THE EFFECT OF BINGE EATING ON OREXIGENIC NEURONS OF THE LATERAL HYPOTHALAMUS

By

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Abstract

Binge eating is a form of disordered eating involving excessive caloric intake in a brief period of time, typically from palatable, calorie-dense foods. The underlying neural mechanisms controlling this behavior are poorly understood, but likely involve both homeostatic and hedonic feeding circuitry. The lateral hypothalamus contains orexin and melanin-concentrating hormone (MCH) neurons, both of which are orexigenic neurons that also play roles in stress, reward and motivation. It is possible that activation of these neurons is responsible for the development or maintenance of binge eating behavior. To test this, we established an animal model of binge eating by providing rats with three schedules of intermittent access to a palatable, high fat diet (HFD): 6 daily exposures (1wDaily), 6 exposures over 2 weeks on Mondays, Wednesdays and Fridays (2wMWF), and 18 exposures over 6 weeks (6wMWF). Rats eagerly consumed the diets upon presentation following repeated exposures, consuming up to a quarter of their daily calories in a one-hour food exposure, indicative of binge-like behavior. Following 1wDaily or 2wMWF feeding, there was no apparent effect of HFD on orexin neurons, but after 6wMWF feeding, orexin neurons were significantly hyperpolarized in the HFD group compared to those in controls. However, we did not observe any changes in MCH neurons. This suggests that long-term, but not short-term intermittent access to a palatable diet high in fat induces changes in orexin neurons. In conclusion, our results suggest that orexin neurons are unexpectedly inhibited by prolonged exposure to feeding patterns that induce binge eating. This may be a homeostatic response to repeated binge eating.
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List of Abbreviations

1wDaily, 1-week daily feeding paradigm

2wMWF, 2-week feeding on Monday, Wednesday and Friday

6wMWF, 6-week feeding on Monday, Wednesday and Friday

ACSF, artificial cerebrospinal fluid

AgRP, agouti related peptide

BED, binge eating disorder

BIBO 3304, \(N-(1R)-1-[[[[4-(((Aminocarbonyl)amino)methyl]phenyl)methyl]amino]carbonyl]-4-[(aminoiminomethyl)amino]butyl]-\alpha\)-phenyl-benzeneacetamide ditrifluoroacetate

BN, bulimia nervosa

CART, cocaine and amphetamine related transcript

D2R, Dopamine receptor D\(_2\)

DA, dopamine

DSM-5, Diagnostic and Statistical Manual or Mental Disorders, Fifth Edition

GABA, gamma amino-butyric acid

GW-3430, 6-(4-chlorophenyl)-3-[3-methoxy-4-(2-pyrrolidin-1-ylethoxy)phenyl]-3H-thieno[3,2-d]pyrimidin-4-one

HFD, high fat diet

MCH, melanin concentrating hormone
MCH1R, melanin concentrating hormone receptor type 1

MCH2R, melanin concentrating hormone receptor type 2

NPY, neuropeptide Y

NPY-Y1, neuropeptide Y receptor type 1

OX1R, orexin receptor type 1

OX2R, orexin receptor type 2

PBS, phosphate buffer solution

POMC, proopiromelanocortin

SB334867, \( N-(2\text{-methyl-6-benzoaxazolyl})-N'1,5\text{-naphthyridin-4-yl urea} \)

VTA, ventral tegmental area

WD, western diet
Chapter 1 - Introduction

1.1 Binge eating

Binge eating refers to episodes of disordered food intake which meet two particular criteria: the first refers to feeding in which an excess of calories are consumed in a much smaller period of time than would be expected for the size of the meal, and the second affectively qualifies the feeding behaviors as accompanied by a feeling of loss of control during the eating. The behavior often occurs when individuals are not hungry or in metabolic need for food intake and when individuals are alone due to embarrassment over the amount of food consumed, and it is accompanied by feelings of shame and guilt (American Psychiatric Association, 2013; Hudson et al., 2007; Kessler et al., 2013). The Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5; 2013) lists binge eating as a criterion for eating disorders including bulimia nervosa (BN), binge eating disorder (BED) as well as eating disorder not otherwise specified, and the presence of the behavior is a risk factor for comorbid mental illnesses as well as multiple forms of physical health problems (Hudson et al., 2007; Javaras et al., 2008).

Binge eating can lead to an increased risk of overweight and obesity, with the number of binge eating episodes per month positively correlated with body mass index, and thus carries similar increased risks to physical health (Striegel-Moore et al., 2009). Those suffering from BN and BED are more likely to suffer from type-II diabetes, and thus are susceptible to further deleterious health effects due to the disease (Raevuori et al., 2014). Compared to women without BN or BED, women with these eating disorders also reported significantly higher incidence of poor or very poor health, as well as reporting higher incidence of specific physical ailments including joint and limb pain, gastrointestinal distress, shortness of breath and abnormal menstruation, which negatively affected normal day to day functioning and quality of life.
BN specifically increases the risk for heart attack and stroke, while both disorders increase the risk for musculoskeletal, pain and cardio-metabolic conditions (Kessler et al., 2013). Furthermore, both eating disorders, and even subclinical binge eating, have significantly higher comorbidity rates for other mental illnesses compared to those without disordered eating. Specifically, rates for mood and anxiety disorders including major depressive disorder, agoraphobia and obsessive-compulsive disorder, impulse control disorders including attention-deficit/hyperactivity disorder, and substance use disorders including alcoholism and drug dependence are significantly higher in this population compared to those without binge-eating disorders (Javaras et al., 2008; Johnson et al., 2001; Kessler et al., 2013).

As a behavioral symptom of mental illness, binge eating is treated with psychosocial therapies including cognitive behavioral therapy, however these therapies are rarely successful six months post-treatment (McElroy et al., 2012). Pharmacological treatments are also available that include targeting many different neurotransmitter systems, including antidepressants, psychomotor stimulants, and opioid-receptor antagonists. However, these pharmaceuticals have their own specific sets of risks and side-effects depending on the drug, and like psychotherapy are not effective in all cases (Arnold et al., 2002; McElroy et al., 2015). Therefore, in order to effectively treat binge eating behavior, it is necessary to further investigate the neurological correlates of this behavior, with a goal of identifying novel therapeutic targets.

1.1.1 Human studies

Human studies of binge eating tend to focus on epidemiology, treatment and the neurobiological correlates and potential underlying mechanisms for the behavior. Binge eating behavior typically manifests in young adulthood, with the age of onset typically ranging from 18 to 20 years for BN, earlier compared to 20 to 25 years for BED (Hudson et al., 2007; Kessler et
Binge eating disorders are considered by some to be “culturally-bound” disorders, in that they are prevalent more in Western societies compared to others. Even so, there are still significant differences across Western nations in their lifetime prevalence, ranging from low incidences of 0.1% for BN and 0.7% for BED in Italy, compared to 2.0% for BN and 4.7% for BED in Brazil (Kessler et al., 2013). One consistency is that the prevalence rates for BED are typically higher than those for BN in most nations. Some studies have demonstrated strong sex differences in the prevalence of these disorders, especially for BN. The female-to-male gender ratio has been estimated as ranging from as low as 5:1 to as high as 20:1 (Pesce et al., 2015; Steinhausen & Jensen, 2015). This gender difference also exists for BED, although the female-to-male ratio is much lower compared to BN, ranging from 2:1 to 6:1 (Ágh et al., 2015). It is possible that these gender differences can partly be explained by societal factors, including more pressure on women than men to be thin, or for women to more often seek help for their maladaptive behaviors (Davis, 2015). However, hormonal influences cannot be excluded, and there is some evidence that feeding patterns and preferences change with the menstrual cycle (Frye & Demolar, 1994; McNeil et al., 2013). For example, ratings of chocolate craving have been found to vary with the menstrual cycle, and cravings were associated with more maladaptive eating habits, including guilt and inability to control intake (Hormes & Timko, 2011).

As previously mentioned, the main treatments for binge eating are therapist-led psychotherapy, medication or a combination of the two. Often, especially in the case of BED, clinically significant recovery is measured both by a reduction of binge eating as well as decreasing body weight, although weight loss may not be relevant in all cases (Mathes et al., 2009; McElroy et al., 2015). Meta-analyses of the effectiveness of certain talk therapies,
including cognitive behavioral therapy and interpersonal therapy, have shown them to be effective in reducing binge frequency compared to wait-list controls. However, some studies report that the therapeutic efficacy to reduce binge eating frequency was less significant at 12-month follow ups compared to that immediately post-treatment, indicating that they are not universally effective over time (McElroy et al., 2015; Wilfley et al., 2002). Antidepressants including fluoxetine and citalopram have been shown to reduce the frequency of binge episodes as well as associated psychiatric concerns, however the rate of drop-out from drug trials remains relatively high, from 16 to 57% (Arnold et al., 2002; McElroy et al., 2003, McElroy et al., 2012). Stimulant drugs such as lisdexamphetamine have also been shown to reduce binge frequency, however as with the antidepressants and other drug interventions, the rates of symptom improvement generally decrease once the drug is discontinued (Mathes et al., 2009). Combining therapy with drug treatment is often more successful than either treatment alone, however due to the number of drugs to choose from, finding the most suitable one for each patient varies from case to case, and again treatment effects often reduce once therapy or medication ceases.

Because treatment is not always successful, it is necessary to further understand the mechanisms underlying binge eating in the brain in order to figure out better treatments. Genetic studies within humans have provided some insights to these putative mechanisms of binge eating behavior based on the correlations with certain biological findings. For example, many studies have focused on the role of genetic variation in the activity of dopamine neurons, as well as dopamine transporters and receptors. Those with either BN or BED have been shown to be more likely to have a certain polymorphism of the dopamine transporter gene compared to those without an eating disorder, implicating the mesolimbic dopamine system with binge eating
behavior (Bello & Hajnal, 2010). However, human studies are yet unable to fully uncover the complex interaction between binge eating behavior and the brain.

1.1.2 Animal studies

Because the interconnected effects of binge eating and neuronal activity cannot be directly measured using human studies, animal models are necessary in order to use accurate, invasive methods of studying the neural associations of binge-like feeding. Animal models allow for the production, investigation and testing of binge-like models of food intake and the correlation of physiological parameters, including effects on the activity of neuronal populations. While it is difficult to measure subjective feelings in animal models that are a part of human binge eating, such as a loss of control or guilt, there are methods that can objectively test for factors such as anxious or depressive behaviors that may accompany binge-like feeding (Corwin & Buda-Levin, 2004). Due to the different behavioral phenotypes of the eating disorders, including variations in feeding frequency or self-imposed fasting, many different models have been generated in order to represent the variety of behaviors observed in each disorder. The common factor in modelling BN or BED is to promote binge-like feeding behavior in animals.

There are three common methods used to promote binge-like feeding behavior in animals, including using food restriction, stress, or intermittent exposure to palatable foods (Avena et al., 2008; Bake et al., 2014; Boggiano et al., 2007; Hagan et al., 2003; Pankevich et al., 2010; Vickers et al., 2015). In food restriction paradigms, animals do not have ad libitum access to any food for a period of time, or a baseline of caloric intake is measured and only a fraction is allotted to each animal (Hagan & Moss, 1997; Pankevich et al., 2010). These models have the benefit of being able to mimic some forms of human eating disorders in which people self-restrict their food intake, only to engage in subsequent binge episodes. However, because food
restriction results in the altered levels of many hunger and satiety signals, including ghrelin, leptin, glucose and a number of neuropeptides in the brain, the subsequent neuronal effects from binge eating may be indiscernible from the effects of food restriction, potentially confounding the results. Thus, food restriction may not be a suitable methodology for investigating specific effects of binge eating, depending on what variables related to binge eating are intended for study (Ahima et al., 1996; Kunii et al., 1999; Zhao et al., 2008).

