The Ventral Pallidum as a Non-homeostatic Feeding Relay

BY

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LIST OF ABBREVIATIONS

Acb	Nucleus accumbens
AcbC	Core of the nucleus accumbens
AcbSh	Shell of the nucleus accumbens
AgRP	Agouti-related peptide
ARC	Arcuate nucleus of the hypothalamus
BMI	Body mass index
CART	Cocaine-and amphetamine-regulated transcript
DAMGO	D-Ala2,N,Me-Phe4,Gly-ol5-enkephalin
DBS	Deep brain stimulation
DMH	Dorsomedial hypothalamic nucleus
LH	Lateral hypothalamus
NMDA	N-Methyl-D-aspartic acid
NPY	Neuropeptide Y
PFC	Prefrontal cortex
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus of the hypothalamus
VMH	Ventromedial hypothalamic nucleus
VP	Ventral pallidum
VPm	Medial ventral pallidum

SUMMARY

In the wild, most animals consume food strictly for survival, and their feeding behaviors follow homeostatic mechanisms. Unlike most animals, humans, laboratory rats and pets engage in non-homeostatic as well as homeostatic feeding. Non-homeostatic feeding is postulated to lead to brain deregulation and disease (Berthoud, 2007; Neel, 1962; Ravussin and Bogardus, 2000; Speakman, 2008; Woods et al., 2004). In this dissertation, I propose that the VPm is a critical brain region that modulates non-homeostatic feeding. Glutamatergic manipulations of the VPm induce feeding in rats fed ad libitum and additionally, modulate activity in hypothalamic areas implicated in the regulation of feeding such as the LH, DMH and PVN. Moreover, these pharmacological manipulations of the VPm which induce feeding are independent of orexin/hypocretin and MCH expressing neurons in the LH. This lack of orexin/hypocretin and MCH involvement suggests that feeding induced in satiated rats by excitation of the VPm does not operate via the traditional hypothalamic mechanisms implicated in the regulation of homeostatic feeding. Furthermore, behavioral experiments indicate an additional difference between feeding induced by excitation of the VPm and homeostatic feeding after fasting. GABAergic manipulations of the VPm induce a preferential increase of fat intake in rats fed ad libitum while 24-h food deprivation does not. These results also suggest that the VPm might have a role in the regulation of fat intake. Finally, my experiments suggest that GABAergic manipulations of the AcbSh can modulate LH activity independently of the integrity of the VPm.

CHAPTER 1. INTRODUCTION

All animals consume food for energy and nutrition. Regulating the precise amount of calories needed to sustain metabolic processes is considered a “homeostatic” behavior. The term homeostasis derives from the two Greek words *ὅμοιος* (similar) and *στάσις* (static or still) and was first described by the ninetieth century French physiologist Claude Bernard by its original name, “*milieu intérieur*,” a term still used today as a synonym for homeostasis. The term homeostasis was coined and further developed by the twentieth century American physiologist Walter Bradford Cannon. In “Lectures on the phenomena of life common to animals and plants” Bernard wrote: "The stability of the milieu intérieur is the condition for the free and independent life" (1974). Importantly, these discussions led to some of the first ideas about the consequences of altering the *milieu intérieur.* In animals, for instance, an energetic deficit caused by lack of food will cause behavioral changes which reorient the animal towards obtaining calories (Hughes, 1965). Only when all the homeostatic needs have been completed can animals direct their behaviors toward endeavors outside of the homeostatic realm and become “free” to do other things. Organisms evolved in a world of constant homeostatic challenges. However, from a relatively recent historical standpoint, some organisms are now facing a new kind of environment, one that includes an overabundance of food, for example, which amply provides for one of the most primary components of metabolic homeostasis. This condition is becoming more common in humans living in developed countries as well as their pet animals.

Arguably, an environment in which all homeostatic needs are satisfied allows humans to pursue more advanced undertakings. However this ample environment can also lead to indulgent excess that may be detrimental to the carefully evolved homeostatic system (Berthoud, 2007; Neel, 1962; Ravussin and Bogardus, 2000; Speakman, 2008; Woods et al., 2004). Indeed, it's possible that many of the chronic diseases affecting the developed world are caused by our own success in providing a comfortable, unchallenged life. Obesity, for example, is a salient example of a problem of epidemic proportions thanks in part to the overabundance of food. If consuming food is, at the core, a homeostatic behavior, it should follow that excessive food consumption would be caused by dysregulation of the structures that control or modulate homeostatic feeding (Appelhans, 2009; Berthoud, 2007; Cason et al., 2010; Volkow and Roy, 2005; Woods et al., 2004; Zheng and Berthoud, 2007). However recent research suggests that not all feeding is related to homeostatic mechanisms (Berthoud, 2006; Corwin and Hajnal, 2005; Lutter and Nestler, 2009; Saper et al., 2002; Stroebe et al., 2008). An example of non-homeostatic feeding of psychopathological value is binge eating attacks displayed by patients with bulimia (Corwin and Hajnal, 2005). Moreover, it seems that there are multiple brain regions, some outside of the classical feeding center, the hypothalamus, that may be driving some types of feeding behaviors considered non-homeostatic and hedonic (Berthoud, 2006; Corwin and Hajnal, 2005; Lutter and Nestler, 2009; Saper et al., 2002).

In this introduction, I briefly discuss what is known about homeostatic feeding and the link to the hypothalamus (Section A). Then, I focus on brain regions involved in the

mediation of non-homeostatic influences in the control of feeding, highlighting connections, and histology, and significant findings (Section B). Finally in Section C, I summarize key unanswered questions that lead to the experiments performed for this dissertation.

A. The hypothalamus and homeostatic control of food intake: the dual center hypothesis

The hypothalamus is often summarized as controlling basic instincts, including the humorous anecdote that this region regulates the “four fs: feeding, fighting, fleeing and reproduction” (Lambert, 2011). Classically, some of the first experiments which linked the hypothalamus to food intake were conducted in animal models in the 1940s. Bilateral, but not unilateral, lesions of the ventromedial hypothalamus (VMH) were found to induce obesity in rats (Hetherington and Ranson, 1940). Interestingly, these lesions appeared to alter the homeostatic regulation of food intake because they permanently altered the animals’ feeding behavior and fat storage. Similarly, though in an opposite response, bilateral lesions of the lateral hypothalamus (LH), a hypothalamic region essential to this dissertation, also alter homeostatic feeding but by causing acute aphagia (Anand and Brobeck, 1951). Together these results led to the proposal of the dual center hypothesis of feeding in which the VMH acted as the satiety center and the

LH as the feeding center (Stellar, 1954) and working together, these regions created the homeostatic drive to eat and fast.

One of the drawbacks of these early studies was technical. Mostly, electrolytic lesions were used which, in addition to eliminating the brain region in question, also damaged fibers of passage as well as neurons thus potentially eliminating important connecting pathways and not just the targeted brain region. For example electrolytic LH lesions affected the pallidofugal pathway as well as the dopaminergic nigrostriatal bundle, and specific lesions of either fiber track alone were found to induce aphagia (Marshall et al., 1974; Morgane, 1961). Additional problems with the dual center hypothesis quickly emerged as chronic effects of the lesions were studied. Animals with electrolytic LH lesions show an initial, severe decrease in body weight (Bernardis and Bellinger, 1993). However, after approximately one week, the LH lesioned animal would then successfully maintain a body weight, albeit lower than a non-lesioned animal, and defend this lowered body weight in response to fasting and re-feeding (Boyle and Keesey, 1975; Keesey and Boyle, 1973). These results indicate that there are other areas involved in the homeostatic control of feeding (Corbett and Keesey, 1980; Vilberg and Keesey, 1990). Overall, the dual center hypothesis was proven insufficient to explain the hypothalamic control of food intake and new regional targets, such as the arcuate nucleus began to be studied for their involvement in the regulation of food intake.

A1. Beyond the dual center hypothesis: the arcuate nucleus

To correct the fiber lesioning effects of electrolytic lesions, pharmacological excitotoxic fiber sparing lesioning techniques were developed. Using these techniques, Olbey (1969) described that lesions of the arcuate nucleus of the hypothalamus (ARC) cause obesity in mice indicating for the first time that the ARC was involved in the regulation of feeding. Moreover, the ARC is ideally positioned to act as a key regulator of feeding because its infundibular location exposes it to circulating levels of gut hormones which carry important peripheral information to the brain pertaining to energy homeostasis (Parker and Bloom, 2012) and has a role in the control of appetite. The ARC contains populations of neurons expressing peptides with opposite feeding effects: the orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) or NPY/AgRP neurons, and the anorexigenic pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART) or POMC/CART neurons (Parker and Bloom, 2012). All these neurons also express receptors for key homeostatic hormones, such as leptin and ghrelin. Circulating leptin, which increases with increasing fat mass, inhibits the orexigenic NPY/AgRP neurons while it activates the anorexigenic POMC/CART neurons thus reducing food intake when the fat reserves are high (Parker and Bloom, 2012). In contrast, high levels of circulating ghrelin, which are elevated during periods of fasting, have the opposite effect and drive food intake (Inui et al., 2004). These ARC neurons in turn project to the LH, as well as the dorsomedial hypothalamic nucleus (DMH) and the paraventricular nucleus (PVN) (Bouret et al., 2004). Though the precise mechanisms are still being studied, mounting evidence points to the ARC, LH, DMH and

PVN forming a homeostatic hypothalamic feeding circuit (Harrold et al., 2012) using some of the same peptides as discussed for the ARC above.

B. Beyond the hypothalamus: striatopallidal mediation of non-homeostatic feeding

More recently, cortico-limbic regions, outside the hypothalamus, have been found to be involved in the modulation of feeding behavior and have been proposed to be central to non-homeostatic and hedonic feeding (Berthoud, 2006; Lutter and Nestler, 2009; Saper et al, 2002). Many of these data are derived from experiments examining motivation, hedonics and reward that arguably occur independent of homeostatic drive (Berridge and Robinson, 2003; Berridge and Valenstein, 1991; Kelley et al., 2002; Levine and Billington, 2004; Levine et al., 1995; Will et al., 2003; Zhang and Kelley, 2000). The medial ventral pallidum (VPm) and the shell of the nucleus accumbens (AcbSh) are two key brain regions which are able to elicit food intake during times of satiation (Maldonado-Irizarry et al., 1995; Rivero-Covelo et al., 2013; Shimura et al., 2006; Smith and Berridge, 2005; Stratford et al., 1998; Stratford et al., 1999; Stratford and Kelley, 1997; Stratford and Wirtshafter, 2004; Stratford and Wirtshafter, 2012; 2013), and it has been suggested that accumbal feeding circuits might be implicated in the regulation of hedonically desirable food (Lutter and Nestler, 2009; Saper et al., 2002). Importantly, the ability of these regions to control feeding points to the complexity of feeding

behavior to modulate the caloric intake beyond merely sustaining metabolic processes. In this section, I examine three key brain regions hypothesized to be involved in a functional feeding circuit: the VPm, the AchSh, and the LH (Stratford, 2007; Stratford and Kelley, 1999; Stratford and Wirtshafter, 2012).

B1. The ventral pallidum

Excitation of the VPm can elicit food intake (Rivero-Covelo et al., 2013; Shimura et al., 2006; Smith and Berridge, 2005; Stratford et al., 1999; Stratford and Wirtshafter, 2013) and is hypothesized to do so by modulating hypothalamic regions, specifically the LH. Activation of the VPm by blocking $GABA_A$ receptors with intracranial bicuculline injections increases food intake without affecting water intake of satiated rats (Stratford et al, 1999; Stratford and Wirtshafter, 2013). Moreover, lesions of the LH attenuate feeding induced by intra-VPm bicuculline injections (Stratford and Wirtshafter, 2013). These results highlight the importance of GABAergic inputs into the VPm in causing pallidal feeding as well as the functional relationship with the LH.

B1a. Anatomy and histology of the ventral pallidum

The pallidal complex is a subcortical heterogeneous structure located in the forebrain. Its dorsal aspect is known as the globus pallidus and its ventral portion as the ventral pallidum (VP) (Heimer and Wilson, 1975). The VP lie ventral to the anterior commissure and is not as well studied as the neighboring globus pallidus. The dorsal pallidum receives afferents from the dorsal striatum; and in a parallel fashion, the VP receives inputs from the ventral striatum. The VP is part of the corticoaccumbo-thalamocortical motor loop of the basal ganglia (Kretschmer, 2000). The pallidum has been defined histochemically by the presence of high levels of the peptides substance P and enkephalin, staining for iron, and the presence of heterogeneous pallidal cell types that include cholinergic and GABAergic projection neurons (Groenewegen et al., 1993). Additionally, it contains neurons that express NMDA, AMPA, D1 and D2 receptors (Albin et al., 1992; Boyson et al., 1986; Page et al., 1995).

