public Policy, UC Berkeley

Honors and Awards

2014 SSIB New Investigator Travel Award
2013-2014 UIC Dean's Scholar Fellowship
2011-2013 Chicago Biomedical Consortium Scholar Award
2011-2013 UIC Center for Clinical and Translational Science Predoctoral Fellowship
2010-2011 Departmental T32 Training Grant in the Neuroscience of Mental Health
2009-2011 UIC Chancellor's Supplemental Research Fellowship

Mentorship

2014- Pawel Bujakowski Psychology 396 – Supervised Undergraduate Research
2013-2014 Brett Palmer, B.S. Psychology 399 – Independent Research
2012-2013 Laura Whiteford, B.A. Honors College Undergraduate Capstone Project
2010-2011 Ashraf Abbas, B.S. Honors College Undergraduate Capstone Project

Teaching Experience

2014 (Fall) Instructor – PSCH 363 – University of Illinois at Chicago
Laboratory in Behavioral Neuroscience

2013 (Spring)	Co-Instructor - BIOS 396 - University of Illinois at Chicago Advanced Topics in Modern Neuroscience
2006 (Spring)	Co-Instructor - History 98 - University of California, Berkeley Musical Styles of the 1900s

Volunteer Service

Lead Coordinator for CBC Scholars "Loop Connections" Seminar (Dec. 2012)
Chicago Biomedical Consortium Scholars UIC Rep and Planning Committee (2012-2013)
Akiba-Schechter Jewish Day School Science Fair Judge (2012)
Chicago Brain Bee Volunteer (2012, 2013, 2014)

Publications

Mccutcheon JE, **Cone JJ**, Sinon CG, Fortin SM, Kantak PA, Witten IB, Deisseroth K, Stuber GD, Roitman MF. Optical suppression of drug-evoked phasic dopamine release. *Frontiers in Neural Circuits* 8:114 (2014). PMID: 25278845

Mietlicki-Baase EG, Reiner DJ, **Cone JJ**, Olivos DR, McGrath LE, Zimmer DJ, Roitman MF, Hayes MR. Amylin modulates the mesolimbic dopamine system to control energy balance. *Neuropsychopharmacology* (in press). PMID: 25035079

Cone JJ, McCutcheon JE, Roitman MF. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *Journal of Neuroscience* 34: 4905-13 (2014). PMID: 24695709

Koranda J, **Cone JJ**, McGehee DS, Roitman MF, Beeler JA, Zhaung X. Nicotinic receptors regulate the dynamic range of dopamine release in vivo. *Journal of Neurophysiology* 111: 103-111 (2014). PMID: 2408939

Ryczko D, Grätsch S, Auclair F, Dubé C, Bergeron S, Alpert MH, **Cone JJ**, Roitman M, Alford S, Dubuc R. Forebrain dopamine neurons project down to a brainstem region controlling locomotion. *Proceedings of the National Academy of Sciences* 110(34) E3235-42 (2013). PMID: 23918379

Cone JJ, Chartoff EH, Potter DN, Ebner SR, Roitman MF. Prolonged high-fat diet reduces dopamine reuptake without altering DAT gene expression. *PLoS One* 8(3): e58251 (2013). PMID: 23516454

Brown HD, McCutcheon JE, **Cone JJ**, Ragozzino ME, Roitman MF. Primary food reward and reward predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum. *European Journal of Neuroscience* 34(12): 1997-2006 (2011). PMID: 22122410

Roitman MF, Wescott S, **Cone JJ**, McLane MP, Wolfe HR. Trodusquemine (MSI-1436) reduces acute food intake without affecting dopamine transporter activity. *Pharmacology, Biochemistry and Behavior* 97(1): 138-143 (2010). PMID: 20478327

Tye KM, Tye LD, **Cone JJ**, Hekkelman EF, Janak PH, and Bonci AB. Methylphenidate facilitates learning-induced amygdala plasticity. *Nature Neuroscience* 13(4): 475-81 (2010). PMID: 20208527

Tye KM*, **Cone JJ***, Schairer W, and Janak PH. Amygdala neural encoding of the absence of reward during extinction. *Journal of Neuroscience* 30(1): 116-25 (2010). PMID: 20053894 ***Co-first author.**

Chaudrhi N, Sahuque LL, **Cone JJ**, and Janak PH. Reinstated ethanol-seeking in rats is modulated by environmental context and requires the nucleus accumbens core. *European Journal of Neuroscience* 28(11): 2288-98 (2008). PMID: 19046372

Commentary

Cone JJ and Roitman MF. Homeostatic circuitry pushes back. *Cell Science Reviews* 6(3): 25-26 (2010).

Research in Progress

Cone JJ, Roitman JD, Roitman MF. Ghrelin regulates phasic mesolimbic signaling evoked by food-predictive stimuli. *Anticipated Submission: 1 month.*

Cone JJ, Fortin SM, McCutcheon JE, Roitman MF. Phasic dopamine signaling tracks the hedonic value of sodium in state-dependent manner. *Anticipated Submission: 4-6 months.*

Oral Presentations

Cone JJ, Fortin SM, Palmer BA, McCutcheon JE, Roitman MF. Phasic dopamine signaling tracks the hedonic value of sodium based on physiological state. *SSIB* 2014.