Binge episodes in humans are often precipitated by stressful life events, thus animal models involving stress exposure are able to mimic these feeding patterns (Kessler et al., 2013). Stress-induced binge eating involves pairing some form of stress with access to highly palatable foods. Foot shock, noise, or maternal separation are some common stressors used in these studies (Boggiano et al., 2007; Iwasaki et al., 2000; Wilson & Cantor, 1987). Like food restriction, experience to stress results in changes in a number of feeding related signals. For example, glucocorticoids that are released during stress interact with insulin and leptin function, both hormones that in part modulate the membrane potential and activity of neurons in the arcuate nucleus of the hypothalamus that regulate feeding behavior (Gyengesi et al., 2010). Downstream effects of stress exposure itself, like food restriction, may confound the effects of binge eating on neuronal populations. Thus, using models of stress-induced binge-like behavior may be inappropriate depending on the variables under investigation.

A third common method to promote binge-like behavior involves using intermittent exposure to palatable foods (Babbs et al., 2012; Corwin et al., 1998; Iemolo et al., 2012). In the majority of these models, animals have free access to standard chow and water throughout the study. In addition, animals are given access to a palatable food, in most cases between 30 and 120 minute episodes, daily, every other day, or three times per week on non-consecutive days
(Corwin et al., 1998; Dimitriou et al., 2000; Wojnicki et al., 2013). After a number of exposures, animals typically exhibit binge-like behavior upon presentation of the food. These intermittent access models have the benefit of reducing the number of variables by omitting factors related to food restriction or stress.

Using animal models of binge eating, it is possible to experimentally tease out neurophysiological correlates of these behaviors in the brain that may be the cause or the result of binge-like eating. The following sections will review physiological and neural evidence gathered from human research and animal models in the study of the central control of normal food intake and binge-like feeding.

1.2 Central control of feeding behavior

1.2.1 Overview

Early research on food intake has identified various putative mechanisms by which it is controlled. For example, the “glucostatic theory” postulates that food intake is governed by the level of available energy for the body. Mayer suggested that when levels of available energy, specifically glucose, falls, a meal is initiated in order to replenish available energy (Mayer, 1955). The “lipostatic theory” suggests that food intake is based not on levels of available glucose, but on levels of energy stored in the form of white adipose tissue (Kennedy, 1953). It has since become apparent that the regulation of energy balance is a complex process which integrates both central and peripheral neurobiological and endocrine signals with the environment. Simplistically, many of these signals can be thought of as either hunger mechanisms promoting food intake or satiety mechanisms that reduce food intake or terminate a meal.
Internal hunger mechanisms include signals coming from the digestive tract as well as concentrations of substances within the blood. The hormone ghrelin, dubbed the “hunger hormone” is secreted by the stomach and duodenum when hungry, whereas its secretion is decreased by increasing levels of glucose, fatty acids and dietary fiber following food intake (Gormsen et al., 2006; Guo et al., 2008; Shiiya et al., 2002). Insulin, a hormone produced by the pancreas, is released following food intake to decrease blood glucose levels and initiate glycogenesis (Berg et al., 2002). Another hormone, leptin, is produced by white adipose tissue at a level proportional to the amount of white adipose tissue in the body (Considine et al., 1996).

These hormonal signals of both satiety and hunger, such as insulin, leptin and ghrelin, are able to act on circumventricular organs outside the blood-brain-barrier or adjacent brain areas where neurons bear receptors for these chemicals (Golden et al., 1997). Following food intake, increases in insulin and leptin activate anorexigenic neurons and inhibit orexigenic neurons in the brain, suppressing appetite (Cowley et al., 2001; Qiu et al., 2014). On the contrary, when energy stores are metabolized in between meals, decreasing levels of insulin and an increase in ghrelin in part stimulate hunger and promote the initiation of food intake.

The specific role of the brain, and in particular the hypothalamus, in food intake was first hypothesized from patients suffering from Fröhlich's syndrome, a disorder characterized by low levels of gonadotropin releasing hormone that results from hypothalamic abnormalities (Copeman, 1927; Morgan, 1959). Patients with the disorder engaged in excessive feeding behavior, suggesting to clinicians that the hypothalamus played a significant role in food intake. Following this, brain lesioning in animal studies revealed regions of the brain involved with feeding. Damage to the ventromedial nucleus of the hypothalamus produced a voracious appetite, leading to it to be termed the “satiety center,” whereas lesions of the lateral
hypothalamic area produced the cessation of feeding behavior, leading to starvation and death (Brobeck et al., 1943). Thus, this led to the lateral hypothalamus being labelled the “feeding center” (Anand & Brobeck, 1951). In the years following these lesion studies, comparably more precise and refined electrical stimulation studies were conducted, in which specific brain regions were stimulated with electrodes. Animals would subsequently eat ravenously if stimulated at the lateral hypothalamus, furthering the idea that particular parts of the brain control certain aspects of ingestive behavior (Hoebel, 1969). Later, these regions of the brain would be found to contain receptors for the peripheral signals of satiety and hunger, thus providing mechanistic evidence as to how the brain and body interact to regulate food intake.

In addition to homeostatic control of food intake, external food cues and psychological factors also play roles in hedonic control of feeding (Lutter & Nestler, 2009; Saper et al., 2002). Central control of homeostatic food intake occurs when metabolic signals indicating the need for energy are up- or downregulated, such as with leptin, ghrelin and other factors. On the other hand, hedonic food intake occurs outside the metabolic need for energy balance. It depends especially on external food cues such as the availability of palatable, calorie-rich food. Cues such as sight, smell or taste of food can stimulate brain regions involved with motivation and reward, promoting food intake for the pleasurable sensations it can provide (Amin & Mercer, 2016; Hussain & Bloom, 2012).

1.2.2 Hypothalamic control of food intake

The hypothalamus is considered to be the homeostatic control center of the body, maintaining such processes as body temperature, fluid balance, sleep-wake cycles, and food intake. It also serves to modify behaviors in order to maintain homeostasis of these processes. The hypothalamus contains multiple nuclei with distinct neuronal populations that produce
unique neuropeptides that function to increase or decrease feeding related sensations and behaviors. As previously mentioned, lesioning and stimulation studies first brought to light the integral role of the hypothalamus in feeding behavior (Brobeck et al., 1943; Hoebel, 1969). These studies highlighted the paraventricular, arcuate and lateral hypothalamic regions as being specific regions related to satiety and hunger. These nuclei have significant reciprocal projections with each other and with other brain regions. Furthermore, the arcuate nucleus is located adjacent to circumventricular organs. This allows these nuclei to regulate feeding behavior and food intake based upon the levels of peripheral signals.

As previously stated, the maintenance of energy homeostasis and food intake can be thought of as a series of competing satiety and hunger signals integrated by the hypothalamus. The arcuate nucleus of the hypothalamus is the first to respond to changes in peripheral signals due to its proximity to circumventricular organs. These first order neurons include the orexigenic neuropeptide-Y and agouti related peptide (AgRP/NPY) expressing and the anorexigenic pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART) expressing neurons (Cowley et al., 2001). Satiety signals such as leptin and insulin are increased following food intake, and decrease the activity of NPY/AgRP neurons and excite POMC/CART neurons (Cowley et al., 2001). Hunger signals like ghrelin have the opposite effect (Wang et al., 2002). In addition, NPY has been shown to inhibit POMC/CART neurons, suggesting that complex interactions between peripheral signals and the interaction of these neuronal populations in part regulate hunger and satiety (Roseberry et al., 2004).

These key neurons of the arcuate nucleus have significant projections to second order neurons in other hypothalamic nuclei, including the lateral hypothalamus (Elias et al., 1999). The lateral hypothalamus contains neurons that produce orexigenic peptides, including the orexins
and melanin-concentrating hormone (MCH). Both the arcuate and lateral hypothalamic nuclei affect one another in promoting food intake. For example, administration of an NPY-Y1 receptor antagonist, BIBO3304, partially attenuated orexin-induced feeding, suggesting that communication between the two nuclei in part regulate food intake (Yamanaka et al., 2000). Furthermore, the lateral hypothalamus lies at the intersection of homeostatic and hedonistic feeding due to the nature of its reciprocal projections within the hypothalamus and outside regions, including significant projections with dopaminergic ventral tegmental area (VTA) neurons as well as the nucleus accumbens (Fadel & Deutch, 2002; Stratford & Kelley, 1999). Animals will lever press in order to receive electrical stimulation of the lateral hypothalamus, and electrical stimulation has also been shown to increase feeding behavior (Huston, 1971; Mogenson & Stevenson, 1966). Together, these are indicative of the dual role of the lateral hypothalamus in both central control of food intake as well as motivation and reward.

1.2.3 Neurobiology of binge eating

The neurobiology of binge eating, unlike normal feeding behavior, may rely less on homeostatic mechanisms and may be modulated primarily by hedonic mechanisms. In terms of homeostatic factors, some have argued that episodes of binge eating are instances in which certain satiety mechanisms do not function properly, leading to a prolonged meal and excessive food intake (Blundell & Finlayson, 2004). Some studies have suggested abnormal levels of neuropeptides related to hunger and satiety, including lower basal or post-prandial levels of anorexic cholecystokinin and peptide YY, and decreased basal or increased post-prandial levels of ghrelin in those suffering from eating disorders compared to the general population (Geliebter et al., 2004; Monteleone et al., 2005). Another study addressing the same parameters had conflicting results, suggesting that the baseline levels of these hormones before a meal are
similar between groups. However, they found increased levels of meal-induced cholecystokinin and peptide YY in a binge eating population compared to weight-matched controls (Munsch et al., 2009). Considering the heterogeneity of feeding behaviors in binge-eating disorders, it is possible that the observed differences in peripheral signals are dependent on specific eating patterns and not binge-eating alone. Despite this, all forms of binge eating share a hedonic force driving the behavior.

The psychological motivation to eat primarily derives from the rewarding sensations provided by foods that are palatable, typically high in sugar and fat. This preference is partly mediated by the mesolimbic dopamine system and the endogenous opioid system. Rewarding sensations have long been associated with dopamine release from neurons in the VTA onto the nucleus accumbens, and palatable foods high in sugar or fat have been shown to stimulate this dopamine release (Avena et al., 2006; Rada et al., 2005; Rada et al., 2012). In addition, it has been shown in animal models that larger amounts of food result in a larger release of dopamine (Meguid et al., 1995). It is possible that the chronic release of dopamine from constant ingestion of foods high in sugar and fat modify the natural rewarding mechanisms of food intake, producing a feed-forward cycle of overeating, perhaps a similar mechanism observed in drug addiction (Alsiö et al., 2012). In addition to the release of dopamine, many studies have demonstrated that the hedonic consumption of sugars and fats is dependent on the activation of mu-opioid receptors, which have been correlated with feelings of pleasure, as well as being a factor involved with the intake of drugs of abuse (Chang et al., 2007; Colantuoni et al., 2001).

Binge eating has often been compared to drug addiction, with the first mention of so-called “food addiction” taking place several decades ago (Randolph, 1956). Many of the DSM-5 criteria for substance use disorders can be applied to those suffering from binge eating disorders,
including: use of larger amounts of drug, or food, over time; physical or psychological problems related to usage including negative affect or hangover effects; or spending excess time either engaging in the behavior or recovering from it, such that other obligations or activities are not met (Meule & Gearhardt, 2014; Smith & Robbins, 2013). This point of view in part led to the development of the Yale Food Addiction Scale, a diagnostic tool used to clinically assess the presence or severity of binge eating behavior in viewing it as an addictive behavior similar to drug abuse or compulsive gambling (Gearhardt, 2009). As previously mentioned, foods high in refined sugars and fats cause excessive dopamine release and activation of mu-opioid receptors in the brain, similar to commonly abused drugs such as nicotine, opiates and alcohol. Those with addictive disorders and those with binge eating disorders have also been shown to share similar genetic traits related to the dopamine system (Wang et al., 2001). In addition, psychosocial stressors often precipitate a binge episode, similar to how stress often precedes a drug binge (Hudson et al., 2007). Therefore, binge eating and addiction have similar behavioral and neurochemical properties.