In terms of neurotransmitters, glutamatergic projections to the VP come from limbic structures such as the central, medial, and basomedial amygdaloid nuclei and the midline thalamic nuclei (Fuller et al., 1987) and dopaminergic ones come from the ventral tegmental area (Klitenick et al., 1992). Neurons in the VP also express $GABA_A$ and $GABA_B$ receptors (Groenewegen and Russchen, 1984) with the main GABAergic input coming from the nucleus accumbens (Groenewegen and Russchen, 1984). The VP is rich in GABA, it has been reported that 85% of the axon terminals that form synapses in this region are GABAergic (Chang et al., 1995).

Electrophysiological studies indicate that pallidal output neurons are inhibited by GABA, mu, kappa, and D2 agonists; in turn their activity is increased by glutamate, substance P, and D1 agonists (Chrobak and Napier, 1993; Maslowski and Napier, 1991; Mitrovic and Napier, 1995; Napier and Potter, 1989;).

B1b. Hodology of the ventral pallidum

The VP receives projections from multiple targets including the medial prefrontal cortex, amygdala, ventral tegmental area, dorsal raphe, and ventral striatum (Gaykema and Zaborszky, 1996; Groenewegen and Russchen, 1984; Hakan et al., 1992; Heimer et al., 1991; Maurice et al.,1997; Mogenson et al., 1987; Nauta et al., 1978; O'Donnell et al., 1997; Peyron et al., 1998; Sesack et al., 1989; Yang and Mogenson, 1985; Yeomans and Pollard, 1993; Zaborszky et al., 1984; Zahm et al., 1985; Zahm, 1989; Zahm et al., 1996). The concentration of projections from cortical and subcortical areas into the VP make this structure a relay for a variety of neural functions such as locomotion, reward and feeding.

VP efferent projections can be divided into two groups: those analogous to the dorsal pallidum, and those efferent projections associated with the limbic system and the hypothalamus. Like the dorsal pallidum, the VP projects to the extrapyramidal motor system including the entopeduncular nucleus, substantia nigra, subthalamic nucleus

and retrorubral area (Bell et al., 1995; Bevan et al., 1996; 1997; Canteras et al., 1990; Churchill et al., 1996; Groenewegen and Berendse, 1990; Groenewegen et al., 1993; Haber et al., 1985; 1993; Hakan et al., 1992; Klitenick et al., 1992; Maurice et al., 1997; Parent et al., 1988; Vives and Mogeson, 1985; Zahm et al., 1985; Zahm and Heimer, 1990). VP efferents are also associated with the limbic system and hypothalamus, including the LH, as well as the mediodorsal thalamus, medial prefrontal cortex, ventral striatum, entorhinal cortex, lateral septum, lateral habenula, ventral tegmental area, raphe nuclei, locus coeruleus and basolateral, lateral and central amygdaloid nuclei (Bell et al., 1995; Bevan et al., 1996; 1997; Canteras et al., 1990; Churchill et al., 1996; Groenewegen and Berendse, 1990; Groenewegen et al., 1993; Haber et al., 1985; 1993; Hakan et al., 1992; Klitenick et al., 1992; Maurice et al., 1997; Parent et al., 1988; Tripathi et al., 2013; Vives and Mogeson, 1985; Zahm et al., 1985; Zahm and Heimer, 1990). As with the afferents into the VP, the efferent projections from the VP enable this structure to modulate a variety of neural functions, from locomotion to reward and food intake.

It is also possible to divide the VP according to its inputs into a dorsolateral and a ventromedial portion (Groenewegen et al, 1993); these areas receive projections from the core and the AcbSh respectively which terminate on GABAergic and cholinergic output neurons (Groenewegen et al, 1993). The topographical organization is also present in the VP outputs, with the dorsolateral VP projecting to the dorsomedial part of the subthalamic nucleus and the VPm projecting to the LH (Groenewegen et al, 1993).

The relationship between the AcbSh, the VPm and the LH is a central theme of this dissertation and it links the striato-pallidal feeding circuit with hypothalamic feeding.

Before it was considered part of a feeding circuit, the VP as a whole was considered part of a putative brain reward circuit. Since the feeding aspects of the VP may be related to the role that this structure has in reward, in the next section I review some of the literature linking the VP and reward.

B1c. Implication of the ventral pallidum in reinforcing behaviors

As part of the basal ganglia, the VP has been historically considered a functional part of the neural motor system (Nauta and Mehler, 1966). In addition to its role in motor modulation, neurons in this area respond to learning and the performance of reward-incentive behaviors (Haber and Knutson, 2010). These data led to the contemporary view of the VP as an important part of a putative neural reward circuit. Neurons in the VP are able to track the value of substances capable of eliciting gustatory sensations (tastants) by increasing firing when a sucrose solution is intraorally infused and decrease firing when a hypertonic salt solution is infused (Tindell et al., 2006). Interestingly, when the same animals are salt depleted, the neurons in the VP react with increased firing to the infusion of hypertonic saline (Tindell et al., 2006), thus neurons in

this region track value and change their response in accordance to the state of the body.

The VP is reciprocally connected with brain regions implicated in the mediation of motivated behaviors like the medial prefrontal cortex, the ventral striatum, and the ventral tegmental area (Gaykema and Zaborszky, 1996; Groenewegen and Russchen, 1984; Hakan et al., 1992; Heimer et al., 1991; Maurice et al.,1997; Mogenson et al., 1987; Nauta et al., 1978; O'Donnell et al., 1997; Peyron et al., 1998; Sesack et al., 1989; Yang and Mogenson, 1985; Yeomans and Pollard, 1993; Zaborszky et al., 1984; Zahm et al., 1985; Zahm, 1989; Zahm et al., 1996). In addition to connectivity, electrical self-stimulation studies show that the VP has a functional role in reward. Electrical self-stimulation of multiple sites within the VP supports operant responding at levels similar to the ones obtained with electrical stimulation of the medial forebrain bundle, a fiber bundle classically considered to be part of a brain reward system (Hernandez et al., 2006; Panagis et al., 1995). Interestingly, in Panagis' study (Panagis et al., 1995), rostro-caudal differences in effectiveness were also reported but they went in the opposite direction of the ones reported by McAlonan (McAlonan et al., 1993), with caudal sites having a lower frequency threshold than rostral stimulation. This result supports the role of the VP in reward, but adds to the evidence for heterogeneity of different VP sites in regards to their support of reinforcement.

In addition to electrical stimulation experiments, lesion studies provided some of the earliest evidence of the functional implication of the VP in reward and perhaps feeding

as well. Excitotoxic lesions of the VP reduce self-administration of heroin or cocaine in rats (Hubner and Koob, 1990), a result that implicates the VP in the mediation of the reinforcing effects of these drugs. In a similar fashion, N-Methyl-D-aspartic acid (NMDA) induced lesions of the VP disrupted the acquisition of amphetamine induced place preference (Hiroi and White, 1993). Moreover, acquisition of conditioned place preference induced by sucrose administration is attenuated by lesions of the rostral VP to a greater extent than by lesions of the caudal VP (McAlonan et al., 1993), indicating the existence of functional differences between regions within the VP. Thus, lesions of the VP affect responses to drugs and natural food rewards like sucrose, implicating the VP in a putative brain reward circuitry as well as in a feeding circuit.

B1d. Implication of the ventral pallidum in feeding

Recently, the VP and specifically the VPm, has been implicated in the regulation of feeding. Activation of the VPm by blocking $GABA_A$ receptors with intracranial bicuculline injections increases food intake with no effects on water intake in satiated rats (Stratford et al, 1999, Stratford and Wirtshafter, 2013). Additionally, VPm activation using bicuculline selectively increases saccharin intake but not water or quinine intake, further implicating the VP in the regulation of the consumption of what some researchers call hedonically valuable food stuffs (Shimura et al, 2006).

Pharmacological manipulations of the VP can also decrease food intake or motivation to work for food. For instance, the $GABA_A$ agonist muscimol injected across the VP reduces sucrose intake (Shimura et al., 2006). In a different study, muscimol into the VP decreased lever presses for preferred foodstuff but increased consumption of a freely available non-preferred food option (Farrar et al., 2008). Similarly, muscimol injections into the VP reduce the intake of high fat chow and suppress the enhanced fat intake elicited by mu-opioid agonist injections in the nucleus accumbens (Taha et al., 2009). Thus, pharmacological inactivation of the VP reduce the motivational value of palatable food.

The behavioral substrate of the hyperphagia induced by excitation of the VP remains uncertain. It is possible that these manipulations increase the perceived palatability of the consumed tastant, thus increasing consumption. Perceived palatability can be studied experimentally by analyzing the rats' licking microstructure (Davis, 1989; 1998), this method provides a detailed profile of the effects that GABA agonists in the VPm have on the rat's licking intake pattern. The GABAergic agonist bicuculline injected into the VPm increases the intake of a 10% sucrose solution leaving initial rate unaffected and decreasing cluster size (Rivero-Covelo et al., 2012). In microstructural literature, faster initial rates of licking and bigger licking cluster sizes are linked to higher perceived palatability (Davis, 1989; 1998). Thus, these results suggest that intra-VPm bicuculline injections increase sucrose consumption but not sucrose's perceived palatability. An alternative method to study palatability is to analyze facial taste reactivity (Berridge, 2000; Berridge and Robinson, 2003), this method reveals that intra-VP bicuculline

injections increase sucrose consumption but do not correlate with facial taste reactions linked with increased palatability (Smith and Berridge, 2005). One of the possible mechanisms for bicuculline in the VPm to increase the total intake of sucrose would be to increase its perceived palatability. Contrary to that hypothesis, we find that VPm induced feeding increases consumption while possibly reducing perceived palatability (Rivero-Covelo et al., 2012; Smith and Berridge, 2005). Therefore, an alternative hypothesis compatible with these results is that activation of the VPm induces feeding in part by blocking satiety signals.

In contrast to intra-VPm bicuculline injections, analysis of facial taste reactivity after activation of opioid receptors in the posterior VP induces both an increased sucrose intake and tongue protrusions in rats, facial patters that correlate with the presentation of palatable tastants (Smith et al, 2009). On the other hand, activation of the same receptors in the anterior VP decreases sucrose intake and tongue protrusions (Smith et al, 2009). These results suggest the existence of an anterior-posterior gradient for feeding effects in the VP elicited by opioid agonist. In juxtaposition to the opioid results, feeding effects elicited by intra-VPm bicuculline injections do not show this anterior-posterior disassociation (Rivero-Covelo et al., 2012; Smith and Berridge, 2005).

B1e. The ventral pallidum and the regulation of macronutrient intake

Non-homeostatic feeding is operationally defined as eating more than what is biologically required, doing so in a brief period of time and consuming specific nutrients in unhealthy quantities (Corwin and Hajnal, 2005). As I have discussed, intra-VPm bicuculline injections induce voracious feeding in a short period of time (Stratford et al., 1999; Stratford and Wirtshafter, 2013) but nothing is known about the effects of pallidal feeding in macronutrient (protein, fat and carbohydrates) selection. If feeding induced by intra-VPm bicuculline injections is a mediator of non-homeostatic food intake, it would be relevant to determine if pallidal feeding alters macronutrient preference, an experiment I address in chapter 4.

B2. The shell of the nucleus accumbens

The involvement of the AcbSh in the regulation of food intake was first supported by the behavioral response to antagonists of excitatory amino acids or $GABA_A$ agonists in the AcbSh. These manipulations elicited a pronounced and specific feeding response in satiated rats (Maldonado-Irizarry et al., 1995; Reynolds and Berridge, 2001; Stratford et al., 1997; Stratford and Kelley, 1997).

B2a. Anatomy and histology of the nucleus accumbens shell

The nucleus accumbens can be subdivided into a core, surrounding the anterior commissure, and a shell (Meredith et al., 1992; Záborszky et al., 1985). The AcbSh spans medial, ventral and lateral to the nucleus accumbens core and has main outputs to the VPm and the LH (Heimer et al., 1991; Zahm and Brog, 1992).