Cone JJ and Roitman MF. Peripheral hormones that signal physiological state regulate phasic dopamine signaling evoked by food via actions in different brain sites. *5th Annual National Clinical and Translational Sciences Predoctoral Programs Meeting* (2013).

Cone JJ and Roitman MF. Physiological state dynamically tunes phasic dopamine signaling evoked by food reward. *Graduate Student Talk Competition at the Chicago Chapter of the Society for Neuroscience Meeting* (2013).

Cone JJ and Roitman MF. Peripheral feeding hormones dynamically tune the dopamine response to food. *4th Annual Chicago Biomedical Consortium Scientific Exchange* (2013).

Cone JJ and Roitman MF. Ghrelin modulates phasic dopamine signals evoked by food and food-predictive stimuli. *4th Annual National Clinical and Translational Sciences Predoctoral Programs Meeting* (2012).

Cone JJ and Roitman MF. Homeostatic feeding signals interact with brain reward circuitry. *3rd Annual Chicago Biomedical Consortium Scientific Exchange* (2012).

Cone JJ and Roitman MF. Ghrelin increases phasic dopamine signals evoked by food reward. *SSIB* (2011).

Cone JJ, Robbins HA, Roitman JD, and Roitman MF. Consumption of a high fat diet affects phasic dopamine release and reuptake in the ventral striatum. *SSIB* (2010).

First Author Abstracts

Cone JJ, Roitman JD, Roitman MF. Ghrelin affects both phasic dopamine and nucleus accumbens neuron responses to food predictive stimuli. *Society for Neuroscience* (2013). **Selected as a "Hot Topic" and featured in meeting press release**

Cone JJ, Roitman JD, Roitman MF. Ghrelin regulates phasic mesolimbic signaling evoked by food stimuli. *SSIB* (2013).

Cone JJ and Roitman MF. Ghrelin modulates phasic dopamine signals evoked by food reward via action in the lateral hypothalamus. Computational and Systems Neuroscience (CoSyne*;* 2013).

Cone JJ and Roitman MF. Ghrelin dynamically modulates phasic dopamine signals evoked by food reward and food-predictive stimuli. *Society for Neuroscience* (2012).

Cone JJ and Roitman MF. Ghrelin modulates phasic dopamine signals evoked by food reward. *Society for Neuroscience* (2011).

Cone JJ, Robbins HA, Roitman JD, and Roitman MF. Consumption of a high fat diet affects phasic dopamine release and reuptake in the ventral striatum. *Society for Neuroscience* (2010).

Cone JJ, Kremin TE, and Janak PH. Simultaneous nucleus accumbens and ventral hippocampus recordings in rats during an operant conditioning task. II: Perievent information. *Society for Neuroscience* (2008).

Cone JJ, Tye KM., and Janak PH. Neural activity in the basolateral amygdala changes with altered reward availability. *Society for* Neuroscience (2007).

Contributing Author Abstracts

McCutcheon JE, **Cone JJ**, Sinon G, Witten IB, Deisseroth K, Stuber GD, Roitman MF. Optical Suppression of phasic dopamine spikes evoked by presynaptic release modulators. *Society for Neuroscience* (2013).

Ryczko D, Dube C, Alpert MH, **Cone JJ**, Auclair F, Roitman M, Alford S, Dubuc R. Conservation of the descending projections from forebrain dopamine neurons to a brainstem region controlling locomotion in vertebrates. Possible implications for Parkinson's disease. *Society for Neuroscience* (2013).

Koranda J, Beeler JA, **Cone JJ**, Roitman MF, Zhuang X. In-vivo characterization of nAChR modulation of dopamine release in the dorsolateral striatum. *Society for Neuroscience* (2012).

Brown HD, McCutcheon JE, **Cone JJ**, Ragozzino ME, Roitman MF. Reward and reward-predictive cues are correlated with regionally selective phasic dopamine signaling in the striatum. *Society for Neuroscience* (2011).

McCutcheon JE, Brown HD, **Cone JJ**, Beeler JA, Ragozzino ME, and Roitman MF. Phasic dopamine signaling evoked by unexpected food reward differs with respect to striatal subregion and preference. *SSIB* (2011).

Roitman MF, Roitman JD, and **Cone JJ.** Consumption of a high fat diet affects phasic dopamine release and reuptake in the nucleus accumbens. *FENS* (2010).

Tye KM, Tye LD, **Cone JJ**, Hekkelman EF, Janak PH, Bonci AB. Methylphenidate (Ritalin) enhances task performance and learning-induced amygdala plasticity via distinct D1 and D2 receptor mechanisms. *Society for Neuroscience* (2009).

Tye KM, Tye LD, **Cone JJ**, Hekkelman EF, Janak PH, Bonci AB. Ritalin enhances learning-induced plasticity via distinct dopamine receptor mechanisms. *Amygdala in Health and Disease, 2nd Gordon Research Conference* (2009).

References

Mitchell F. Roitman, Ph.D.
Associate Professor
University of Illinois at Chicago, Department of Psychology
Phone: (312)-996-3113
Email: mroitman@uic.edu

Patricia H. Janak, Ph.D.
Professor
Ernest Gallo Clinic and Research Center
University of California, San Francisco
Phone: (415) 502-7382
Email: patricia.janak@ucsf.edu

Jamie D. Roitman, Ph.D.
Assistant Professor
University of Illinois at Chicago, Department of Psychology
Phone: (312)-355-1438
Email: jroitman@uic.edu