However, binge eating also has aspects dissimilar to those of substance use disorders. The operational definitions of the disorders draw differences between the time frames of either a substance or food-related binge episode. Where drug use may be a near constant daily behavior, binge eating takes place in discrete periods of time, often lasting only a few hours maximum (Gearhardt et al., 2011). Furthermore, withdrawal and tolerance are two of the diagnostic criteria for substance dependence, whereas these criteria are not part of those for eating disorders in the DSM-5 (American Psychiatric Association, 2013). Nonetheless some studies have suggested that in animals trained to binge on palatable foods, administration of naloxone, an opioid receptor antagonist, produces measurable withdrawal symptoms such as tremor and anxiety, whereas
these effects were not present in control animals (Colantuoni et al., 2002). Others have suggested that not all people engaging in binge eating do so in the same way, classifying some people's binge eating as more “compulsive” or “addictive” in nature compared to that of others. For example, some people experience withdrawal-like symptoms such as negative affect, difficulty sleeping and inability to concentrate when not bingeing (Cassin & von Ranson, 2007). In summary, despite some differences, biological and psychological characteristics are highly similar between binge eating and substance dependence, which may suggest that these disorders are more alike than different.

1.3 Orexin and MCH

The lateral hypothalamus contains the cell bodies of both orexin and MCH-producing neurons that mediate both reward and food intake based upon connections with other brain regions. Thus, it is highly likely that these neurons play a significant role in binge eating that involves hedonic control of food intake. In the adult rat, orexin neurons are located approximately 2.3mm to 3.7mm posterior from the bregma, and MCH neurons from approximately 2.1mm to 3.9mm (Nambu et al., 1999; Sapin et al., 2010). Both orexin and MCH neurons are found in the lateral hypothalamic area as well as the perifornical area, whereas MCH neurons also extend into the anterior regions of the zona incerta and more medial regions (Hahn, 2010). These neurons project widely throughout the brain in order to regulate arousal, sleep, reward, stress, motivation and feeding behavior (Barson et al., 2013). A brief overview of some of the connections between these neurons and others regulating food intake and reward can be seen in Figure 1.
Peripheral signals influencing food intake, such as circulating leptin and ghrelin, activate or inhibit anorexigenic and orexigenic neurons in the arcuate nucleus, respectively. Orexigenic neuropeptide Y/agouti related peptide (NPY/AgRP) neurons of the arcuate nucleus inhibit orexin neurons, as do local MCH neurons in the lateral hypothalamus. In turn, orexin neurons project to NPY/AgRP neurons. Food intake stimulates the release of dopamine from the ventral tegmental area onto the nucleus accumbens, and both regions share projections to and from the lateral hypothalamus, where dopamine can activate or deactivate orexin neurons depending on the
concentration of dopamine release, whereas orexin has excitatory effects on dopaminergic neurons.
1.3.1 Orexin

The orexin, or hypocretin, peptides were independently discovered by two groups nearly twenty years ago (de Lecea et al., 1998; Sakurai et al., 1998). The name orexin derives from the Greek *orexis*, for appetite, while hypocretin is a portmanteau of hypothalamus and secretin, another neuropeptide that in part regulates water homeostasis. Orexins are produced by cleavage of the prepro-orexin peptide, resulting in orexin A and orexin B peptides. Orexin receptors are G-protein coupled receptors, OX1R and OX2R. Orexin A has equal affinities for both receptor types, whereas orexin B has a higher affinity for OX2R than OX1R. Orexin peptides are typically excitatory in nature, with activation of both orexin receptors resulting in an increase in intracellular calcium (Sakurai et al., 1998). While orexin neurons are only located in the lateral hypothalamus, they send extensive projections throughout the cerebrum and brainstem in order to regulate many functions. For example, orexins’ projections to noradrenergic neurons of the locus coeruleus in part maintain arousal, and projections to the limbic system mediate mood (Hagan et al., 1999; Winsky-Sommerer, 2004). Injections of orexin A into the lateral ventricles have also been shown to promote anxiety-like behaviors compared to saline control, implicating orexin activity with the stress system (Suzuki et al., 2005).

Orexins have been shown to play a positive role in food intake, particularly the intake of palatable foods. Injection of orexin-A directly into hypothalamic nuclei including the lateral hypothalamus and paraventricular nucleus, as well as the nucleus accumbens, have been shown to promote intake of standard chow in sated rodents (Dube et al., 1999; Thorpe & Kotz, 2005). This effect has also been demonstrated when the food is palatable, and specifically increased consumption of a high fat food over a high carbohydrate food (Clegg et al., 2002). Furthermore, intraperitoneal administration of the OX1R antagonist SB-334867 preferentially decreases intake
of palatable high fat food in sated rats compared to vehicle injection, implicating that orexin activity may be necessary for hedonic food consumption (Choi et al., 2010).

The role of orexins in modulating feeding behavior is also partly based on orexin neurons’ connections with other hypothalamic neurons regulating feeding. Orexins activate orexigenic NPY/AgRP neurons of the arcuate nucleus, whereas NPY inhibits orexin neuron firing (Fu, 2004; Yamanaka et al., 2000). NPY/AgRP neurons are directly responsive to peripheral signals such as leptin and ghrelin, thus orexin neurons are also downstream targets of these peripheral signals of hunger and satiety.

Orexin neurons also have projections to the VTA and the nucleus accumbens. Orexin peptides activate dopaminergic neurons of the VTA, as well as neurons of the nucleus accumbens shell, suggesting a role for orexins in reward and motivation (Korotkova et al., 2003; Mukai et al., 2009). On the other hand, our lab previously demonstrated that dopamine modulates GABAergic transmission to orexin neurons, disinhibiting them at low concentrations and inhibiting activity at higher concentrations (Linehan et al., 2015).

Given its connection with the VTA dopaminergic system, it is no surprise that orexins have been shown to play a role in substance dependence. Orexin-A injection results in reinstatement of drug seeking in addiction models, including alcohol and nicotine dependence (Lawrence et al., 2006; Plaza-Zabala et al., 2010). Conversely, orexin knock-out mice display a lower intensity of morphine dependence and opioid antagonist-induced withdrawal symptoms following repeated morphine exposure than wildtype mice, implicating orexin neurons in the development of substance dependence (Georgescu et al., 2003).
Similar effects have been noted for palatable food intake. Orexin injection into the fourth ventricle increases progressive ratio response for sucrose in rats, whereas injection of the OX1R antagonist SB334867 prevented the acquisition of a high-fat-induced conditioned place preference (Kay et al., 2014). Additionally, SB334867 administration reduced the cue-induced reinstatement of sucrose-seeking in food restricted rats (Cason & Aston-Jones, 2014). Finally, in a mouse model of binge-like consumption of sucrose or saccharin solutions, intraperitoneal administration of SB334867 significantly decreased the intake of these solutions without altering arousal as measured by locomotion (Alcaraz-Iborra et al., 2014). Considering the similarities between substance dependence and binge eating and the role orexins play in both food intake and drug dependence, these findings suggest a role for orexin activity in binge-like feeding behavior.

1.3.2 Melanin-concentrating hormone (MCH)

MCH was first discovered in the pituitary of teleost fishes as a factor that prevents changes in skin pigment by acting as an antagonist of alpha-melanocyte stimulating hormone (Kawauchi et al., 1983). Following this, the peptide was identified in the hypothalamus of rodents, and was found to be the natural ligand of an orphan receptor (Chambers et al., 1999). There are two isoforms of MCH receptor identified, MCH1R which is found in all vertebrates, and MCH2R which is found only in certain mammalian species, including humans, dogs, ferrets and others (Tan et al., 2002). MCH has been demonstrated as playing a role in sleep wake cycles, depressive behaviors, and food intake (García-Fuster et al., 2012; Qu et al., 1996; Verret et al., 2003). MCH receptor activation generally results in decreased neuronal activity, and MCH has been shown to reduce the activity of neighboring orexin neurons (Gao & Van Den Pol, 2001; Rao et al., 2008). However, repeated intracerebroventricular MCH administration resulted in an
increase in NPY gene expression, suggesting that MCH may activate other orexigenic neurons of the hypothalamus (Della-Zuana et al., 2002).

Like orexin neurons, MCH neurons project widely across the brain, although the effects of MCH can be considered complementary, and sometimes opposite, to those of orexin. Where orexin promotes energy expenditure and wakefulness, the MCH system has been shown to have a negative effect on energy expenditure, as can be seen in the hyperactive phenotypes of animals with MCH knockout (Alon and Friedman, 2006). Indeed, MCH neurons are relatively silent during wakefulness and display spontaneous firing activity during REM sleep (Hassani et al., 2009). However, both peptide systems have been linked to states of stress. Administration of MCH produces anxiety-like behaviors in rats as measured by behaviors in the elevated plus maze, whereas the MCH1R antagonist GW3430 attenuates the anxiogenic effects of MCH when co-administered, and produces anxiolytic effects when administered alone (Smith et al., 2005). Furthermore, MCH neurons have also been implicated in reward and motivation. The nucleus accumbens shell, a prominent structure in reward and addiction, has a dense concentration of MCH1R receptors, strongly suggesting a role in reward (Saito et al., 2001). Activation of the MCH1R receptor has been shown to potentiate cocaine-induced increases in locomotion, and blockade or knockout of these receptors results in reduced response to cocaine and conditioned place preference (Chung et al., 2009).

Also similar to orexin neurons, MCH neuron activation increases palatable food consumption, and palatable foods in turn activate MCH neurons (Gomori et al., 2003). While MCH has been shown to promote food intake, antagonism of MCH1R has been shown to reduce the conditioned responses and reinstatement for sucrose-seeking (Karlsson et al., 2012). Also, compared to wildtype controls, MCH1R knockout mice display decreased levels of overeating.
when presented with a conditioned stimulus for sucrose availability, suggesting MCH plays a role in non-homeostatic feeding (Sherwood et al., 2015). Considering the roles that both orexin and MCH neurons have in food intake and addictive behaviors, it is possible that both groups of neurons play significant roles in the development or maintenance of binge-like feeding behaviors.

1.4 Rationale and Objectives

Because binge eating is a hedonic form of feeding that parallels addiction, it likely involves the reward system and food intake control center. Orexin and MCH neurons are important regulators of food intake and the reward system. Therefore, I hypothesized that these neural populations are functionally altered in a rat model of binge-like feeding in a manner that promotes binge eating. This thesis tested this hypothesis by examining the electrophysiological properties of both orexin and MCH neurons in rats displaying binge-like feeding on palatable diets.

Objective 1 – Establishing an animal model of binge eating

In order to investigate any possible correlations of altered function of orexin or MCH neurons in binge-like feeding, a reliable, robust animal model of binge eating is necessary. Because both food restriction and physical stress may have direct and indirect effects on orexin and MCH neurons irrespective of binge eating, I utilized an animal model of binge eating using intermittent access to palatable foods.

Objective 2 – Characterize orexin and MCH neurons in the binge eating model

To determine functional changes of these neurons associated with binge eating, in-vitro electrophysiological recordings were performed. This research may represent the first
electrophysiological study of orexin and MCH neurons following induction of binge-eating behavior in rats. Any associated changes may represent unique mechanisms that underlie binge eating, which can lead to the development of novel treatments for binge eating disorders.
Chapter 2 - Materials and Methods

All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care, under the approval of the Memorial University Institutional Animal Care Committee.

2.1 Animal Models

Male and female 3 to 4 week old Sprague-Dawley rats (40-100g) were obtained from the breeding colony of Memorial University. Rats were singly housed and acclimatized for 3 days before the feeding paradigms started in a temperature and light controlled room (12:12h light-dark schedule, with lights on from 8:00 am to 8:00 pm) within the animal care facility in the Health Sciences Centre. Chow (Prolab RMH 3000) and water were provided ad-libitum. For ad libitum feeding, chow was placed on the food hopper.

2.1.1 Intermittent palatable diet feeding paradigms

1-week daily feeding (1wDaily) paradigm: following acclimatization, rats were allowed in-cage access from 2:00 pm to 3:00 pm on 6 to 11 consecutive days to one of three diets: standard chow (29.8% protein, 13.4% fat, 56.7% carbohydrate; 3.08kcal/g), a high fat diet (HFD) (Research Diets D12492, 20% protein, 60% fat, 20% carbohydrate; 5.24kcal/g), and a “western diet” (WD) high in sugar and fat (Test Diet AIN76-A, Western Diet, 15.5% protein, 40.1% fat, 44.4% carbohydrate; 4.49kcal/g).