As with the rest of the striatum, the most common neuronal type in the AcbSh is the medium spiny neuron, comprising up to 90% of the neurons in the region (Meredith et al, 1993). Medium spiny neurons are output GABAergic cells but they also have axonal collaterals that could inhibit other local neurons. A small part of the neuronal population in the striatum is comprised of cholinergic large aspiny neurons. The axonal collaterals of these cells terminate on medium spiny neurons. The medium aspiny cells are a third type of neuron present in the AcbSh; these are interneurons and are believed to use somatostatin as a neurotransmitter. Finally, the GABAergic small aspiny cells encompass the fourth type of striatal neurons. These interneurons innervate the medium spiny neurons and regulate their activity (Meredith, 1999).

B2b. Hodology of the shell of the nucleus accumbens

The AcbSh receives glutamatergic projections from multiple brain regions including the ventromedial prefrontal cortex, midline thalamic area, the basolateral amygdala, and the ventral subiculum (Christie et al., 1987; Fuller et al., 1987). The main GABAergic input comes from the VPm (Groenewegen and Russchen, 1984). The LH sends orexigenic projections while both the LH and the arcuate nucleus send CART efferents to the AcbSh (Peyron el at., 1998; Yang et al., 2005). The ventral tegmental area and the dorsal raphe comprise the dopaminergic projections to the AcbSh, while the median and dorsal raphe nuclei are the source of 5HT (Brog et al., 1993; Compan et al., 1996; Stratford and Wirtshafter, 1990; Vertes, 1991). The nucleus of the tractus solitarius sends norepinephrine projections (Berridge et al., 1997; Delfs et al., 1998).

The main output target of the GABAergic medium spiny neurons located in the AcbSh is the VPm (Zahm and Brog, 1992). Unlike the core, the AcbSh projects directly to the LH (Heimer et al., 1991). Additionally, the VPm also projects to the LH (Groenewegen et al, 1993; Tripathi et al., 2013). Thus, the AcbSh can influence the LH through a direct projection or through a VPm relay. To date, the functional differential importance of the direct and indirect AcbSh to LH projections remains unclear. In this dissertation I conduct experiments aimed at elucidating the functional nature of this circuit (chapter 3).

As with the VPm, the AcbSh is also considered part of a putative brain reward circuit. Since the role of the AcbSh in modulating motivated behaviors and reward has been

linked to its role as a mediator in non-homeostatic influences in feeding, in the next section I review the role of the AcbSh in reward.

B2c. Implication of the nucleus accumbens shell in reinforcing behaviors

For more than two decades, multiple research groups demonstrated a link between the nucleus accumbens and dopamine with reinforcing behaviors and an effort to obtain a reward (see for example: Carlezon and Thomas, 2009; Salamone et al., 2007; Sesack and Grace, 2010). The most common effect observed in the nucleus accumbens when a stimuli of positive hedonic value is provided is an inhibition in the firing of the striatal medium spiny neurons. The range of stimuli capable of eliciting this transient inhibition is quite broad and includes self-administration of cocaine (Peoples and West, 1996), heroin (Chang et al., 1997), ethanol (Janak et al, 1999), sucrose (Nicola et al., 2004), food (Carelli et al., 2000), or a sucrose-predicting cue (Roitman et al., 2005). Conversely, aversive tastants induce excitation in the nucleus accumbens, suggesting that some neurons in this structure monitor motivational value (Roitman et al., 2008). This accumbal role in monitoring the value of different stimuli implicates the accumbens in mediating non-homeostatic feeding by initiating feeding in the presence of tastants of high hedonic value independently of the homeostatic energy requirements.

A general pattern of inhibition in the presence of positive hedonic stimuli and excitation in the presence of an aversive one has been shown to be present in the AcbSh.

Neurons in the AcbSh show a characteristic increase in activity when the rats are intraorally infused with an aversive hypertonic solution but show a decrease when the same solution is provided to the same rats after being sodium depleted (Loriaux et al., 2011). As it has been mentioned before, VP neurons show a similar, albeit inversed, pattern of responses to saline solutions (Tindell et al., 2006). Given that the AcbSh send a mostly GABAergic projection to the VPm (Zahm and Brog, 1992), it makes sense for these two value tracking results to be inversed. In other words, an increase in accumbal activity will increase pallidal GABAergic release and thus decrease activity in the VP and vice versa. These two results together indicate that both the AcbSh and the VP can track the value of stimuli and that they can be affected by the homeostatic state of the animal. It is likely that this ability to track value according to the homeostatic state of the animal is related to the role of the AcbSh and the VPm in mediating non-homeostatic influences in feeding.

B2d. Implication of the nucleus accumbens shell in non-homeostatic feeding

In addition to its role in reward, a recent line of research has implicated the ventral striatal regions as brain areas involved in the regulation of food intake. The main output of the AcbSh is the VPm (Zahm and Brog, 1992) that projects to the LH (Groenewegen et al, 1993; Tripathi et al., 2013), a hypothalamic region involved in the regulation of food intake (see *fig. 1*). Given the amount of data implicating the VPm in the regulation of food intake (Rivero-Covelo et al., 2013; Stratford et al., 1999; Smith and Berridge,

2005; Stratford and Wirtshafter, 2013) and given the close connectivity between the AcbSh and the VPm (Churchill et al., 1990; Nauta et al., 1978; Heimer et al., 1991; Zahm and Heimer, 1990), the AcbSh is in a unique position to affect the motivational regulation of food intake.

The involvement of the AcbSh in the regulation of food intake was first supported by the effects observed by injecting antagonists of excitatory amino acids or $GABA_A$ agonists in the AcbSh (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997). These manipulations elicited a pronounced and specific feeding response in satiated rats (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997) and demonstrated the involvement of striatal regions in the regulation of food intake.

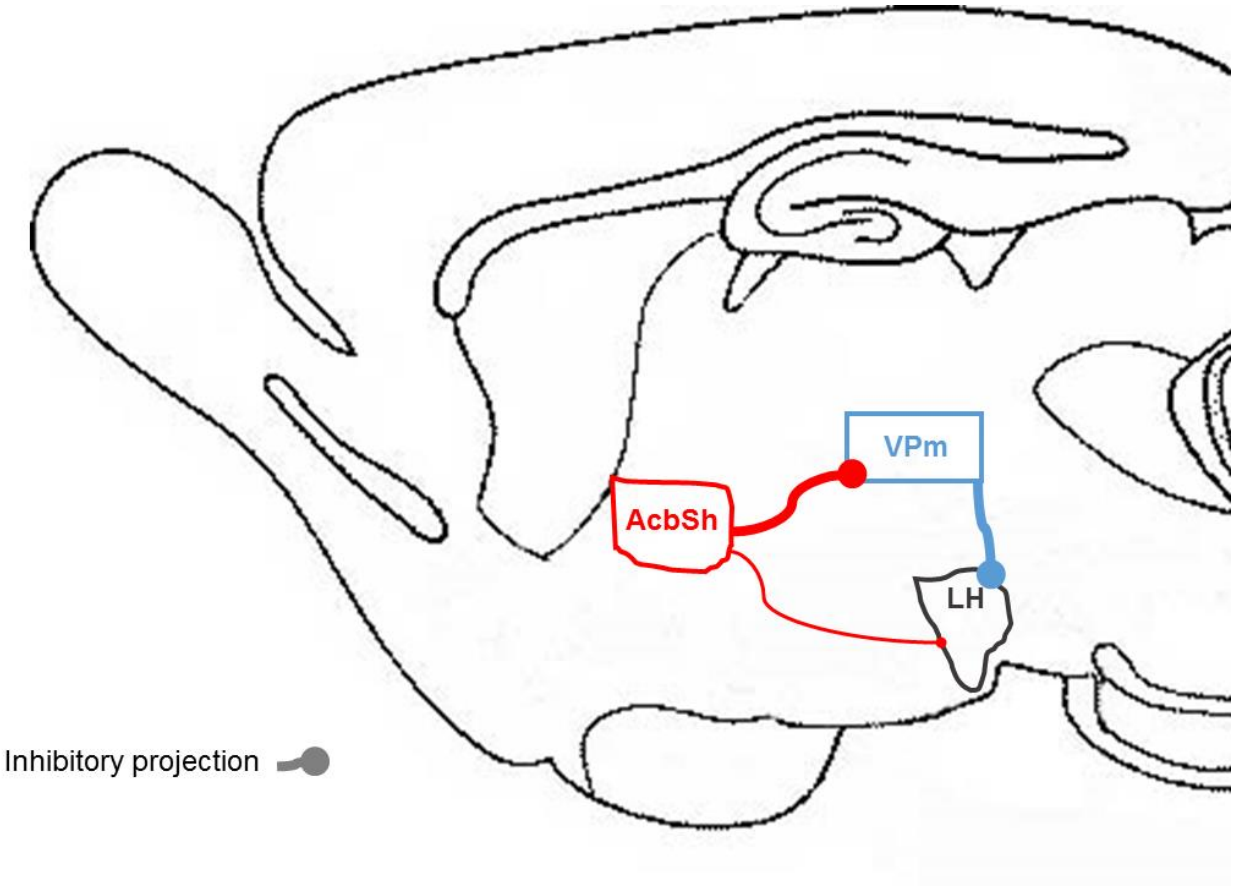

Figure 1. Schematic representation of the proposed striato-pallidal-hypothalamic feeding circuit. The AcbSh sends a major GABAergic projection to the VPm and a minor one, presumed to be GABAergic as well, to the LH. The VPm also projects to the LH. I propose that the striatal and pallidal efferences to the LH mediate the feeding effects observed by manipulations of the AcbSh and the VPm.

Specifically, intra-AcbSh injections of the $GABA_A$ agonist muscimol increased food intake in satiated rats and indicated that the ventromedial AcbSh is the most sensitive area to this manipulation (Stratford and Kelley, 1997). Similarly, injections of the AMPA and Kainate receptor antagonist DNQX into the AcbSh induced a similar increase in food consumption (Maldonado-Irizarry et al., 1995) while injections of the same

compound in neighboring areas such as the accumbens core, anterior dorsal, posterior dorsal, ventromedial, dorsomedial, and ventrolateral striatum failed to elicit food intake in satiated rats (Kelley and Swanson, 1997). These results circumscribed the feeding effect to the AcbSh and not to other striatal regions.

The demonstration that pharmacological inactivation of the AcbSh induces feeding in satiated rats does not prove that the AcbSh is directly implicated in the regulation of food intake. An alternative explanation for this effect could be that AcbSh inactivation increases the intake of any edible material or even increases gnawing behavior. To test these hypotheses, the AcbSh was inactivated using DNQX, which increased the intake of both a liquid and solid diet but did not increase the consumption of water or gnawing behavior (Stratford et al., 1998). Additionally, intra-AcbSh of muscimol do not increase water intake (Stratford and Kelley, 1997). Taken together, these results show that inactivation of the AcbSh leads specifically to an increase in food intake.

Another set of experiments focused on the pharmacology of the feeding induced by pharmacological manipulation of the AcbSh. NPY expressing neurons in the hypothalamus have been linked to the initiation of food intake (Hanson and Dallman, 1995). Because of the link to NPY, it is possible that the feeding effect observed by inactivating the AcbSh is in part induced by the release of NPY. In fact, feeding induced by muscimol injections into the AcbSh can be blocked by intraventricular injections of Y1 and Y5 receptor antagonists (Stratford and Wirtshafter, 2004), which suggest that the release of NPY is required to observe feeding induced by intra-AcbSh muscimol

injections. These results also implicate the hypothalamus in the expression of food intake induced by infusions of $GABA_A$ into the AcbSh since NPY expressing neurons are mainly located in the arcuate nucleus (Morris, 1989). Furthermore, NPY injections into the LH induce a strong feeding but not drinking response in rats (Stanley et al., 1985).

Additional research has focused on the motivational effects of AcbSh induced feeding. In this fashion, dopaminergic agonists, like amphetamine, and GABA agonists, like muscimol, injected into the AcbSh have a clear motivational effect when animals are trained to press a lever to obtain food (Wirtshafter and Stratford, 2010). Instead, when rats can press a lever to obtain water, only amphetamine, but not muscimol, into the AcbSh increase lever pressing (Covelo et al., 2012). This indicates that the motivational effects of the GABA agonist muscimol in the AcbSh are specific to food and that the role of dopamine in the AcbSh seems to be related to effort allocation (Covelo et al., 2012). Thus, GABAergic agonists in the AcbSh specifically increase both food intake and the motivational value of food. It is indubitable that this relationship between the drive to eat and the increased value of food is involved in AcbSh mediated non-homeostatic feeding behaviors.