2-week Monday, Wednesday, Friday feeding paradigm (2wMWF): Following acclimatization, rats had 1-hour in-cage access from 2:00 pm to 3:00 pm to a known amount of one of two diets, chow or HFD on Mondays, Wednesdays and Fridays (MWF) for a total of 6 to 9 in-cage food exposures.
6-week MWF feeding paradigm (6wMWF): This paradigm followed the same weekly schedule as the 2wMWF feeding paradigm, except rats were given at least 18, up to 26 in-cage food exposures.

For all animals, the in-cage diets were removed following the one hour exposure and weighed with a digital scale in order to assess the amount of the diet consumed. Daily chow intake from the food hopper was also measured at this time. Caloric intake from the in-cage food and chow in the hopper were calculated from the weight of food that disappeared and the caloric density of each food. Total daily caloric intake was the sum of calories consumed from the two sources of food. A percentage of caloric intake from intermittent exposure to in-cage food was calculated by dividing the calories consumed from intermittent feeding by the total daily caloric intake. This percentage of total caloric intake consumed during the 1-hour feeding was used as a measure of binge-feeding intensity. Initial body weights were measured upon arrival and final body weights for each rat were recorded prior to sacrifice.

2.2 In-vitro electrophysiology

2.2.1 Slice Preparation

Each rat was sacrificed the morning following their final 1-hour food exposure. Rats were anaesthetized with isoflurane and decapitated, and the brains removed, trimmed, and placed in a vibratome (Leica Microsystems VT1000S) chamber filled with ice-cold buffer solution perfused with 95%O₂/5%CO₂, containing (in mM): 87 NaCl, 2.5 KCl, 25 NaHCO₃, 7 MgCl₂, 1.25 NaH₂PO₄, 25 glucose, 30 sucrose, 3 pyruvic acid, 1 ascorbic acid, 0.5 CaCl₂. Slices were coronally sectioned at 250µm and then incubated for 30 minutes in 32-34°C artificial cerebrospinal fluid (ACSF) perfused with 95%O₂/5%CO₂, containing the following (in mM):
119 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 25 NaHCO₃, 2.5 glucose, 1 ascorbic acid, 0.5 CaCl₂. Following this, slices were maintained at room temperature in ACSF perfused with 95%O₂/5%CO₂ until patch clamp electrophysiology.

2.2.2 Whole-cell patch-clamp

Hemisected slices were placed in a recording chamber with ACSF bubbled with 95%O₂/5%CO₂ perfused at 1.5-2.5 mL/min at 26-28°C under an infrared-differential interference contrast microscope (DM LFSA Leica Microsystems). Whole-cell patch-clamp experiments were conducted using a Multiclamp 700B amplifier and pClamp 9.2 and 10.3 software (Molecular Devices). The recording pipettes had a tip resistance between 3-6MΩ when filled with internal solution containing the following (in mM): 123 K-gluconate, 2 MgCl₂, 8 KCl, 0.2 EGTA, 10 HEPES, 4 Na₂ATP, 0.3 Na-GTP, 7.3pH. The internal solution also included 2.2mM biocytin for post-hoc immunohistochemistry.

Voltage-clamp mode at -70mV was used to form a gigaohm seal before breaking into the cell, at which point membrane capacitance, membrane resistance, and access resistance were measured and recorded. Neurons with membrane resistance below 200MΩ or access resistance above 20 MΩ were excluded from analysis. Current-clamp mode was used to identify putative orexin and MCH neurons by their electrophysiological responses to hyperpolarizing and depolarizing current steps ranging from -200pA to 200pA in 50pA increments. These recordings were also used to assess resting membrane potential, latency to first spike, threshold, and elicited action potential frequency. A current ramp protocol was also used which injected current from 0pA to 100pA or 200pA over five seconds. Resulting responses were used to assess rheobase, or the minimum current injection necessary to elicit an action potential, and elicited action potential
frequency. Recordings were digitized at 20kHz in Clampex, and at 1kHz in Axoscope, and stored for off-line analysis.

2.2.3 Post-hoc immunohistochemistry of recorded cells

Following patch-clamp, brain slices were placed in 10% formalin (Sigma Aldrich) at least overnight, then washed three times for ten minutes in phosphate buffer solution (PBS). Primary antibodies were prepared by diluting anti-Orexin A goat polyclonal IgG (1:2000 dilution; Santa Cruz Biotechnology) and anti-MCH rabbit polyclonal IgG (1:2000 dilution; Phoenix Pharmaceuticals) in PBS with 0.3% Triton-X (Fisher Scientific). After washing, slices were placed in a 96-well plate and submerged in 70µL of the primary antibody solution, covered, and kept at 4°C for 72 hours. Following incubation with primary antibodies, slices were washed again in PBS. Secondary antibody cocktail contained 1:500 donkey anti-goat Alexa Fluor 488 (Invitrogen), 1:500 donkey anti-rabbit Alexa Fluor 594 (Invitrogen) and 1:500 streptavidin Alexa Fluor 350 (Life Technologies). Slices were incubated in secondary antibodies in dark, overnight at 4°C. After incubating in secondary antibodies, sections were again washed in PBS and mounted onto glass slides with Dako Fluorescent Mounting Medium (Dako, Agilent Technologies). Slices were then assessed under a fluorescence microscope to determine whether biocytin-labelled cells were positive for orexin A or MCH peptide. A small number of patched cells without clear biocytin-labelling (24 of 152 orexin neurons, or 15.8%; and 9 of 51 MCH neurons, or 17.6%) were included in the analysis based upon their electrophysiological responses to hyperpolarizing and depolarizing currents.
2.3 Data Analysis

Electrophysiological data was analyzed using Clampfit 9.2 and 10.3, (Molecular Devices). All data are expressed as mean ± standard error, where n represents the total number of cells per group. Statistical analysis was performed for feeding, body weight and electrophysiological data using appropriate tests in GraphPad Prism 6 software (GraphPad Software). Student’s t tests, one-way or repeated measures two-way ANOVA were used to analyze group data. Multiple comparisons were made using the Sidak test where appropriate. Statistical significance was chosen as p<0.05.
Chapter 3 - Results

3.1 Behavioral responses to intermittent palatable food exposures

3.1.1 Feeding Behavior of male 1wDaily access group

In order to determine whether a 1-hour intermittent feeding paradigm induces binge-like feeding behavior, rats were given 1h-access daily to control diet (chow, n=11), 60% high fat diet (HFD, n=16) or western diet (WD, n=6), and caloric intake from these diets was measured daily. Compared to the chow group, the HFD group consumed more calories during the one-hour feeding period on each day of intermittent exposure, whereas the WD group consumed more calories from day 2 onward, and this amount increased over elapsed days (Fig 2A, main effect of diet p<0.0001; main effect of elapsed days p<0.0001; interaction, p<0.0001; two-way ANOVA). The groups consumed different amounts of calories from chow from the food hopper (Fig. 2B, main effect of diet, p<0.0001; main effect of elapsed days, p<0.05; interaction p<0.001; two-way ANOVA). Multiple comparisons revealed that control and WD groups consumed a comparable amounts of hopper chow during the study, however the HFD group consumed less chow on the first (p<0.05), third (p<0.001), fourth (p<0.001), fifth (p<0.001) and sixth days (p<0.0001) day compared to chow control. This decrease is likely to be compensatory, because as mentioned above, the amount of HFD consumption significantly increased with repeated daily exposures within groups, whereas the chow group consistently consumed similar amount of calories during the 1h period on consecutive days. All groups consumed a similar amount of total calories throughout the 6-day feeding period, but this amount changed with time (Fig. 2C, effect of diet, p>0.05; main effect of elapsed days, p<0.0001; interaction, p>0.05, two-way ANOVA). Both the HFD and WD groups consumed a substantial proportion of their daily caloric intake from the palatable diets during the 1-hour exposure compared to chow control, and this proportion
increased over time (Fig. 2D, main effect of diet, p<0.0001; main effect of elapsed time, p<0.0001; interaction, p<0.0001; two-way ANOVA). We compared the percent of total daily intake during the 1-hour food exposure during the latter half of the food exposure between the three groups as a measure of the plateau of binge-like feeding. There was a significant difference between the groups (Fig. 2E, p<0.0001, chow mean=5.1±2.7%, HFD mean=27.1±8.5% and WD mean=17.9±2.9%, one-way ANOVA), and multiple comparisons revealed a difference between chow and HFD (p<0.0001), chow and WD (p<0.01) and HFD and WD (p<0.05). This suggests that both HFD and WD access groups engaged in binge-like behavior, and it was more robust in the HFD group.

Initial body weights were taken for a subsample of the chow (n=10) and HFD (n=9) group, and there was no significant difference between the groups (Fig. 3A, chow=86.4±3.1g, HFD=74.8±5.2g, p>0.05, Student’s t-test). Body weight gain was also compared between the two groups, and again there was no significant difference between groups (Fig. 3B, chow=87.2±6.8g, HFD=91.6±11.5g, p>0.05, Student’s t-test).
Figure 2. 1wDaily intermittent palatable diet access induces binge-like feeding

A. Caloric intake from food provided in-cage for 1h. B. 24h intake of chow provided *ad libitum* via a food hopper. C. Daily total calorie intake was calculated as a sum of 24h-intake of chow provided *ad libitum* and 1h-intake of chow, HFD or WD. D. Percentage of calories consumed during the 1h access to chow, HFD or WD with respect to total daily caloric intake. E. Average of the proportion of caloric intake from 1h access to chow, HFD or WD with respect to total caloric intake from their respective plateaus. Chow, black closed circles (n=11), HFD, gray closed circles (n=16) and WD, open circles (n=6). * for control vs HFD, *p<0.05, **p<0.01, ****p<0.0001 + for control vs WD, +p<0.05, ++p<0.01, +++p<0.001, +++++p<0.0001
Figure 3. 1wDaily access to HFD does not alter body weight gain

A. Initial body weights for a subsample of rats in 1wDaily chow (n=10) and HFD (n=9) groups.

B. Body weight gain was calculated as initial weight subtracted from final weight before sacrifice.
3.1.2 Feeding behavior of male 2wMWF feeding group (3 access/week)

Previous literature suggests that both daily intermittent diet access (Bake et al., 2014; Kelley et al., 2003) and diet access three times per week (MWF) (Corwin et al., 1998; de Jong et al., 2013) are able to induce binge-like behavior. Some studies suggest that intermittent access in MWF schedule results in greater binge-like behavior (Babbs et al., 2012; Corwin et al., 1998; Dimitriou et al., 2000). Therefore, we decided to use an alternative feeding schedule to assess whether it can produce more robust binge-like behavior as measured by the percent of total daily calories from HFD, or whether the time course of behavioral responses changes. We chose to test HFD and exclude WD from this paradigm, since HFD produced stronger binge-like behavior than WD in the 1wDaily feeding model. To test for any differences between the patterns of HFD exposure, i.e. daily or three times a week on non-consecutive days, the number of intermittent HFD access periods was matched to that of the 1-week group.

The chow (n=4) and HFD-fed (n=4) groups consumed a different amount of calories during the 1h feeding period, which increased over time (Fig. 4A, main effect of diet, p<0.01; main effect of number of exposures, p<0.0001; interaction, p<0.0001, two-way ANOVA). Multiple comparisons revealed that the amount of caloric consumption during the 1h period significantly increased with repeated exposures, resulting in significantly different caloric intake during the 1h feeding period from the second exposure onward. Both groups consumed a similar number of calories from chow from the hopper during the study (Fig. 4B, effect of diet, p>0.05; effect of number of exposures, p>0.05; interaction, p>0.05; two-way ANOVA), and total daily caloric intake was not different between the chow control and HFD groups (Fig. 4C, effect of diet, p>0.05; effect of number of exposures, p>0.05; interaction, p>0.05; two-way ANOVA). The HFD group consumed a larger percentage of their calories during the 1-hour food exposure,
which increased with successive exposures (Fig. 4D, main effect of diet, p<0.01; main effect of number of exposures, p<0.01), interaction, p<0.01; two-way ANOVA). The chow group consistently consumed fewer calories during the 1h exposure.