Interestingly, muscimol injected into the AcbSh increases the consumption of a 10% sucrose solution, but fails to increase initial rate of licking and cluster size, increasing instead the number of clusters and the time spent drinking the sucrose solution (Wirtshafter et al., 2012). These results are similar to our observation that intra-VPm

bicuculline injections increase sucrose intake while reducing cluster size (Rivero-Covelo et al., 2012). One possible explanation is that inactivation of the AcbSh, or activation of the VPm, produces insensitivity to postingestive feedback. A weak postingestive satiety is a possible mechanism for weight gain and obesity, and could play an important role in the etiology of obesity (Blundell and Cooling, 2000).

The AcbSh is not a homogenous structure and further research has showed a rostral-caudal gradient in the behavioral effects of AcbSh inactivation using muscimol. Thus, rostral injections caused increased food intake, positive conditioned place preference, and increased positive hedonic reactions to sucrose infusions (Reynolds and Berridge, 2002). This study provided evidence of a bivalent organization of the AcbSh.

As discussed before, the AcbSh projects to the LH (Heimer et al., 1991), a classical hypothalamic feeding center, both directly and indirectly through the VPm (Churchill et al., 1990; Groenewegen et al, 1993; Heimer et al., 1991; Nauta et al., 1978; Tripathi et al., 2013; Zahm and Heimer, 1990) making the AcbSh-VPm-LH a functional feeding circuit (Stratford, 2007; Stratford and Kelley, 1999; Stratford and Wirtshafter, 2012) mediating non-homeostatic influences on feeding. Thus, it is possible for the AcbSh to exert its influence in food intake through the LH. Indeed, the immediate early gene, c-fos, is expressed in the LH after injecting muscimol into the AcbSh and injections of NMDA antagonists into the LH block AcbSh induced feeding (Stratford and Kelley, 1999). Similarly, intra-AcbSh injections elicit Fos expression in the LH (Baldo et al., 2004; Straford, 2005; Zheng et al., 2003). These results suggest that inactivation of the

AcbSh induces activation of the LH and that excitation of the LH is necessary to observe AcbSh induced feeding. However, the functional connectivity between the AcbSh and the LH remains unclear since the AcbSh could influence the LH directly and/or indirectly through its main output the VPm that also projects to the LH.

B2e. The accumbens shell and the regulation of macronutrient intake

Extra-hypothalamic areas have been implicated in the regulation of macronutrient intake. Injections of the mu-opiod agonists into the nucleus accumbens induce preferential fat versus carbohydrate intake in rats (Zhang et al., 1998). Unlike the specificity seen with mu-opioid agonists, injections of the $GABA_A$ agonist muscimol into the AcbSh equally increases the intake of both carbohydrates and fat (Basso and Kelley, 1999).

B3. The lateral hypothalamus

As indicated previously, pharmacological manipulations of the AcbSh and VPm induce feeding in satiated rats and I propose are mediators of non-homeostatic feeding. Importantly, both the AcbSh and the VPm are interconnected and project to the LH, a classic hypothalamic hunger center.

B3a. Anatomy and histology of the lateral hypothalamus

The LH is one of the multiple nuclei located in the hypothalamus and has been classically implicated in the regulation of food intake and arousal (Bernardis and Bellinger, 1996).

The LH can be divided into anterior, tuberal, and posterior regions (Saper et al., 1979). Among the LH cell populations involved in the homeostatic regulation of food intake, it is possible to distinguish between those with an orexigenic effect and those with anorexigenic one. Among the orexigenic peptides are orexin/hypocretin and MCH expressing neurons and amid the anorexigenic peptides are neurotensin and CART expressing cells (Parker and Bloom, 2012). It is worth noting that the orexigenic and anorexigenic populations described here are a small proportion of the cells in the LH (Parker and Bloom, 2012).

B3b. Hodology of the lateral hypothalamus

Extrahypothalamic afferents to the LH include the AcbSh and the VP, but also the prefrontal, orbitofrontal, insular and olfactory cortex, nucleus of the tractus solitarius and

parabrachial nucleus (Groenewegen et al., 1993; Haber et al., 1985; Horst et al., 1989; Simerly, 1995). In turn, the LH projects to the AcbSh and VP in addition to the orbitofrontal, prelimbic, sensorimotor, motor and piriform cortex, hippocampus, locus coeruleus and nucleus of the tractus solitarius (Kokkotou et al., 2001; Saito et al., 2001; Zheng et al., 2005). From this pattern of connectivity, it is obvious that the LH is a brain area capable of eliciting strong behavioral events.

B3c. Lateral hypothalamus and feeding

Classically, the LH has been considered the “hunger center” of the brain (Bernardis and Bellinger, 1996). The first functional connection between the LH and feeding came from Delgado and Anand (1953) who reported that electrical stimulation of the LH induces feeding in cats. These results were later confirmed in rats (Margules and Olds, 1962), monkeys (Aou et al., 1991) and even humans (Quaade et al., 1974). Additionally, bilateral LH lesions produce hypophagic rats (Bernardis and Bellinger, 1996). Similar to electrical stimulation but more physiologically relevant, injections of excitatory amino acids, $GABA_A$ antagonists, or activation of mGluR1 and/or mGluR5 glutamate receptors in the LH elicit food intake (Charles et al., 2013; Stanley et al., 1985; 1993). Conversely, $GABA_A$ agonists injected in the same location suppress homeostatic feeding (Stanley et al., 2011). Electrical recordings in LH have shown that neurons in this hypothalamic region respond to feeding, although they can also be affected by

other factors such as circadian rhythms (Ono et al, 1986). Thus, the relationship between LH and feeding is firmly established.

B3d. Relationship between the medial ventral pallidum, the shell of the nucleus accumbens and the lateral hypothalamus

In terms of its connectivity with the ventral striatal-pallidal system, both the AcbSh and the VPm project to the LH (Churchill et al., 1990; Groenewegen et al, 1993; Heimer et al., 1991; Nauta et al., 1978; Tripathi et al., 2013; Zahm and Heimer, 1990). Also, as mentioned, feeding can be induced in satiated rats by pharmacologically inactivating the AcbSh (Stratford and Kelley, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998; Stratford and Wirtshafter, 2004) or activating the VPm (Rivero-Covelo et al., 2013; Shimura et al., 2006; Smith and Berridge, 2005; Stratford et al., 1999; Stratford and Wirtshafter, 2013) and it is very likely that the LH is an important output of both feeding effects. As a result of this common output, the nature of the functional relationship between these three structures remains unclear. For instance, the AcbSh may induce food intake via LH modulation from both the AcbSh itself and the VPm. An alternative mechanism would require only VPm inputs to the LH. To shed some light over this controversy, feeding elicited in satiated rats by unilateral intra-AcbSh muscimol injections can be attenuated by ipsilateral LH or VPm lesions (Stratford and Wirtshafter, 2012). Therefore, the VPm and the LH are necessary to observe feeding induced by

infusion of $GABA_A$ agonists into the AcbSh. What remains to be elucidated is the functional circuitry between these three structures.

C. Summary

Glutamatergic manipulations of the VPm could induce feeding in rats fed ad libitum. The VPm receives inputs from multiple neurotransmitters including GABA and glutamate (Maurice et al.,1997; Sesack et al., 1989; Vives and Mogenson, 1985; Zaborszky et al., 1984; Zahm and Brog, 1992). Excitation of the VPm by GABAergic blockade induces voracious feeding (Stratford and Kelley, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998; Stratford and Wirtshafter, 2004). To date, the effects of glutamatergic manipulations of the VPm in feeding have not been studied in rats. In chapter 2, I study the role of glutamatergic manipulations in VPm stimulated feeding.

Excitation of the VPm might modulate the neurons in the LH. Feeding in satiated rats can be induced by injections of $GABA_A$ antagonists or mu-opioid agonists into the VPm (Stratford et al., 1999; Smith and Berridge, 2005; Stratford and Wirtshafter, 2013). Additionally, activation of the LH using excitatory amino acids elicits feeding in satiated rats (Hettes et al, 2010), and unilateral LH lesions attenuate VPm activation induced feeding in the side ipsilateral to the lesion (Stratford and Wirtshafter, 2013). Finally, the VPm projects to the LH (Groenewegen et al, 1993; Tripathi et al., 2013). Taken

together, these results suggest that the LH might be downstream of VPm stimulated feeding. In chapter 2, I study the effect of intra-VPm NMDA injections on Fos expression in the LH and other hypothalamic regions involved in the homeostatic regulation of food intake.

Glutamatergic manipulations of the VPm might activate the orexin/hypocretin and/or MCH orectic systems. The orexin/hypocretin and MCH expressing neurons located in the LH constitute an important part of the brain orectic or anabolic pathway (Obici, 2009). Because of the orexigenic nature of these neurons, it is logical to hypothesize that these neural populations might have a role in feeding induced by excitation of the VPm. In chapter 2, I study if the LH neurons activated by intra-VPm NMDA injections express orexin/hypocretin or MCH.

The VPm might constitute a functional relay between the AcbSh and the LH. As I have illustrated in fig. 1, the VPm sits between the AcbSh and the LH and both the VPm and the AcbSh project to the LH (Groenewegen et al, 1993; Heimer et al., 1991; Tripathi et al., 2013). Unilateral intra-AcbSh muscimol injections induce ipsilateral Fos expression in the VPm and the LH (Pulman et al., 2012; Stratford, 2005), supporting the idea of a functional connection between the AcbSh, the VPm and the LH. However, it is unclear if the AcbSh alone can modulate activity in the LH independently of the VPm or if the indirect AcbSh-VPm-LH projection is also necessary for the AcbSh to modulate neural excitation in the LH. To clarify the functional role of the VPm in the AcbSh-VPm-LH

circuit, in chapter 3, I study Fos expression in the LH after intra-AcbSh muscimol injections in rats with VPm lesions.

Excitation of the VPm might alter macronutrient preference. Changes in macronutrient selection have been mostly described after pharmacological manipulations of hypothalamic targets (Clegg et al., 200; Leibowitz et al., 1990; Nagase et al., 2002; Naleid et al., 2007;Stanley et al., 1985; Tempel et al., 1988). One of the few non-hypothalamic regions that has been shown to alter macronutrient preference is the nucleus accumbens. Mu-opioid manipulations of the accumbens have been shown to induce preferential fat over carbohydrate intake in rats (Zhang et al., 1998). In contrast, intra-AcbSh muscimol injections elicit similar increases in fat and carbohydrate intake (Basso and Kelley, 1999). Given the presence of GABAergic projections from the accumbens to the VPm (Churchill et al., 1990; Nauta et al., 1978; Heimer et al., 1991; Zahm and Heimer, 1990), it is possible that the VPm might affect macronutrient intake in a fashion similar to that observed after mu-opioid agonists in the accumbens. In chapter 4, I study the effects that intra-VPm bicuculline injections have on macronutrient preference. Additionally, I compare the effects of feeding induced by excitation of the VPm and feeding induced by 24-h fasting on macronutrient preference. Finally, given the experimental differences between my design and that of Basso and Kelley (1999), also in chapter 4, I study the effects that intra-AcbSh muscimol injections have on macronutrient preference.

CHAPTER 2. EFFECTS OF INTRA-VENTRAL PALLIDUM N-METHYL-D-ASPARTIC ACID INJECTIONS ON FOOD INTAKE, HYPOTHALAMIC FOS EXPRESSION AND OREXIN/HYPOCRETIN AND MELANIN CONCENTRATING HORMONE EXPRESSING NEURONS.

Pharmacological activation of the medial ventral pallidum (VPm) using the $GABA_A$ antagonist bicuculline (Stratford et al., 1999, Stratford and Wirtshafter, 2013) induces food intake in ad libitum fed rats. To date, the role of glutamatergic compounds in feeding induced by stimulations of the VPm in satiated rats remains unknown. Excitation of the VPm using N-Methyl-D-aspartic acid (NMDA) was attempted in anesthetized rats with the goal of studying Fos expression (Turner et al, 2008). Using that protocol, intra-VPm NMDA injections failed to induce Fos (a marker of neural excitation) in the LH, suggesting that the VPm and the LH might not be functionally related. However, the lack of Fos expression in the LH could be attributed to the anesthesia used in this procedure. Using a similar protocol, but in awake rats, could yield novel data that would increase our understanding of the VPm feeding circuit.