To better characterize the feeding behavior, we asked whether 1h bingeing on HFD influenced the chow intake on the same day as well as subsequent days. Specifically, we compared the total chow consumed on binge days (1h exposure to HFD) with those on the subsequent day (non-binge day). The rats consumed a similar amount of chow from the hopper on binge day compared to the subsequent day (Fig. 5A, effect of day, p>0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05; two-way ANOVA). Overall, 1h access to HFD had no significant effect on total calories consumed (Fig. 5B, no effect of day, p>0.05; main effect of number of exposures, p<0.0001; interaction, p<0.01; two-way ANOVA), however multiple comparisons revealed that the total calories consumed on the day of the sixth intermittent access were greater than those of the following day (p<0.05).

In order to assess the effect of binge-like feeding on body weight, body weights were measured at day one and on the final day. There were no differences in initial body weight of chow (67.2g±1.8g) and HFD groups (67.2g±0.8g) (Fig. 6A, p>0.05, Student’s t-test), or in the total body weight gain of chow (247.5g±15.1g) and HFD (269.0g±16.6g) groups (Fig. 6B, p>0.05, Student’s t-test).
Figure 4. 2wMWF intermittent HFD access induces binge-like feeding.

A. Caloric intake from food provided in-cage for 1h. B. 24h intake of chow provided *ad libitum* via a food hopper. C. Daily total calorie intake was calculated as a sum of 24h-intake of chow provided *ad libitum* and 1h-intake of chow or HFD. D. Percentage of calories consumed during the 1h access to chow or HFD with respect to total daily caloric intake. Chow, closed black diamonds (n=4); HFD, open grey diamonds (n=4). *p<0.05, **p<0.01, ***p>0.001, ****p<0.0001. Black arrowheads represent 1h in-cage food exposures.
Figure 5. 2wMWF in-cage chow or HFD access does not alter food intake between binge day and the subsequent day

A. For HFD group, total daily caloric intake on binge day consisted of calories from hopper chow plus calories from in-cage HFD. Total caloric intake on the subsequent day was only calories from hopper chow. B. Chow intake from the hopper on binge day versus the subsequent day. Binge day, open grey diamonds, closed line; subsequent day, closed black squares and dashed line. **p>0.01.
Figure 6. 2wMWF access to HFD does not alter body weight gain

A. Initial body weights for 2wMWF chow (n=4) and HFD (n=4) groups. B. Body weight gain was calculated as initial weight subtracted from final weight before sacrifice.
3.1.3 Comparison of behavioral response to different frequency of intermittent palatable food exposures

Literature suggests that greater intervals of intermittent feeding result in greater binge-like behavior (Corwin et al., 1998; Wojnicki et al., 2013), so we compared these two groups with different feeding intervals to determine whether binge eating was more intense in 2wMWF paradigm with longer intervals between feeding episodes than the daily schedule.

We found no difference overall in the calories consumed between one-week and two-week groups (Fig. 7A, main effect of feeding schedule, p>0.05; main effect of number of exposures, p<0.0001, interaction, p<0.01; two-way ANOVA), although multiple comparisons revealed a significant difference on the sixth day, with rats on 1-week daily feeding paradigm (13.7±1.4kcal) consuming less than those on two-week MWF paradigm (24.7±3.9kcal) (p<0.01). Nonetheless, there was no significant difference between the two groups in the total percent of calories obtained from HFD (Fig. 7B, main effect of feeding paradigm, p>0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05; two-way ANOVA). Furthermore, multiple comparisons revealed no differences between the groups (p>0.05 on each number of exposure). This suggests that by the end of their respective intermittent feeding paradigms, both groups engaged in binge-like behavior of a similar magnitude.
Figure 7. Two schedules of intermittent HFD access promote similar feeding behaviors

A. Total calories from HFD in the 1wDaily (n=16) and 2wMWF (n=4) groups. B. Percent of daily calories from HFD in the 1wDaily and 2wMWF groups. 1wDaily HFD, closed grey circles; 2wMWF HFD, open grey diamonds. **p>0.01.
3.1.4 Feeding behavior of male 6wMWF (3 access/week)

Next, we decided to determine whether more repetition would lead to a more severe binge eating behavior. This time, we extended the MWF paradigm to six weeks, so that the number of intermittent accesses to HFD increased from six to eighteen.

The HFD group consumed more calories during the intermittent feeding than control group, and HFD intake increased over time (Fig. 8A, main effect of diet, p=0.0001; main effect of number of exposures, p<0.0001; interaction, p<0.0001; two-way ANOVA). However, the HFD group consumed less chow than the control group overall (Fig. 8B, main effect of diet, p<0.05, main effect of number of exposures, p<0.0001; interaction, p<0.0001; two-way ANOVA). As a result, both chow control and HFD group consumed a similar amount of total calories throughout the experiment (Fig. 8C, effect of diet, p>0.05; main effect of number of exposures, p<0.0001; interaction, p<0.01; two-way ANOVA). Again, the HFD group progressively consumed a larger proportion of calories during the 1h intermittent food access than control group (Fig. 8D, main effect of diet, p<0.0001; main effect of number of exposures, p<0.01; interaction, p<0.01; 2-way ANOVA) which plateaued after the third exposure.

Like the 2wMWF group, we compared the total calories and calories from chow consumed on the feeding day to the day after in the HFD group. We found that they consumed less calories from chow on the binge day (Fig. 9A, main effect of day, p<0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05 two-way ANOVA), but the total calories consumed was similar on binge days as the subsequent days (Fig. 9B, effect of day, p>0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05; two-way ANOVA). Thus, even though the animals display binge-like behavior upon HFD exposure, they are able to...
compensate for the excess calories consumed from HFD by reducing chow intake, suggesting that their homeostatic control is intact.

We also compared the initial body weights and body weight gain (Fig. 10B) between chow (n=7) and HFD (n=7) 6wMWF groups. There was no difference in initial body weights (Fig. 10A, chow=77.2±2.6g, HFD=70.0±3.9g, p>0.05, Student’s t-test) or in body weight gain (Fig. 10B, chow=434.8±15.1g, HFD=382.1±30.2g, p>0.05, Student’s t-test).
Figure 8. 6wMWF intermittent HFD access induces binge-like feeding in male rats.

A. Caloric intake from food provided in-cage for 1h.  B. 24h intake of chow provided ad libitum via a food hopper.  C. Daily total calorie intake was calculated as a sum of 24h-intake of chow provided ad libitum and 1h-intake of chow or HFD.  D. Percentage of calories consumed during the 1h access to chow or HFD with respect to total daily caloric intake. Chow (n=7), open white circles; HFD (n=7) closed blue circles. *p<0.05, **p<0.01, ***p>0.001, ****p<0.0001. Black arrowheads represent 1h in-cage food exposures.
Figure 9. 1h access to HFD has a short-term inhibitory effect on chow intake

A. For the HFD (n=7) group, total daily caloric intake on binge day consisted of calories from hopper chow plus calories from in-cage HFD. Total caloric intake on the subsequent day was only calories from hopper chow. B. Chow intake from the hopper on binge day versus the subsequent day. Binge day, closed blue circles and closed line; subsequent day, open squares and dashed line. *p>0.05.
Figure 10. 6wMWF access to HFD does not alter body weight gain in male rats

A. Initial body weights for 6wMWF chow (n=7) and HFD (n=7) groups. B. Body weight gain was calculated as initial weight subtracted from final weight before sacrifice.
3.1.5 Comparison of behavioral response to different number of intermittent palatable food exposures

In order to assess whether the magnitude of binge-like feeding would increase with a greater number of HFD exposures, we compared the percent of daily intake from HFD during the last three exposures for the 1wDaily, 2w- and 6wMWF groups. We chose the final three exposures because the intake was the greatest at these time points. There was no significant difference in the proportion of total calories coming from HFD between any group (Fig. 11, p>0.05, 1wDaily mean=27.1±8.5%, 2wMWF mean=25.1±6.5%, 6wMWF mean=26.8±6.1%, one-way ANOVA), suggesting that each schedule produced similar plateau of binge-like behavior.
Figure 11. 1wDaily, 2wMWF and 6wMWF intermittent access to HFD promote similar magnitudes of binge eating

Mean percentage of calories consumed during the 1h access from HFD with respect to total daily caloric intake in the last three HFD exposures for 1wDaily (27.1%, n=16), 2wMWF (25.1%, n=4) and 6wMWF (26.8%, n=7).
3.1.6 Feeding behavior of female 6wMWF group (3 access/week)

The literature suggests that binge-like behavior occurs more often in females than males (Hudson et al., 2007; Kessler et al., 2013). However, it is unclear whether this is due to psychosocial factors intrinsic to humans or biological differences between males and females. Therefore, we sought to determine whether there are any sex differences in binge-like behavior in our rat model.

Using the 6-week, three access a week paradigm, we found that the female HFD group consumed significantly more calories during intermittent HFD access than female control chow group and the calories consumed increased over time (Fig. 12A, main effect of diet, p<0.0001; main effect of number of exposures, p<0.0001; interaction, p<0.0001; two-way ANOVA). In contrast, the control group consumed significantly more calories from standard chow over 24h, than the HFD group (Fig. 12B, main effect of diet, p<0.05; main effect of number of exposures, p<0.0001; interaction, p<0.001; two-way ANOVA). Taken together, female control (n=7) and HFD group (n=6) consumed a similar amount of total daily calories (Fig. 12C, main effect of diet, p>0.05; main effect of number of exposures, p<0.0001; interaction, p<0.001; two-way ANOVA), but the HFD group consumed a higher proportion of daily calories during the intermittent feeding period than control group (Fig. 12D, main effect of diet, p<0.001; main effect of number of exposures, p<0.0001; interaction, p<0.01; two-way ANOVA).

The female HFD group consumed similar amounts of calories from hopper chow on binge days as subsequent days (Fig. 13A, main effect of day, p>0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05; two-way ANOVA). Furthermore, they consumed similar amounts of total calories on the binge days as the subsequent days (Fig. 13B, main effect of day, p>0.05; main effect of number of exposures, p<0.0001; interaction, p<0.05; two-way ANOVA).
ANOVA). This indicates that while HFD animals consumed similar amounts of chow on binge days as well as the subsequent days, overall they consumed less chow than the control group. These results contrast with those found in the male 6wMWF group, suggesting that intermittent HFD access affects male and female feeding behavior differently.

Like the males, we compared the initial body weights and body weight gain between female chow (n=6) and HFD (n=7) 6wMWF groups. There was no difference in initial body weights (Fig. 14A, chow=68.4±5.2g, HFD=72.8±1.5g, p>0.05, Student’s t-test) or in body weight gain (Fig. 14B, chow=194.7±7.4g, HFD=193.8±12.0g, p>0.05, Student’s t-test).

In order to assess any sex-specific differences in feeding and binge-like behavior, we compared the male and female 6wMWF HFD groups. HFD males and females consumed a similar number of calories from HFD during 1-hour food exposure (Fig. 15A, effect of sex, p>0.05; main effect of number of exposures, p<0.0001; interaction, p<0.05; two-way ANOVA). HFD males consumed more hopper chow (Fig. 15B, main effect of sex, p<0.01; main effect of number of exposures, p<0.0001; interaction, p<0.0001; two-way ANOVA), and more total daily calories (Fig. 15C, main effect of sex, p<0.01; main effect of number of exposures, p<0.0001; interaction, p<0.0001; two-way ANOVA) than HFD females. Nonetheless, males and females consumed a similar proportion of their daily calories from HFD on intermittent access days (Fig. 15D, effect of sex, p>0.05; main effect of number of exposures, p<0.0001; interaction, p>0.05; two-way ANOVA). Thus, the intensity of binge eating is similar between sexes.
Figure 12. 6wMWF intermittent HFD access induces binge-like feeding in female rats.

A. Caloric intake from food provided in-cage for 1h.  
B. 24h intake of chow provided *ad libitum* via a food hopper.  
C. Daily total calorie intake was calculated as a sum of 24h-intake of chow provided *ad libitum* and 1h-intake of chow, HFD or WD.  
D. Percentage of calories consumed during the 1h access to chow or HFD with respect to total daily caloric intake. Chow (n=7), open white triangles; HFD (n=6) closed red triangles. *p<0.05, **p<0.01, ***p>0.001, ****p<0.0001. Black arrowheads represent 1h in-cage food exposures.
Figure 13. 1h access to HFD does not alter total daily food intake in female rats

A. For the HFD group, total daily caloric intake on binge day consisted of calories from hopper chow over 24h plus calories from in-cage HFD during the 1-hour access. Total caloric intake on the subsequent day was only calories from hopper chow. B. 24-h chow intake from the hopper on binge day versus the subsequent day. Chow (n=7), open white triangles; HFD (n=6) closed red triangles. Binge day, solid line; subsequent day, dashed line.
Figure 14. 6wMWF access to HFD does not alter body weight gain in female rats

A. Initial body weights for 6wMWF chow (n=7) and HFD (n=6) groups. B. Body weight gain was calculated as initial weight subtracted from final weight before sacrifice.
Figure 15. 6wMWF exposure to HFD induces a similar magnitude of binge eating in male and female rats

A. Caloric intake from food provided in-cage for 1h.  B. 24h intake of chow provided *ad libitum* via a food hopper. C. Daily total calorie intake was calculated as a sum of 24h-intake of chow provided *ad libitum* and 1h-intake of chow, HFD or WD. D. Percentage of calories consumed during the 1h access to chow or HFD with respect to total daily caloric intake. Male (n=7), closed blue circles; Female (n=6) closed red triangles. *p<0.05, **p<0.01, ***p>0.001, ****p<0.0001. Black arrowheads represent 1h in-cage food exposures.
3.2 Electrophysiological properties of lateral hypothalamic neurons in binge eating animal model

Using the animal model established above, we investigated the electrophysiological changes in orexin and MCH neurons associated with binge eating.

3.2.1 Identification of orexin neurons

Orexin neurons are localized in the lateral hypothalamus and perifornical area, and characterized by their relatively large size compared to other neurons in the same area. Our lab has previously established a reliable method of identifying orexin neurons in young rats aged 3 to 4 weeks based on certain electrophysiological properties and post-hoc immunohistochemistry (Belanger-Willoughby et al., 2016; Linehan et al., 2015; Parsons et al., 2012). Specifically, Orexin neurons could be identified by H-currents upon application of hyperpolarizing currents, rebound depolarization often capped by action potentials after removal of hyperpolarizing currents, as well as spontaneous activity in the absence of current injection. The present study found the same electrophysiological characteristics in orexin neurons from rats six and eleven weeks old (Fig. 16A). A majority of cells that displayed these characteristics were confirmed to be immunopositive for orexin A (120 of 145 cells) and were included in the analysis (Fig. 16B). The remaining 25 neurons (17.2%) were not successfully filled with biocytin, however the latter subgroup was also included in the analysis because they displayed electrophysiological responses to hyperpolarizing and depolarizing currents typical of orexin neurons.

3.2.2 Properties of Orexin neurons in 1wDaily access group

Using a series of positive and negative step current injections in current clamp mode, we analyzed electrophysiological parameters in order to detect any differences between the three
diet groups under the daily intermittent access paradigm. Resting membrane potential did not differ between the control (-47.2±4.1mV, n=17 cells from 7 rats), HFD (-46.6±3.1mV, n=24 cells from 8 rats), or WD (-45.9±3.9mV, n=10 cells from 4 rats) groups (Fig. 16C, p>0.05, one-way ANOVA). Furthermore, no significant differences were found in spontaneous action potential frequency between the three groups (Fig. 16D, p>0.05, one-way ANOVA). In response to positive current injections, there was no overall difference in latency to the first spike between the groups (Fig. 16E, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p<0.05; two-way ANOVA). However, multiple comparisons revealed that the 50pA current injection was shorter in orexin neurons from HFD group compared to control group (p<0.05). There was no change in the threshold (Fig. 16F, p>0.05, one-way ANOVA) or the number of elicited action potentials (Fig. 16G, effect of diet, p>0.05; main effect of current injection; interaction, p>0.05; two-way ANOVA). Together, these results suggest that one week of intermittent HFD access does not affect basal excitability of orexin neurons, but may increase their sensitivity to depolarizing stimuli.
Figure 16. Daily intermittent access to HFD, but not WD, alters excitability of orexin neurons

A. Above: Representative traces for orexin neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Fluorescent immunohistochemistry showing neuron labelled with biocytin, orexin A peptide and overlay. C. Resting membrane potential averaged from nine traces before current injection. D. Spontaneous action potential frequency in absence of current injection. E. Latency to first spike following injection of positive current. F. Average threshold to first spike obtained from positive current
injection. \textit{G.} Elicited action potential frequency following application of positive current. *p<0.05.
3.2.3 Properties of orexin neurons in 2wMWF access group

In orexin neurons from the rat groups given intermittent access to 1h HFD or chow three times a week for two weeks, resting membrane potential did not differ significantly between the control and HFD group (-46.0±3.7mV, n=19 cells from 4 rats and -47.6±3.2mV, n=23 cells from 4 rats, respectively, Fig. 17B, p>0.05, Student’s t-test), and spontaneous action potential frequency was not different between the two groups (Fig. 17C, p>0.05, Student’s t-test).

Moreover, latency to first spike (Fig. 17D, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA), firing threshold (Fig. 17E, p>0.05, Student’s t-test) and action potential frequency (Fig. 17F, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA) during positive current injections did not differ significantly between the groups.

Additionally, a current ramp was used to further assess differences in the excitability of orexin neurons. There was a significant difference in rheobase between control and HFD groups (Fig. 18B, p<0.05, Student’s t-test), but there was no difference in action potential frequency during the ramps (Figure 18C, p>0.05, Student’s t-test).
Figure 17. 2wMWF intermittent access to HFD does not alter orexin neuron excitability

A. Above: Representative traces for orexin neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Resting membrane potential averaged from nine traces before current injection. C. Spontaneous action potential frequency in absence of current injection. D. Latency to first spike following injection of positive current. E. Average threshold to first spike obtained from positive current injection. F. Elicited action potential frequency following application of positive current.
Figure 18. More positive current is necessary to invoke the first action potential in orexin neurons following 2wMWF intermittent access to HFD

A. *Above*: Representative trace for chow orexin neuron following application of current ramp. 
*B Middle*: Representative trace for HFD orexin neuron following application of current ramp.  
*Below*: Diagram illustrating current ramp protocol for orexin neurons.  
B. *Rheobase* for orexin neurons in 2wMWF chow and HFD groups. Rheobase was defined as the minimum current necessary to elicit an action potential.  
C. *Action potential frequency* of orexin neurons following application of current ramp. *p<0.05.*
3.2.4 Properties of orexin neurons in male and female 6wMWF access groups

In male rats that underwent 6 weeks of three weekly intermittent access to HFD, the resting membrane potential of orexin neurons was significantly hyperpolarized compared to the control group (HFD: -50.2 ±1.1, n=6 cells from 4 rats, control: -46.8±0.6, n=13 cells from 4 rats, Fig. 19B, p<0.01, Student’s t-test) although no difference in spontaneous action potential frequency was found (Fig. 19C, p>0.05, Student’s t-test). There was no overall difference in latency to 1st spike in orexin neurons between control and HFD males (Fig. 19D, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA). Multiple comparisons revealed that latency to first spike was longer in orexin neurons from the HFD group at 50pA current injection compared to control condition (p<0.01). There were no significant differences in the threshold between the two groups (Fig. 19E, p>0.05, Student’s t-test) or frequency of action potentials elicited by positive current injections (Fig. 19F, effect of diet, p>0.05; main effect of current injection, p<0.0001; no interaction, p>0.05; two-way ANOVA).

In females, there was also a significant difference in the resting membrane potential of orexin neurons between control (-46.51 ± 0.6570, n=19 cells from 4 rats) and HFD (-48.32 ± 0.6159, n=14 cells from 3 rats, Fig. 20B, p<0.05, Student’s t-test). However, there were no differences in spontaneous action potential frequency (Fig. 20C, p>0.05, Student’s t-test), latency to first spike (Fig. 20D, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA), threshold (Fig. 20E, p>0.05, Student’s t-test), or elicited action potential frequency (Fig. 20F, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA).
We also analyzed the membrane resistance of orexin neurons in males and females in the 6wMWF groups. In orexin neurons from male rats, there was a significant negative correlation between resting membrane potential and membrane resistance, such that the more hyperpolarized the cells were, the higher the membrane resistance (Fig. 21A, $R^2=0.2091$, $p<0.05$, linear regression). However, there was no significant difference in membrane resistance between cells from chow and HFD males ($p>0.05$, Student’s t-test). In orexin neurons from female rats, there was no correlation between resting membrane potential and membrane resistance (Fig. 21B, $R^2=0.0012$, $p>0.05$, linear regression). Nonetheless, there was a difference in membrane resistance between cells from chow and HFD females ($p<0.01$, Student’s t-test). This may indicate an inherent difference between male and female orexin neurons, where channels closing hyperpolarize male orexin neurons regardless of diet, and intermittent HFD opens channels that hyperpolarize female orexin neurons.

Overall, these results suggest that long-term, but not short-term binge-like feeding on HFD decreases orexin neuron excitability by hyperpolarizing them, and this may affect males and females via distinct mechanisms.
Figure 19. 6wMWF intermittent access to HFD hyperpolarizes and decreases excitability of orexin neurons in male rats

A. Above: Representative traces for orexin neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Resting membrane potential averaged from nine traces before current injection. C. Spontaneous action potential frequency in absence of current injection. D. Latency to first spike following injection of positive current. E. Average threshold to first spike obtained from positive current injection. F. Elicited action potential frequency following application of positive current. *p<0.05.
Figure 20. 6wMWF intermittent HFD access hyperpolarizes orexin neurons in female rats

A. Above: Representative traces for orexin neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Resting membrane potential averaged from nine traces before current injection. C. Spontaneous action potential frequency in absence of current injection. D. Latency to first spike following injection of positive current. E. Average threshold to first spike obtained from positive current injection. F. Elicited action potential frequency following application of positive current. *p<0.05.
Figure 21. The correlation between resting membrane potential and membrane resistance by diet condition differs between sexes in 6wMWF groups

A. Scatterplot illustrating the negative correlation between resting membrane potential and membrane resistance in orexin neurons from male rats. B. Scatterplot illustrating that there is no correlation between resting membrane potential and membrane resistance in orexin neurons from female rats.
3.2.5 Properties of orexin neurons in adolescent versus adult male controls

It is unknown whether the electrophysiological properties of orexin neurons undergo developmental changes between adolescence and adulthood. As such, it is possible that developmental changes may be interacting with the diet manipulation to affect orexin neurons. Therefore, we decided to compare the parameters of orexin neurons between control males in 1wDaily, 2wMWF and 6wMWF conditions that are four, six and eleven weeks of age, respectively. There was no significant difference in resting membrane potential (Fig. 21A, p<0.05, Student’s t-test) or spontaneous action potential frequency (Fig. 21B, p<0.05, Student’s t-test). There were no overall differences between the groups in latency to 1st spike (Fig. 21C, effect of age, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA,) although multiple comparisons showed the latency to first spike was longer in 1wDaily compared to 2wMWF controls at 50pA current injection (p>0.05). There were no significant differences in threshold (Fig. 21D, p<0.05, Student’s t-test) and elicited action potential frequency (Fig. 21E, effect of age, p<0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA) were not significantly different between the three groups. This demonstrates that there may be developmental changes in orexin neurons’ electrophysiology between these ages.
Figure 22. Orexin neuron activity may undergo developmental changes in rats from 4 to 11 weeks of age in male rats

A. Resting membrane potential averaged from nine traces before current injection. B. Spontaneous action potential frequency in absence of current injection. C. Latency to first spike following injection of positive current. D. Average threshold to first spike obtained from positive current injection. E. Elicited action potential frequency following application of positive current. *p<0.05.
3.2.6 Identification of MCH Neurons

Since we found a change in the resting membrane potential of orexin neurons following 6wMWF feeding, we chose to investigate MCH neurons since they are located in similar regions and also associated with binge eating and addictive behaviors (Haemmerle et al., 2015; Karlsson et al., 2012; Smith et al., 2005). Morphologically, they are similar to orexin neurons in size, in that they are typically larger than other cell types located in the same areas. Our lab has shown previously that MCH neurons display unique electrophysiological responses to a series of hyperpolarizing and depolarizing current step pulses that are distinct to those seen in orexin neurons in animals from 3 to 4 weeks old (Alberto et al., 2011; Belanger-Willoughby et al., 2016). In the present study, we found the same characteristics in older animals, including a lack of $I_h$ upon hyperpolarizing currents, accommodation of action potential frequency in response to depolarizing currents, and no spontaneous activity at baseline (Fig. 22A). Out of 49 cells that showed these characteristics, 43 cells were confirmed to be immunopositive for MCH peptide (87.8% success, Fig. 22B). The other 6 cells were not successfully filled with biocytin, but were included in the analysis based on their electrophysiological responses to hyperpolarizing and depolarizing currents, which were typical of MCH neurons.