Anatomically, the VPm projects to the lateral hypothalamus (LH) (Groenewegen et al, 1993; Tripathi et al., 2013), a structure classically implicated in the regulation of food intake. Because of the known neuroanatomical connections between the VPm and the LH, I hypothesized that excitation of the VPm will induce a parallel excitation in the LH indicating a functional connection between these two regions involved in the feeding response observed from VPm stimulation. Moreover, the LH contains neurons that

express orexin/hypocretin or melanin-concentrating hormone (MCH), both important neurotransmitters in the orectic pathway that promote feeding (Obici, 2009). I hypothesize that pharmacological activation of the VPm will in turn recruit LH cell populations expressing orexin or MCH.

Here, I test the hypothesis that intra-VPm NMDA injections will induce food intake in awake, ad libitum fed rats (Section A) and induce Fos expression in lateral hypothalamic neurons, including those containing orexin and MCH (Section B).

A. Unilateral injections of N-Methyl-D-aspartic acid into the medial ventral pallidum induce feeding in rats fed ad libitum

Injections of $GABA_A$ antagonists like bicuculline or mu-opioid agonists like D-Ala^2-N-Me-Phe^4-$Glycol^5$-enkephalin (DAMGO) into the VPm have been shown to increase food intake in free-feeding rats (Stratford et al., 1999; Smith and Berridge, 2005). Here, I test the hypothesis that feeding can also be induced in satiated rats by injecting NMDA unilaterally in the VPm. NMDA is an amino acid that binds selectively to the NMDA receptor thus partially mimicking the excitatory actions of glutamate. Moreover, bilateral NMDA microinjections into the VP have been reported to elicit feeding in pigeons (Da Silva et al., 2003). I hypothesize that excitation of neurons in the VPm by NMDA receptor activation will lead to feeding in a similar fashion to the effect elicited by $GABA_A$ antagonists like bicuculline.

A1. Subjects, materials and methods

Animals & surgery: Nine Sprague-Dawley rats (Charles River), weighing between 290 and 350 grams at the time of the surgery. I used standard, flat skull stereotaxic techniques and sodium pentobarbital (50 mg/kg) as the anesthetic agent. The rats were chronically implanted with bilateral 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, Va) aimed 2 mm dorsal to the VPm in the following coordinates (in mm) from Paxinos and Watson (2007): AP: -0.2, ML: ±1.8, DV: -6.7. The guide cannulae were held in place using stainless steel screws and acrylic dental cement. A bilateral stainless steel dummy cannula was inserted inside each cannula to help maintain patency. The subjects were allowed to recover for at least a week before behavioral testing.

Testing apparatus: The test chambers consisted of plastic cages (L 43 X W 22 X H 21 cm) with automated pellet dispensers (Med-Associates, St Albans, VT, USA) attached to them. These dispensers automatically delivered a single 45 mg pellet of food (Precision Dustless pellets; Bio-Serve, Frenchtown, NJ, USA) whenever a pellet was removed from the hopper by the rat, no operant behavior was required. The rats were acclimated to the test chambers by placing them inside for 60 minutes for five consecutive days before any drug injections. To increase exploration and association of

the hopper with the food, prior to the very first acclimation day the rats were food deprived overnight. The rats were fed ad libitum for the remainder of the experiment.

Injection protocol: To complete the acclimation period, the rats were exposed to the injection procedure and the injectors were dropped but no injection was performed. After this last step, in three nonconsecutive days the rats were restrained, the obturators removed and 28-gauge injection cannulae, extending 2 mm beyond the tip of the guide, were inserted into each guide cannula. Counterbalanced unilateral injections of vehicle (sterile PBS) or NMDA (0.112 μg and 45 μg /0.5 μl) were made at a rate of 0.33 μL/min using motor-driven microsyringe pumps connected to the injection cannulae with water-filled polyethylene tubing. After the infusion, the injection cannulae were left in place for an additional 60 s to minimize leakage up the cannula track. The obturators were then replaced and the rats were placed in the test cages for 60 min. Their food intake was automatically recorded. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UIC Institutional Animal Care and Use Committee. These animals were then used in the next experiment (section B).

A2. Results

The responses to unilateral injections of vehicle or NMDA are displayed in fig. 2. The highest dose of NMDA induced an increase in food intake compared with the vehicle condition. This result was supported by a one way repeated measures ANOVA in which

there was a significant overall effect ($F_{2,16}$ = 5.395, P < 0.05). Post-hoc comparisons using the Least Significant Difference test indicate a significant difference between vehicle and NMDA 0.45 μg conditions (p < 0.05).

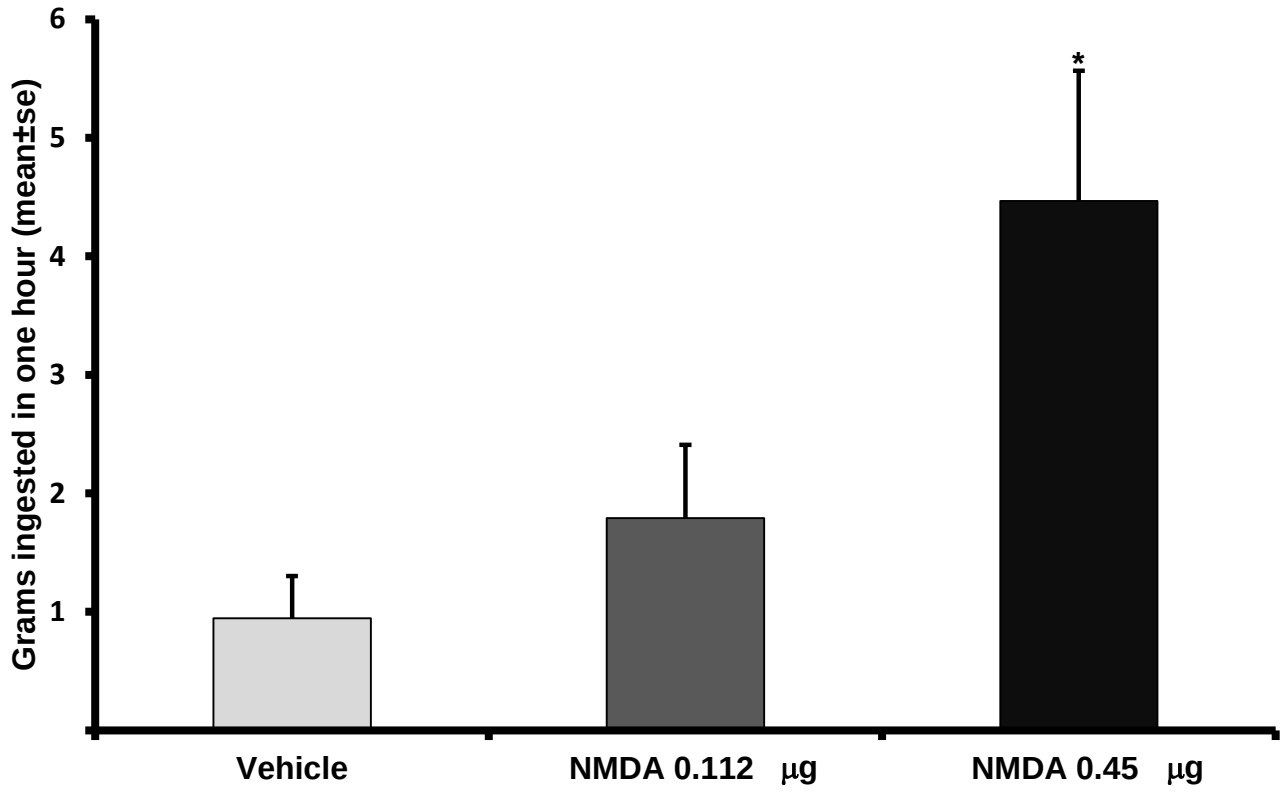

Figure 2. Unilateral injections of NMDA (0.45 μg) into the VPm induce a significant increase of food intake in rats fed ad libitum. *p < .05 vs. vehicle.

A3. Discussion

Here I show for the first time that feeding can be induced in rats fed ad libitum by unilateral injections of NMDA into the VPm. This result suggests that glutamatergic neurotransmission is involved in VPm induced feeding. To date, manipulations of the VPm have been shown to induce feeding in rats by blocking $GABA_A$ receptors in the VPm using bicuculline (Stratford et al., 1999), stimulating mu-opioid receptors using DAMGO (Smith and Berridge, 2005) and now by stimulating the ligand-gated ion channel NMDA with N-Methyl-D-aspartate. It is unlikely that the feeding effects induced by these different neurotransmitters in the VPm are unrelated. I speculate that under normal conditions there is a balance between opioid, GABA and glutamate sources projecting to the VPm. The VPm would then act as a mediator of these influences, some of them non-homeostatic in nature, to regulate food intake. Indeed, recent fMRI studies indicate that high body mass index (BMI) correlates with lower VP activation when non preferred food is presented (Yokum et al., 2011) and that the VP activity is modulated by assumptions about the pleasantness of food (Simons et al., 2013). These results suggest a link between VP activity and judgment of food value. A role for the VP in evaluating food value has been described in rats (see chapter 1, section B1c). Neurons in the VP change their firing rates according to the value of different tastants (Tindell et al., 2006). Taken together, it is possible that a disturbance in the balance of neurotransmitters converging into the VP might lead to increased food consumption.

In the next section, I study how NMDA injected into the VPm, a manipulation that I know now induces feeding, affects neuronal excitation in various hypothalamic regions.

B. Unilateral injections of N-Methyl-D-aspartic acid into the medial ventral pallidum induce activation of the lateral hypothalamus and other hypothalamic regions but does not involve neurons expressing orexin/hypocretin or melanin-concentrating hormone

The lateral hypothalamus (LH) has been classically implicated in the control of food intake (Bernardis and Bellinger, 1996) and its activation, using excitatory amino acids, elicits feeding in satiated rats (Hettes et al, 2010). The LH contains both orexin/hypocretin and MCH expressing neurons. These peptides constitute an important part of the brain orectic or anabolic pathway (Obici, 2009) and are believed to induce food intake through homeostatic pathways (Harrold et al., 2012).

It has been proposed that the AcbSh, VPm and LH form a functional feeding circuit (Stratford et al., 1999; Stratford, 2007; Stratford and Wirtshafter, 2012). This feeding circuit hypothesis is supported by the fact that unilateral injections of muscimol into the AcbSh induce Fos expression, a marker of neural excitation, in the VPm and LH (Pulman, et al., 2012; Stratford, 2005). Similarly, LH lesions attenuate feeding induced by the stimulation of the AcbSh and VPm (Stratford and Wirtshafter, 2012; 2013). Thus, I consider the LH the final relay station of feeding induced in satiated rats by pharmacological stimulation of the VPm. Given that VPm activation induces feeding in

rats fed ad libitum and that the VPm projects to the LH, a brain structure implicated in the control of food intake, I expect the mechanism for VPm induced feeding to include excitation of the LH.

The LH is not an isolated feeding structure in the hypothalamus. The LH is interconnected with other hypothalamic appetite regulating structures: the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial hypothalamic nucleus (VMH) and the dorsomedial hypothalamic nucleus (DMH) (Harrold et al, 2012). I hypothesize that pharmacological excitation of the VPm might excite some of these hypothalamic regions as well as the LH.

Furthermore, given the presence of key feeding neurotransmitters in the LH (e.g. orexin/hypocretin and MCH), I hypothesize that the functional connection between the VPm and the LH will involve at least partial stimulation of the orexin and/or MCH expressing neurons.

To study the relationship between the VPm and the LH, I use unilateral excitatory injections. Unilateral injections present the advantage of allowing a clearer interpretation of the data, especially when working with a structure like the LH that can be activated by arousal, stress and circulating hormones or glucose (Bahjaoui-Bouhaddi et al., 1994; Bonaz et al., 1993; Briski and Gillen, 2001; Cai et al., 2001; Chastrette et al., 1991; Elmquist et al., 1998; Lawrence et al., 2002; Moriguchi et al., 1999; Niimi et al., 1995; Roberts et al., 1995; Silveira et al., 1993; Turton et al., 1996). Following the work of

Stratford (2005), the rationale behind this procedure is that each VPm projects ipsilaterally to the LH on each side of the brain. Because of this ipsilateral VPm-LH projection, bilateral excitation observed in the LH after a unilateral injection of NMDA in the VPm is unlikely to reflect lateralized connections between the VPm and the LH (Stratford, 2005). Bilateral excitation in the LH after a unilateral intra-VPm injection is more likely the result of other factors such as stress or animal handling, thus it can be considered noise. In contrast, the presence of LH excitation ipsilateral to the injected side would imply that excitation of the VPm causes excitation of the LH (Stratford, 2005).