3.2.7 MCH neuron properties in six-week males and females

In MCH neurons from male 6wMWF groups, the resting membrane potential was not significantly different between control and HFD (-59.5±4.0mV, n=17 cells from 4 rats, and -57.1±4.9mV, n=10 cells from 3 rats, respectively, Fig. 22C, p>0.05, Student’s t-test). Likewise, the latency to first spike (Fig. 22D, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA), action potential threshold (Fig. 22E, p>0.05, Student’s t-test) and the frequency of elicited action potentials also revealed no significant
differences (Fig. 22F, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA). A current ramp from 0pA to 200pA revealed no significant differences in rheobase (Fig. 23B, p>0.05, Student’s t-test) or action potential frequency (Fig. 23C, p>0.05, Student’s t-test), between groups.

Similarly, in MCH neurons from the female 6wMWF group, there was no significant difference in the resting membrane potential of MCH neurons between control (-57.5±2.5mV, n=10 cells from 3 rats) and HFD (-59.5±1.6mV, n=11 cells from 3 rats, Fig. 24B p>0.05, Student’s t-test). Likewise, there was no difference between the two groups in the latency to first spike (Fig. 24C, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA), the threshold to fire (Fig. 24D, p>0.05, Student’s t-test) or frequency of elicited action potentials (Fig. 24E, effect of diet, p>0.05; main effect of current injection, p<0.0001; interaction, p>0.05; two-way ANOVA). A current ramp from 0 pA to 200 pA revealed no significant differences between rheobase (Fig. 25B, p>0.05, Student’s t-test) or action potential frequency (Fig. 25C, p>0.05, Student’s t-test).
Figure 23. 6wMWF intermittent access to HFD has no apparent effect on MCH neuron excitability in male rats

A. Above: Representative traces for MCH neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Fluorescent immunohistochemistry showing neuron labelled with biocytin, MCH peptide and overlay. C. Resting membrane potential averaged from nine traces before current injection. D. Latency to first spike following injection of positive current. E. Average threshold to first spike obtained
from positive current injection. F. Elicited action potential frequency following application of positive current. Chow (n=19), HFD (n=10).
Figure 24. Current ramps do not reveal any effect of 6wMWF intermittent access to HFD on MCH neuron excitability in male rats

A. Above: Representative trace for male chow MCH neuron following application of current ramp. Middle: Representative trace for male HFD MCH neuron following application of current ramp. Below: Diagram illustrating current ramp protocol for MCH neurons, showing a gradual current injection from 0pA to 200pA. B. Rheobase for male MCH neurons in 6wMWF chow and HFD groups. C. Action potential frequency of MCH neurons following application of current ramp.
Figure 25. 6wMWF intermittent access to HFD has no apparent effect on MCH neuron excitability in female rats

A. Above: Representative traces for MCH neurons following application of current steps. Below: Diagram illustrating current injection protocol for traces above. B. Resting membrane potential averaged from nine traces before current injection. C. Latency to first spike following injection of positive current. D. Average threshold to first spike obtained from positive current injection. E. Elicited action potential frequency following application of positive current. Chow (n=10), HFD (n=12).
Figure 26. Current ramps do not reveal any effect of 6wMWF intermittent access to HFD on MCH neuron excitability in female rats

A. Above: Representative trace for female chow MCH neuron following application of current ramp. Middle: Representative trace for female HFD MCH neuron following application of current ramp. Below: Diagram illustrating current ramp protocol for MCH neurons. B. Rheobase for female MCH neurons in 6wMWF chow and HFD groups. C. Action potential frequency of MCH neurons following application of current ramp.
Chapter 4 - Discussion

The current thesis had two main goals. The first was to characterize how intermittent palatable diet access affected the feeding behavior of rats. We found that two palatable diets, one high in fat and sugar (WD) and one high in fat (HFD), produced different intensities of binge-like behavior, and that three intermittent HFD access paradigms with different intervals and number of access produced a similar degree of binge-like behavior. Furthermore, there were no significant differences in binge-like behavior between any HFD rats, indicating similar binge behavior between adolescent and adult, and male and female rats. The second goal was to uncover any potential effects that these feeding paradigms had on orexigenic neurons of the lateral hypothalamus. Short-term palatable diet access (6 intermittent episodes), either daily or three times per week appeared to have no effect on the excitability of orexin neurons. Conversely, long-term intermittent HFD access (total of 18 episodes, three times a week) hyperpolarized orexin neurons, but not MCH neurons.

4.1 Induction of binge-like feeding following intermittent access

The definition of binge eating in animal models is poorly defined and often varies between studies (Avena et al., 2008 chapter; Corwin et al., 2008). In the current study, food intake during a 1h exposure was significantly higher in HFD and WD rats compared to chow controls, and accounted for a significant proportion of the animals’ total daily caloric intake. Similar results have been found for other high-fat food exposure paradigms, in which animals consumed approximately 50% of their daily calories in 1h (Wojnicki et al., 2006) or 2h (Corwin et al., 1998; Dimitriou et al., 2000). This can be considered binge-like feeding (Babbs et al., 2012; Corwin, 2004; Corwin et al., 1998), as it covers the DSM-5's criterion of eating a much larger amount of food in a shorter period of time than would be expected (American Psychiatric
Association, 2013). This model of intermittent exposure to palatable diets has the benefit of being more clinically relevant to cases of eating disorders that feature normal eating habits and restricted intake of palatable foods, followed by binges on palatable foods when they are consumed, compared with studies involving stress or total food restriction to induce binge-like feeding.

4.2 1wDaily intermittent access

All three groups of rats, chow control, HFD and WD exposed rats, consumed similar amounts of total daily calories, including *ad libitum* chow and food consumed during 1h food exposure. This is in agreement with other studies of daily binge-like feeding showing that chow control groups and palatable diet access groups consume a similar amount of total calories (Avena et al., 2008; Corwin et al., 1998). The HFD group consumed less calories from chow compared to both chow control and WD groups, which may be a compensatory mechanism. Animals sometimes decrease their chow intake in response to binge-like feeding to maintain a homeostatic baseline of caloric intake (Avena et al., 2008; Corwin et al., 1998; Vickers et al., 2015). That the HFD but not WD group reduced chow intake is likely due to the calories consumed from the diets; HFD rats consumed more calories during the intermittent feeding than WD group, so they decreased their baseline chow intake to accommodate for the extra calories. This is dissimilar to previous research indicating that access to a sweet-fat diet results in a decrease in chow intake (Berner et al., 2008). Since rats consumed more calories from the HFD than the WD, this suggests that HFD produced greater binge-like feeding. It is possible that palatability or physical factors of the foods, including the texture or taste, or the higher caloric density of HFD compared to chow (5.24kcal/g for HFD compared to 3.16kcal/g for chow, and 4.49kcal/g for WD) may have contributed to this observed difference, as other studies that used
high fat and sweet-fat diets have not reported a difference in binge-like feeding between these styles of diets (Berner et al., 2008; Bocarsly et al., 2011). However, it is possible that high fat versus high sugar and fat diets have different mechanisms governing their ingestion. For example, Berner and colleagues have found that administration of baclofen, a GABA\textsubscript{B} receptor agonist, preferentially decreases binge-like intake of a fatty food, has no effect on sucrose bingeing, and increases intake of a sugary, fatty diet (Berner et al., 2008).

### 4.3 Three different intermittent access models produce similar binge-like behavior

Previous studies have demonstrated that the more limited the access is to a palatable fatty food, the more it is consumed when it becomes available. Corwin et al. (1998) compared adult male rats on their consumption of Crisco when it was available, daily for 2h, three times per week for 2h or \textit{ad libitum}, and found that the highest rates of consumption occurred when it was available only three times a week. In a paradigm comparing a chow-only group to three schedules of shortening access, including 2h daily, 2h MWF or \textit{ad libitum}, total calories consumed during the 2h exposures increased above control and \textit{ad libitum} by the second week. Additionally, during the exposure period calorie consumption in the 2h MWF group surpassed that of the 2h daily group by the end of the second week (Dimitriou et al., 2000). These findings are in contrast to our own, where both the 1wDaily and 2wMWF groups consumed a similar proportion of their daily calories from HFD. Because this was the case, we wondered whether increasing the number of intermittent exposures would result in an increase in the proportion of HFD consumed. However, extending the intermittent exposures at a rate of 3 times a week for 6 weeks did not result in further increase in the percent of daily caloric intake from HFD.

Feeding behaviors appear to vary between other studies of binge eating as well. Davis and colleagues (2007) compared the daily caloric intake of a chow control group of rats with
groups given 2h access to Crisco or another HFD either daily or every 3 days, in addition to *ad libitum* chow and water. They found that the group given daily access consumed a similar number of calories as the chow group, whereas the group receiving access every 3 days consumed significantly more calories on the exposure day, and significantly less calories on the day following exposure to Crisco or HFD. Berner and colleagues (2008) compared a chow control group with rats given either *ad libitum* or 2h daily or MWF access to a sweet-fatty diet, and found their intake escalated over repeated exposures before plateauing in caloric intake by approximately the fourth exposure. However, in our study the MWF group consumed a similar number of calories on the subsequent days as the binge day. This illustrates that food intake between binge studies is variable and dependent upon specific methodology.

Specific variables may be able to account for these observed differences between studies, including our own. The intermittent diets used for our experiments were not identical to those used in others. For example, we used rodent-formulated sweet-fat and high-fat diets whereas in the experiments briefly discussed above, Corwin (1998) and Davis (2007) and colleagues used Crisco. In the current study, we used a 1h access to palatable diet paradigm, however Davis (2007) and Dimitriou (2000) used a 2h access paradigm. Finally, the time of day may have played a role. In our experiments, palatable diet access was given six hours before onset of the dark phase. In the experiment discussed above, Berner and colleagues (2008) provided their sweet-fat diet during the dark phase. Taken together, these methodological differences may account for these variations between the current study and the results cited from the literature.
4.4 Comparison of adolescent and adult male feeding behavior

Specific diets and feeding paradigms vary greatly between studies, though many studies consistently make use of older animals to study binge-like feeding. Our feeding paradigms started when rats were juvenile, between 21 to 28 days old and weighed between 40g to 100g. However, much of the literature referenced cite older ages, including 60 days (Corwin et al., 1998, Dimitriou et al., 2000), 90 days (Boggiano et al., 2007), or weights from 200g to 250g at the start of their studies. Bekker and colleagues (2014) compared two rat strains, one prone to overeating and obesity and one as a lean control, and compared the feeding behavior of adolescents (35-40 day old) and adults (105-115 days old) within and between these two strains. In their feeding paradigm, rats were given access to vanilla Ensure, a sweet-fat liquid meal replacement. They found that both strains of adolescent rats increased their intake of Ensure more than the adult rats following repeated exposures. As for chow intake, adult rats of both strains decrease their chow intake to compensate for excess caloric intake from bingeing, whereas both groups of adolescent rats consume more chow weekly after repeated exposures to Ensure. Our results are mostly inconsistent with this finding. In none of our experiments did the WD or HFD binge eating groups increase 24h chow intake relative to their respective control groups. Neither the 2wMWF nor the 6wMWF male or female HFD rats consumed a different amount of total calories on the HFD exposure day compared to the day after. Differences in feeding behavior between different strains of rats, and age at the start of feeding manipulation may be able to account for these differences.