For measurement purposes, in this experiment I divide the LH into the perifornical LH and LH (fig. 4). Of note, most of the orexin/ hypocretin cells and MCH cells are located within the perifornical region (Burt et al., 2011; Valassi et al., 2008).

Here, I find increased Fos expression in the perifornical LH and LH after unilateral intra-VPm NMDA injections. This result supports my hypothesis of a functional feeding circuit between these brain regions. Additionally, I find increased Fos in the PVN and the DMH but not in the VMH or ARC. These results suggest that the VPm might affect other hypothalamic feeding targets besides the LH. However, contrary to my hypothesis, orexin/hypocretin and MCH neurons do not appear to be involved in this behavior, a result I discuss further in the discussion section below.

B1. Subjects, materials and methods

Animals & surgery. Twenty rats were anesthetized using sodium pentobarbital (50 mg/kg), placed in a rat stereotaxic apparatus, and surgically implanted with double guide cannulae aimed 2 mm above the VPm. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UIC Institutional Animal Care and Use Committee.

Injection protocol. At least two weeks after surgery, injectors extending 2 mm from the tip of the guide cannula were inserted to unilaterally inject the rats with NMDA (45 μg/0.5ul) and a contralateral vehicle injection, randomizing the injection side (experimental group, n = 9); or with vehicle in both sides (control group, n = 8). In the case of the control group, which received bilateral saline injections, one side was randomly chosen as the vehicle injection and the other saline side was randomly considered the “drug” injection. After the injections, the rats were placed back in their home cages without food or water for 90 minutes.

Tissue harvest. After the 90 min period in the home cage post-injection, the rats were deeply anesthetized using sodium pentorbarbital (150 mg/kg) and perfused transcardially with 50 ml of 0.15-M saline followed by 200 ml of 10% buffered formalin at pH 6.5, then 300 ml of 10% buffered formalin at pH 9.0. The brains were extracted, placed in 10% buffered formalin at pH 9.0. for 60 minutes and then stored in PBS with 20% sucrose for at least 48 h.

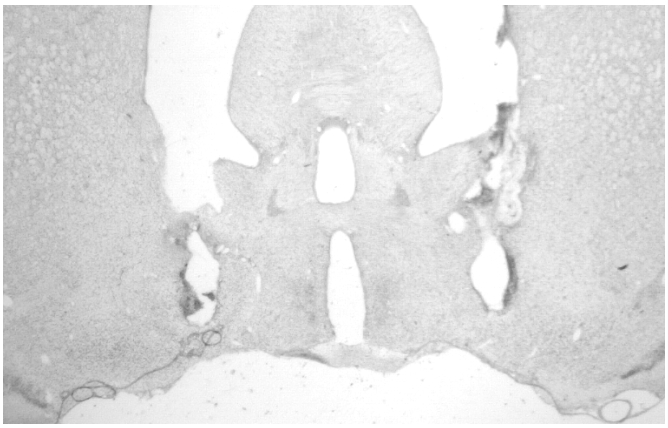

Figure 3. Cresyl violet stained section showing a typical cannula placement in the VPm

Brain sectioning. The brains were frozen cut in 35μm serial coronal sections, stored in cryoprotectant and processed for Fos expression, Fos/Orexin and Fos/MCH double labeling. Serial sections were taken through the LH and a 1 in 3 series processed for Fos alone, another 1 in 3 for Fos and orexin, and another 1 in 3 for Fos and MCH. Sections through the LH were analyzed at four AP levels listed here by their distance from bregma in mm: -1.6, -2.6, -3.6 and -4.2. Sections trough the perifornical LH were analyzed at three AP levels: -1.6, -2.6 and -3.6. Additionally, sections through the paraventricular nucleus (PVN, AP -1.6), dorsomedial hypothalamus (DMH, AP -2.6), ventromedial hypothalamus (VMH, AP -2.6) and arcuate nucleus (ARC, AP -2.6) were also processed and analyzed for Fos immunostaining. A schematic representation of the sampling areas is presented in fig. 4.

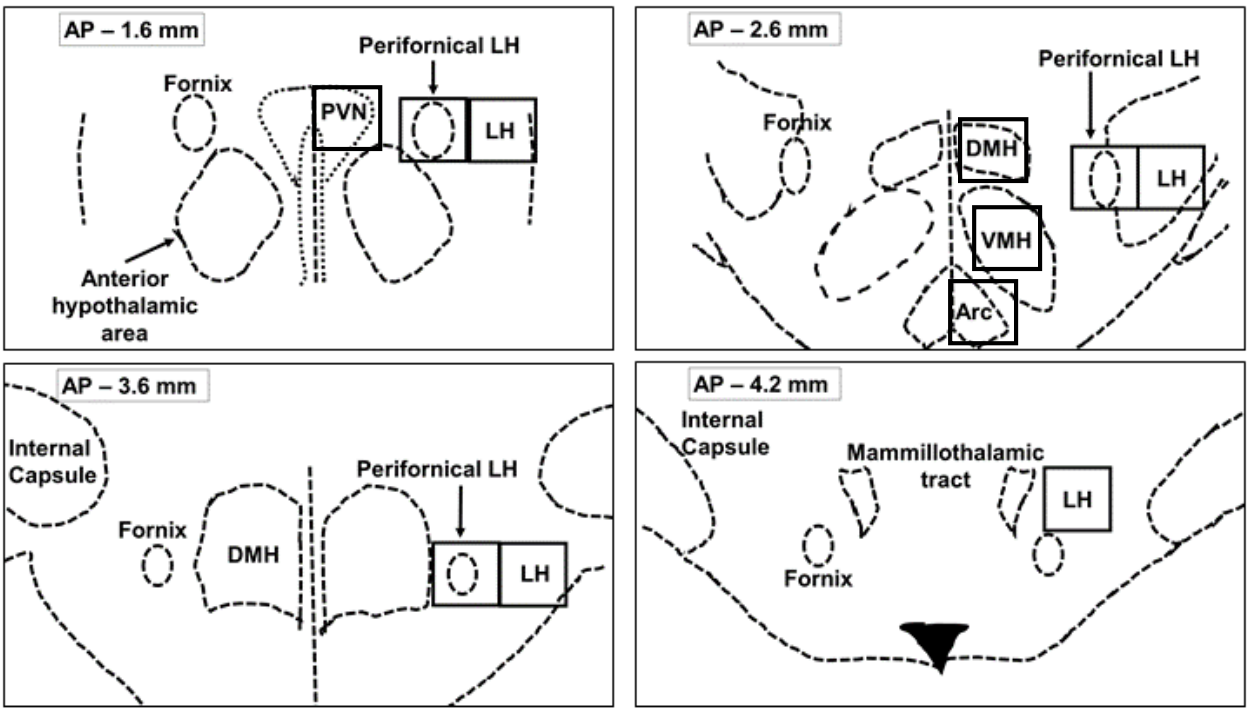

Figure 4. Schematic representation of the hypothalamic regions studied. The LH was divided in two regions: the perifornical LH surrounding the fornix and the LH, lateral to the perifornical field. The squares around the perifornical LH and the LH indicated the approximate size of the microscopic fields. Modified from Stratford (2005).

Fos labeling. Sections for Fos were rinsed in 0.01 M phosphate-buffered saline (PBS, pH7.2) and incubated on a rotary shaker table for 44 h at 4^0 C in a polyclonal rabbit anti-Fos primary antibody (Calbiochem; San Diego, CA) diluted 1:15,000 with 0.01 M PBS containing 4% normal goat serum (NGS; Vector Laboratories, Burlingame, CA). After that, the sections were rinsed in PBS and incubated in the biotinylated goat anti-rabbit secondary (Vector Laboratories, Burlingame, CA) antibody (diluted 1:200 with PBS

containing 4% NGS) for 90 min at room temperature, rinsed in PBS and incubated in the avidin– biotin complex solution for 90 min. Following another series of rinses in PBS, the peroxidase was visualized by incubating the tissue for 5 min in the nickel-enhanced chromogen solution from a Vector 3,3V diaminobenzidine tetrahydrochloride peroxidase substrate kit. The sections were mounted on chrome–alum-coated slides, air-dried, cleared in xylene, and coverslipped with Permount.

Fos/orexin labeling. Sections for Fos/orexin double staining were rinsed in 0.01 M phosphate-buffered saline (PBS, pH7.2) and incubated on a rotary shaker table for 44 h at 4^0 C in a polyclonal rabbit anti-Fos primary antibody (Calbiochem, San Diego, CA) diluted 1:7,500 and a goat anti-orexin primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:500 with 0.01 M PBS containing 2% normal donkey serum (NDS; Vector Laboratories, Burlingame, CA) and 1% bovine serum albumin. After that, the sections were rinsed in PBS and incubated in biotinylated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) (diluted 1:200 with PBS containing 2% NDS) for 90 min at room temperature, rinsed in PBS and incubated in the avidin– Cy3 (Jackson ImmunoResearch, West Grove, PA) diluted 1:300 and alexa fluor 488 donkey anti-goat (Jackson ImmunoResearch, West Grove, PA) diluted 1:200 in PBS containing 2% NDS solution for 90 min at room temperature. Following another series of rinses in PBS, the sections were mounted on chrome–alum-coated slides, air-dried and coverslipped with 90% glycerol 10% PBS 1% n-propyl gallate.

Fos/MCH labeling. Sections for Fos/MCH were rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.2) and incubated on a rotary shaker table for 44 h at 4^0 C in a polyclonal goat anti-Fos primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:500 and a rabbit anti-MCH primary antibody (Phoenix Pharmaceuticals, Burlingame, CA) diluted 1:8000 with 0.01 M PBS containing 2% normal donkey serum (NDS; Vector Laboratories, Burlingame, CA). After that, the sections were rinsed in PBS and incubated in biotinylated donkey anti-goat secondary antibody (diluted 1:200 with PBS containing 2% NDS) for 90 min at room temperature, rinsed in PBS and incubated in the avidin– Cy3 diluted 1:300 and alexa fluor 488 donkey anti-rabbit (Jackson ImmunoResearch, West Grove, PA) diluted 1:200 in PBS containing 2% NDS solution for 90 min at room temperature. Following another series of rinses in PBS, the sections were mounted on chrome–alum-coated slides, air-dried and coverslipped with 90% glycerol 10% PBS 1% n-propyl gallate.

Cell counting. Neuronal counting of Fos expressing cells was done automatically using a Leica Q500MC analyzer, with the detection parameters adjusted so as to yield results similar to those obtained with manual counts. All double staining for Fos and orexin or Fos and MCH containing cells were be counted manually on fluorescent images captured with image-J (Rasband, 1997-2014) by an experimenter blind to the experimental condition. The total number of Fos expressing neurons was first determined and then the number of orexin/hypocretin or MCH expressing cells in the field. Double labeled cells were visualized by superposing a layer containing the Fos marked cells over a layer containing the orexin or MCH marked cells. Since Fos is a

nuclear protein and orexin/hypocretin and MCH are expressed in the cell's soma, double labeled neurons appeared as cells with positive staining in both the nucleus and the soma. I analyzed the percentage of orexin cells which contained Fos and, similarly, the percentage of MCH cells that contained Fos.

B2. Results

Placement verification. The placement of the cannulae was assessed using cresyl violet staining and the tips mapped into a rat brain atlas (Paxinos and Watson, 2007). The AP ranged between 0 - 1mm. A representative histological picture is presented in fig. 3. The histological data indicated that in three rats (one experimental and two controls) the injectors' tips terminated outside of the brain, these animals were rejected and their results are not included here.