4.5 Comparison of male and female binge-like behavior

The human incidence of eating disorders is generally higher in females than males (Hudson et al., 2007; Kessler et al., 2013). Additionally, in a study comparing adult male and
female rats on MWF consumption of a sweet-fat diet, female rats were found be significantly more likely to be binge-eating prone compared to male rats (Klump et al., 2013). Another study classified Sprague-Dawley males as being resistant to binge eating, whereas Sprague-Dawley females were classified as being prone to binge eating (Hildebrandt et al., 2014). Therefore, we compared the effect of intermittent feeding paradigm on female and male rats using the 6wMWF feeding paradigm. However, we did not find any differences in the magnitude of binge-like feeding in the 6wMWF HFD female group compared to the male group. While the notion of binge-eating proneness versus resistance may represent useful phenotypic or genotypic distinction between animals, the number of animals used in our studies was too low to reliably distinguish between these feeding phenotypes. Our findings indicate that male and female rats engage in similar binge-eating behavior when exposed to our specific feeding paradigm.

While eating disorders are often diagnosed more in woman than in men, some studies suggest that there are more similarities than differences between the sexes in binge-eating behavior. Striegel-Moore and colleagues found that significantly more women than men met the most conservative criteria for binge-eating, although when partial diagnoses were included this only accounted for one more female case of binge-eating for every 50 women compared to every 50 men (Striegel-Moore et al., 2009). Thus, it may be that men are underrepresented in the statistical analysis of binge eating. The current study may reflect this, as we found no sex differences in binge-like behavior or frequency between male and female rats.

4.6 Intermittent feeding and body weight

Because binge-eating is often a risk factor or comorbid condition with obesity, we examined the weight gain of rats that experienced intermittent HFD exposure-induced binge eating, and compared to that of animals that did not binge eat. There were no significant
differences between any of the control and HFD groups, which is in agreement with previous studies. A possible reason for this may be partly due to the macronutrient levels in the diets provided, as intermittent high fat or sweet diets alone do not promote weight gain (Avena et al., 2008; Corwin et al., 1998; Dimitriou et al., 2000), although sweet-fat diets do (Berner et al., 2008). Thus, it may be possible that the WD used during our 1wDaily paradigm, which is a sweet-fat diet, could have promoted weight gain if it were available for the same duration as our 6wMWF paradigm. In many binge-eating models animals reduce their calorie intake following an intermittent feeding episode as we have seen in our study, which maintains calorie intake, and thus body weight, similar to that of controls. These animal models thus may resemble an aspect of certain forms of binge-eating seen in human populations where people restrict their food intake in between binges in order to maintain an ideal weight.

4.7 Orexin neurons of young and adult rats exhibit similar electrophysiological properties

Our lab has repeatedly demonstrated that orexin neurons from young rodents (3 to 4 week old) can be identified by their unique responses to hyperpolarizing and depolarizing current injections (Belanger-Willoughby et al., 2016; Linehan et al., 2015; Parsons et al., 2012). The work in this thesis has demonstrated that the same electrophysiological characteristics are seen in orexin neurons from older animals (10 to 11 weeks old), further solidifying that these are reliable characteristics for identifying these unique neural populations in rats.

Furthermore, comparing the electrophysiological properties between four, six and eleven week old rats in the chow condition, we found that there were no significant differences in any of the parameters we measured, except for a difference in latency to first spike at the 50pA injection between four and six week old rats. This difference was not found between the four and eleven
week old rats, thus it may be possible that it reflects a transient developmental change. Future studies may be able to further examine other developmental changes that occur in orexin neurons, as these may be responsible for changes in locomotory or feeding behaviors that are necessary for physical or behavioral development.

4.8 Long-term, but not short-term HFD intermittent access hyperpolarizes orexin neurons

Despite the fact that there were no observable differences in the binge-like behavior between the 1wDaily, 2- and 6wMWF groups in terms of the proportion of total calories consumed from HFD, our results suggest that there is a time-dependent change in the excitability of orexin neurons. Specifically, there are no changes in the resting membrane potential or spontaneous action potential frequency of orexin neurons in the short-term access paradigm (1wDaily and 2wMWF), whereas orexin neurons are hyperpolarized following long-term intermittent access to HFD. This effect was seen in both the male and female 6-week MWF rats. This suggests that the changes in orexin neurons in response to intermittent HFD access only occur with long-term, repeated exposures.

These results may be in conflict with a number of other studies showing that orexin neuron activity is related to the consumption of palatable foods or appetitive substances. In fact, some have reported that binge-like consumption of either palatable foods or other salient reinforcers such as ethanol result in an increase in orexin activity or expression. Orexin mRNA expression was found to be increased 30, 60 and 90 minutes following a sucrose solution binge compared to controls that drank water (Furudono et al., 2006). Likewise, an increase in orexin and OX2 receptor mRNA in the paraventricular nucleus of the thalamus was found following ethanol consumption (Barson et al., 2015), and long-term fructose bingeing in food-deprived rats
resulted in an increase in orexin-A release and orexin activity as measured by co-localization of c-fos (Rorabaugh et al., 2014).

However, decreases in orexin expression have also been reported following consumption of sweet solutions and ethanol. Using drinking-in-the-dark procedures, an alternative method of inducing binge-like consumption, up to four exposures to sucrose or saccharin solutions, but not water, decreased orexin mRNA expression in the lateral hypothalamus (Alcaraz-Iborra et al., 2014; Carvajal et al., 2015). Furthermore, short-term ethanol or sucrose bingeing also decreased the number of orexin-A labelled neurons in the lateral hypothalamus (Olney et al., 2015). However, of note, the authors of these studies suggest that the observed decrease in orexin expression may reflect a temporary, adaptive decrease in orexin production following increased activity that occurs post-ingestion of these substances. Whether this decrease in orexin expression is analogous to the hyperpolarization observed in the current study remains unknown.

It is apparent that short- and long-term bingeing paradigms may affect orexin neurons differently. For example, chronic ethanol consumption has been shown to decrease orexin mRNA expression (Morganstern et al., 2010), and chronic ad libitum HFD consumption reduces the number of orexin-A positive neurons in mice (Nobunaga et al., 2014). These observations may agree with the hyperpolarization of orexin neurons we observed in the long-term bingeing groups but not the short-term groups. Together, these studies may highlight the difference in how orexin neurons respond to salient, rewarding stimuli in short-term versus the long-term bingeing.

4.9 No observable effect of intermittent HFD on MCH neurons

We found no observable effect of intermittent HFD on MCH neuron activity. Levels of MCH peptide have been shown to be unaffected by chronic HFD consumption (Nobunaga et al.,
2014), but elevated levels are found in rats prone to overconsuming HFD (Morganstern et al., 2010), and higher levels of blood triglycerides following a fatty meal are positively correlated with MCH peptide levels (Karatayev et al., 2009). Orexin and MCH neurons are known to be reciprocally connected (Guan et al., 2002), and to project to and from some of the same brain regions including the nucleus accumbens (Haemmerle et al., 2015; Mukai et al., 2009) and arcuate nucleus (Della-Zuana et al., 2002; Yamanaka et al., 2000). It is possible that these intermittent feeding paradigms are affecting the activity of these other nuclei as well. If long-term intermittent palatable food access is affecting orexin neurons, it is possible that MCH neurons are being modulated indirectly in some ways that are not evident in the parameters we examined. Nevertheless, the difference in the effects observed between orexin and MCH neurons despite their proximity towards one another, reciprocal connections and shared efferents highlights the complexity of neural networks governing food intake and motivated behaviors.

4.10 Potential mechanisms of altered orexin activity

The mechanism underlying the hyperpolarization of orexin neurons observed in these experiments remains unknown. Some possibilities underlying this change include alterations in presynaptic excitatory or inhibitory transmission or postsynaptic changes including modulation of receptors, channels or ionic pumps. One speculation is that intermittent palatable food access promotes changes in the brain similar to those of drug abuse, hence the popularity of the term “food addiction.” Alterations in the dopaminergic system have been noted in drug addicted and obese individuals, as both conditions show a decrease in the function of dopamine D2 receptors (Noble, 2000; Stice et al., 2009), which has also been demonstrated in obesity-prone rats (Geiger et al., 2009). Furthermore, D2 receptor activation has been shown inhibit GABAergic transmission to orexin neurons (Linehan et al., 2015). It may be possible that the chronic
downregulation of D2 receptors that occurs in addictive states, obesity or binge-eating results in an increase in GABA signaling to orexin neurons, possibly contributing to the hyperpolarization of orexin neurons. To test this possibility, future studies could measure dopamine-induced changes in inhibitory transmission onto orexin neurons under feeding conditions that induce binge eating.

4.11 Physiological implications of decreased orexin activity

The administration of orexin (Choi et al., 2010) or orexin receptor antagonists (Cason & Aston-Jones, 2014; Choi et al., 2010; Piccoli et al., 2012) in control animals has been found to increase and decrease palatable food intake, respectively. However, orexin neurons mediate a number of other physiological behaviors, including mood, sleep-wake cycles, arousal and motivation. Thus, the decrease in orexin excitability seen in the HFD group may have more effects than on feeding behavior. It is possible that this group of rats may exhibit lower levels of arousal or locomotion, as can be observed with high doses of orexin receptor antagonists (Winrow et al., 2011). Orexin deficiency results in the sleep disorder narcolepsy (Chemelli et al., 1999), so hyperpolarization of these cells may result in a hypersomnolent phenotype compared to controls. Those with binge-eating disorders have higher rates of mental illness, most notably depression, which results in lower levels of arousal, sleep problems and decreased motivation and sensations of pleasure (DSM-5, 2013; Hudson et al., 2007). It is possible that a decrease in orexin excitability in response to long-term binge eating may be at least partly responsible for these effects in humans suffering from binge-eating.
4.12 Limitations of the current study

The current study was conducted in order to assess the effects of intermittent palatable diet access on orexigenic neurons in the lateral hypothalamus. It was conducted in light of the deleterious effects that binge-eating has on physical and mental health in human populations. However, binge-eating has many different presentations in eating disorders, and a single animal model cannot represent the variability of the human conditions. As our paradigms did not make use of food restriction, stress, or other variables that are commonly present in various eating disorders, whether the effect on orexin or MCH neurons remains the same or not under different binge-inducing conditions is worth exploring in the future. This would help to better understand the complex interplay between binge eating and the activity of these neurons.

The current studies used a diet high in fat to promote binge-like food intake. Although the WD used in our 1wDaily paradigm represents a sweet-fat diet, our rats did not binge on this as much as the HFD. Regardless, other studies have made use of sweet or sweet-fat diets to induce bingeing behavior, and a sweet-fat diet may more accurately represent the kinds of foods that people commonly binge on. As mentioned previously, chronic HFD reduced the total number of orexin-A labelled neurons in mouse hypothalamus (Nobunaga et al., 2014) whereas chronic fructose bingeing increased orexin neuron activity (Rorabaugh et al., 2014). Studying the effect of binge-like feeding of a sweet-fat diet on orexin activity may bridge the gap between these studies.

The current study made extensive use of patch-clamp electrophysiology, using coronal brain slices. This procedure necessarily severs many of the connections between the neural populations of the brain, which may not be representative of the environment of these neural populations in vivo. However, this also suggests that the differences observed in orexin neurons
are intrinsic to these neurons, or due to changes in the local network or from intact synaptic connections from more distal brain regions. This is beneficial in order to identify the possible mechanisms by which these cellular changes are occurring.

4.13 Conclusion

To the best of my knowledge, this thesis represents the first electrophysiological analyses of orexin and MCH neurons in an animal model of binge eating. The results suggest that long-term repetition of intermittent access to high fat diet decreases the activity of orexin neurons, supporting past research describing a decrease in orexin peptide following long-term binge-like consumption of palatable foods or other reinforcers. Orexin neurons participate in many physiological behaviors, so a decrease in their excitability associated with binge-eating may also result in impairments in many functions outside of food intake. These impairments may coincide with the symptoms found in comorbid mental illnesses often associated with binge-eating disorder, including major depression and anxiety disorders. While the specific roles orexin and MCH play in the development or maintenance of binge eating remain unknown, future research on these neural populations may provide unique treatment options for binge eating.
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