Intra-VPm NMDA injections increase Fos expression in the perifornical LH. A 2 X 2 X 3 (Group X Side X Level) repeated-measures ANOVA was conducted to analyze the number of Fos positive neurons in the three different perifornical levels studied here (APs: -1.6, -2.6 and -3.6; fig. 4). Neither the group (bilateral saline or saline and NMDA) nor the side (saline or NMDA) effects were significant ($p>.05$). There was a significant level effect (distance from bregma in mm: -1.6, -2.6 and -3.6) ($F_{(2,14)} =4.58$, $p<.02$) indicating higher levels of Fos signal at more caudal locations, this factor did not interact

with any other factor included in this analysis. The side X group interaction was significant as well ($F_{(1,14)}$ =9.51, p<.01) showing that the difference in Fos expression between the saline and the NMDA sides is larger in the NMDA treated rats than it is in the control animals. In this region, the results show that Fos staining was higher in the side ipsilateral to the NMDA injection than in the saline injected side (fig. 5). The group X side X level interaction was not significant (p>0.05), indicating that the group X side effect was not moderated by level.

Intra-VPm NMDA injections increase Fos expression in the LH. A similar 2 X 2 X 4 (Group X Side X Level) repeated-measures ANOVA was performed to analyze the number of Fos positive neurons in the four different LH levels studied here (APs: -1.6, -2.6, -3.6 and -4.2; fig. 4). Neither the group (bilateral saline or saline and NMDA), the side (saline or NMDA) nor the level (distance from bregma in mm: -1.6, -2.6, -3.6 and -4.2) effects were significant (p>.05). As with the perifornical region, the analysis showed a significant ($F_{(1,42)}$ =15.66, p<.001) side X group interaction indicating that the difference between the vehicle side and the NMDA injected side was higher in the NMDA injected rats than in the controls. In the LH, Fos staining was significantly greater ipsilateral to the NMDA injected side than the saline injected side (fig. 6). Although the effect appeared smaller at -3.6, the group X side X level interaction was not significant (p>0.05), indicating that the group X side effect was not significantly moderated by level.

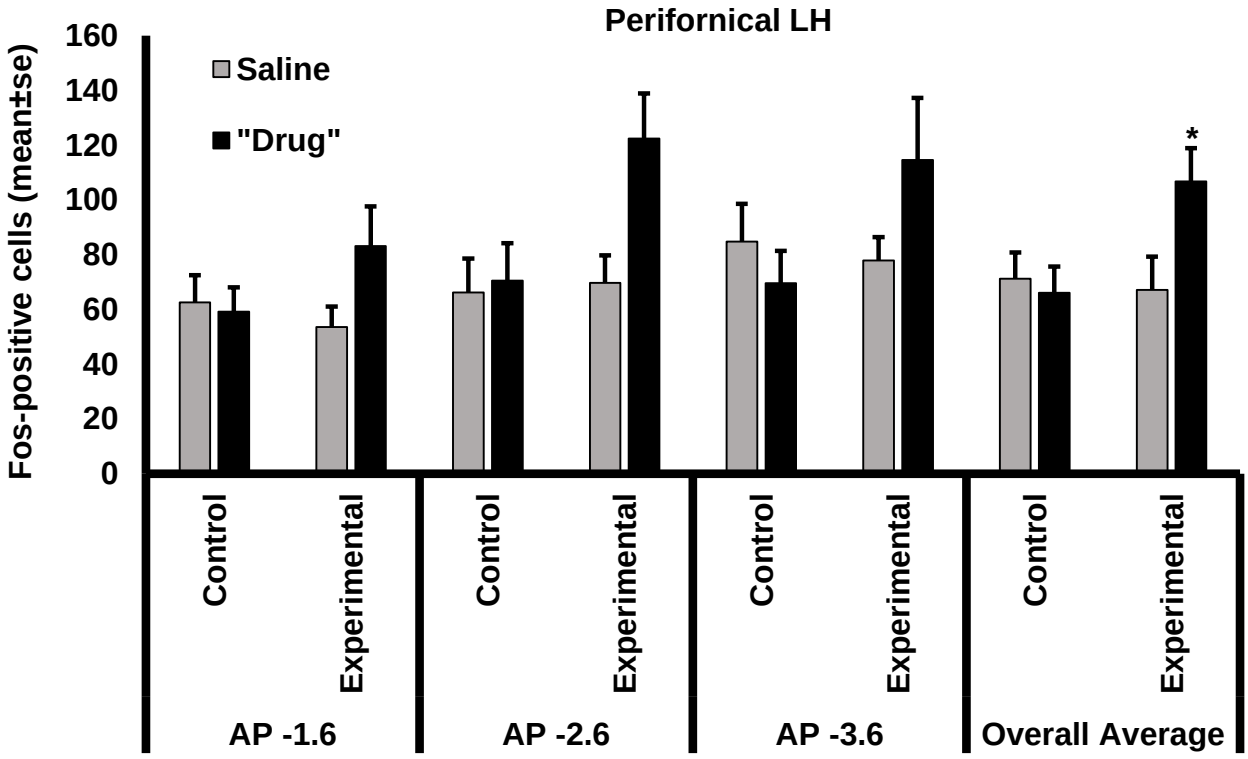

Figure 5. Control rats received saline injections on the "drug" side whereas experimental rats received NMDA 0.45 μg on this side. Injections of NMDA into the VPm significantly increased the number of Fos-positive cells in three different AP levels as well as in the overall Drug x Side interaction. *p < .05 vs. saline.

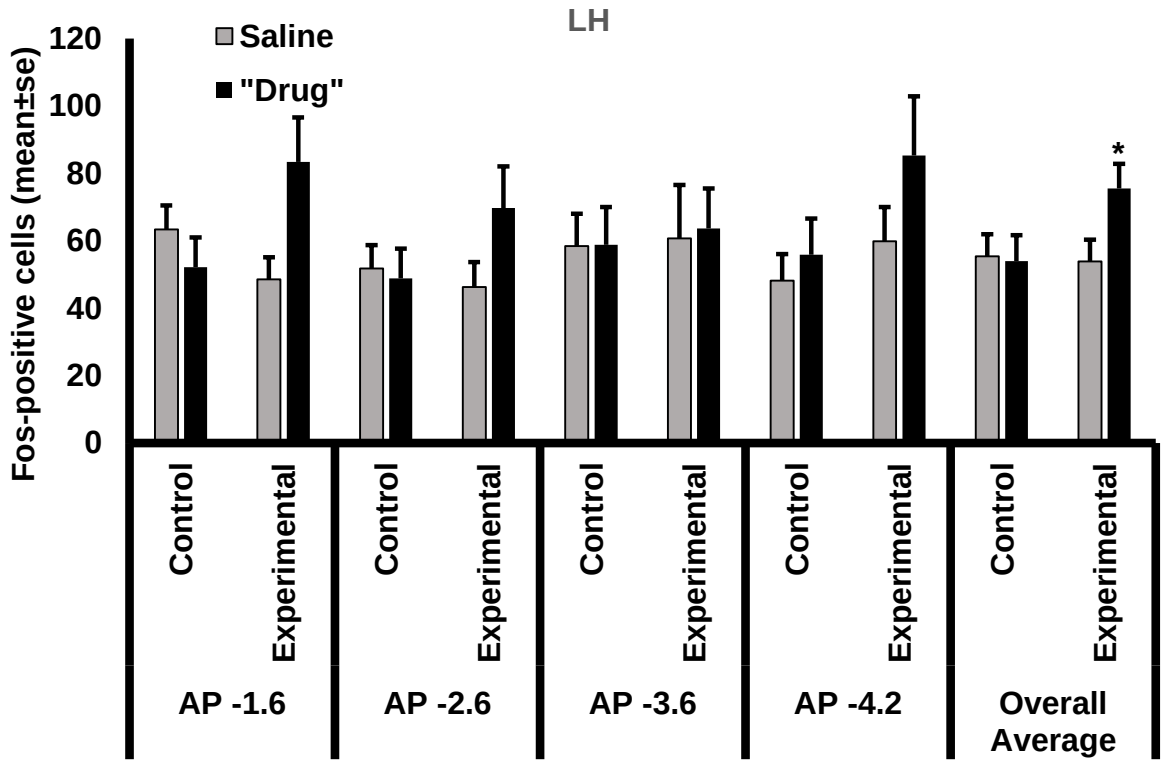

Figure 6. Control rats received saline injections on the "drug" side whereas experimental rats received NMDA 0.45 μg on this side. The NMDA injections increased Fos staining as seen in the overall Drug x Side interaction. This effect was not significantly moderated by level (see text for details). *p < .05 vs. saline.

Statistical analysis of other hypothalamic regions. To analyze the effects of NMDA injections on Fos staining in the PVN, VMH, DMH and ARC, four independent 2 X 2 (Group X Side) ANOVAs were conducted.

Intra-VPm NMDA injections increase Fos expression in the PVN. In the PVN, the factor group was not significant (p>0.05). There was a significant side effect ($F_{(1,14)}$ =12.15, p<.01) and also a significant side X group interaction ($F_{(1,14)}$ =5.15, p<.05) indicating that

the PVN ipsilateral to the NMDA displayed more Fos staining than the vehicle injected side in the NMDA injected rats but not in the controls (fig. 7).

Absence of increased Fos expression in the VMH. In the VMH the ANOVA failed to detect significant effects of any of the factors or interactions ($p>0.05$), suggesting that NMDA injections did not affect Fos staining in this region (fig. 8).

Intra-VPm NMDA injections increase Fos expression in the DMH. In the DMH the ANOVA indicated a significant ($F_{(1,14)} =10.1$, $p<.01$) group effect, this suggests the possibility that NMDA increases Fos staining in the DMH ipsilaterally and contralaterally to the injection side in NMDA injected rats. Post-hoc comparisons indicate that there is no significant difference ($p>0.05$) between the vehicle-injected side in NMDA injected animals and the vehicle side in vehicle injected rats. The analysis also indicated a significant ($F_{(1,14)} =7.16$, $p<.02$) group X side interaction in the DMH, this interaction indicates that NMDA increases DMH Fos more on the side of the NMDA injection than on the contralateral side with a non-significant trend to produce some contralateral increase (fig. 9).

Absence of increased Fos expression in the ARC. In the ARC, the ANOVA failed to detect significant differences between the groups, this suggests that there are no differences in Fos staining between the drug side and the vehicle side in control and experimental animals (fig. 10).

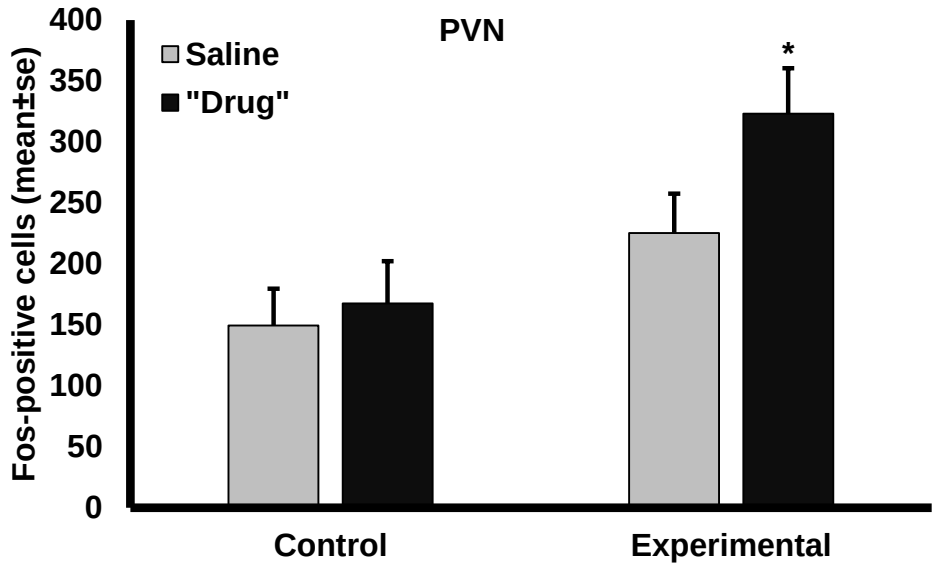

Figure 7. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. Injections of NMDA into the VPm significantly increased Fos staining in the PVN compared with contralateral saline injections. *p < .05 vs. saline.

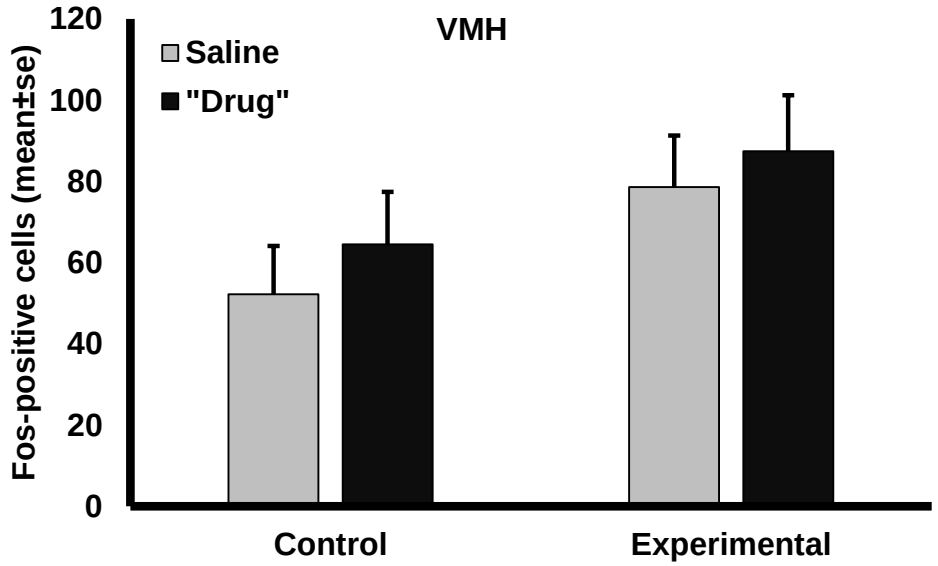

Figure 8. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. In the VMH, I observed no differences between the unilateral intra-VPm NMDA and contralateral intra-VPm saline injections in NDMA injected rats.

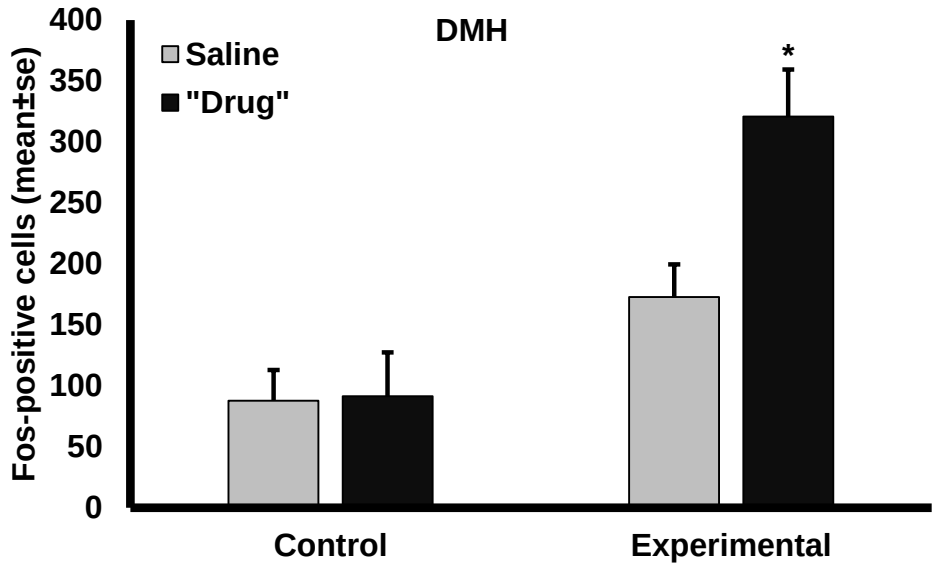

Figure 9. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. Intra-VPm NMDA injections significantly increased Fos staining in the DMH compared to contralateral saline in the NMDA injected rats but not in the bilateral saline injected controls. *p < .05 vs. saline.

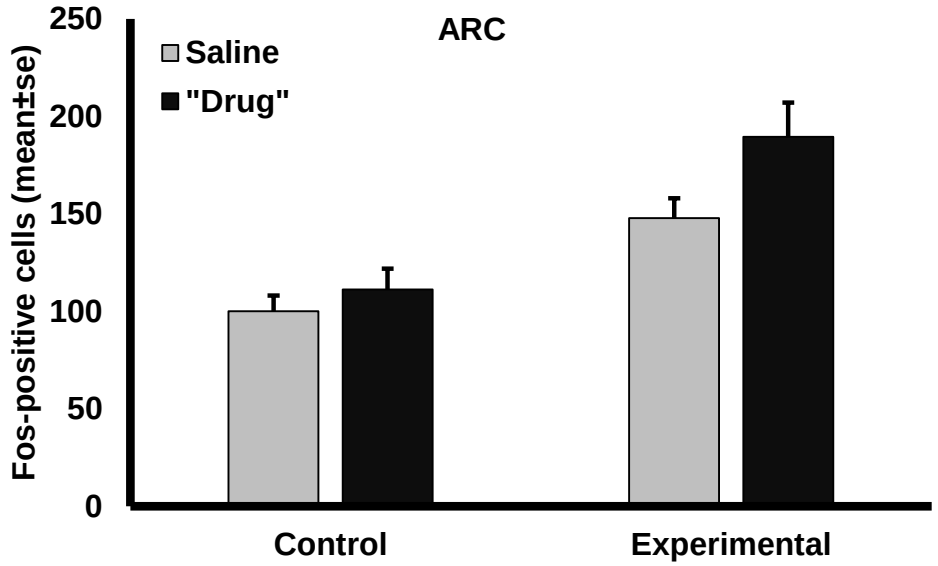

Figure 10. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. In the ARC, I observed no differences between intra-VPm NMDA and contralateral intra-VPm saline injections in NDMA injected rats.

Absence of increased Fos expression in the Fos/orexin and Fos/MCH double stained cells. After calculating the percentage of double labeled Fos/orexin and Fos/MCH cells, two independent 2 X 2 (Group X Side) ANOVAs were conducted. The results indicate that none of the analyzed factors were significant ($p>0.05$). These results suggest that there are no differences in Fos/Orexin and Fos/MCH staining between the NMDA injected side and the vehicle-injected side (Figs. 11 and 12).

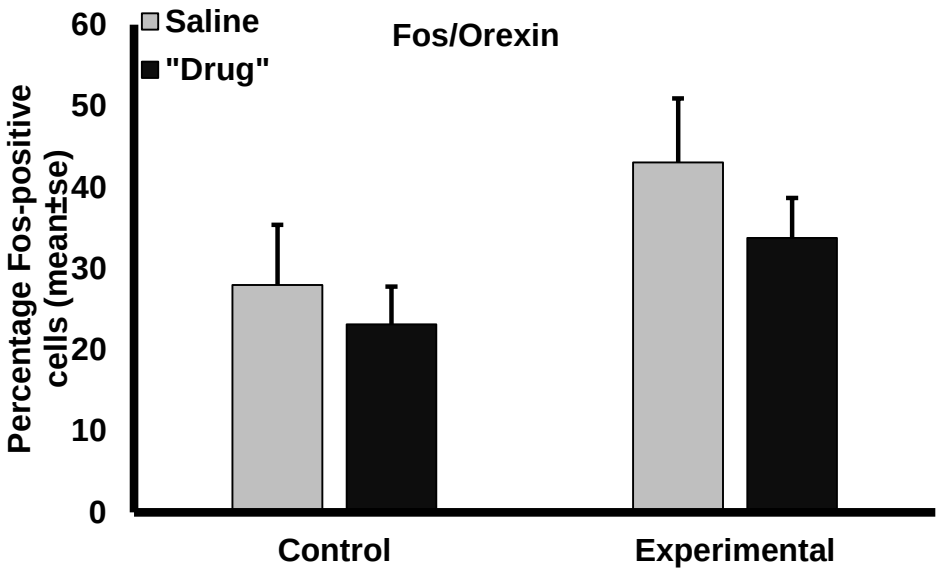

Figure 11. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. Injections of vehicle or NMDA into the VPm did not significantly change the percentage of double labelled Fos/orexin neurons.

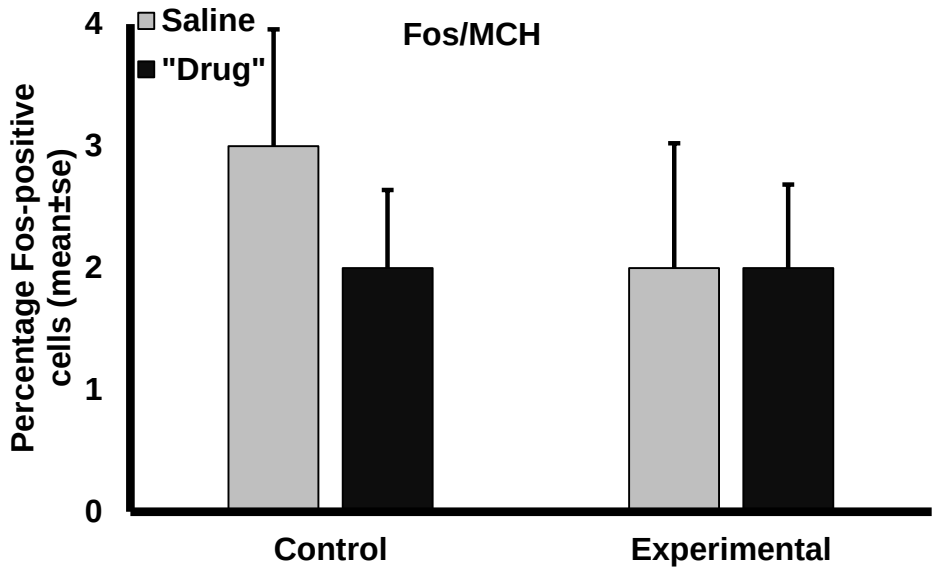

Figure 12. Control rats received saline injections on the “drug” side whereas experimental rats received NMDA 0.45 μg on this side. Injections of vehicle or NMDA into the VPm did not significantly change the percentage of double labelled Fos/MCH neurons.

B3. Discussion

Here I present data demonstrating that unilateral intra-VPm NMDA injections increase Fos expression in the perifornical LH and the LH, the PVN and the DMH but not in the VMH or ARC. These results suggest that: a) as predicted, the VPm and the LH are functionally linked, b) the VPm stimulated feeding effects might be linked to the PVN and the DMH, hypothalamic regions that regulate food intake, c) the VMH and the ARC are not affected by intra-VPm NMDA injections and thus not part of the VPm functional circuit. Furthermore, given that we failed to observe an increase in Fos in orexin/hypocretin and MCH, these results imply that: d) the orexin/hypocretin system is

not involved in feeding induced by intra-VPm NMDA injections and e) feeding induced by intra-VPm NMDA injections is independent from the MCH system. I conclude this section with a comparison between VPm- and AcbSh-stimulated feeding in terms of Fos expression (f) and a general summary (g).

a) The VPm and the LH are functionally linked. Given that the VPm projects to the LH (Groenewegen et al., 1993; Haber et al., 1993; Tripathi et al., 2013; Zahm et al., 1985), my results demonstrate that the same manipulations of the VPm that cause feeding modulate the LH. It is likely that VPm-stimulated feeding takes place in part through the recruitment of hypothalamic areas involved in the homeostatic control of food intake like the LH. This hypothesis is supported by the fact that LH lesions attenuate VPm induced feeding (Stratford and Wirtshafter, 2013).

These results are consistent with the pattern of Fos expression observed after electrical stimulation of the VPm (Panagis et al, 1997) but are different from the effects of pharmacological excitation using NMDA (Turner et al, 2008) in anesthetized rats. The effects of generalized anesthesia on neuronal excitation likely explain the discrepancy between Turner's report and ours.

Ipsilateral manipulations of the AcbSh and the VPm induce Fos expression in the LH. Previous studies have shown that unilateral injections of $GABA_A$ agonists in the AcbSh, a ventral striatal region that projects to both the VPm and the LH (Groenewegen and Russchen, 1984; Heimer et al., 1991; Walaas and Fonnum, 1979; Zahm et al., 1985),

induce ipsilateral Fos immunoreactivity in the LH and the VPm among other brain areas (Pulman et al., 2012; Stratford, 2005). Here, I report a similar pattern of activation which includes increased Fos expression in the LH. It is likely that the similarities are responsible for the common phenotypical aspects of AcbSh- and VPm-stimulated feeding, namely, voracious feeding in rats fed ad libitum.

The entire functional relationship between the AcbSh, the VPm and the LH remains unclear. Considering that the AcbSh projects to both the VPm and the LH (Groenewegen and Russchen, 1984; Heimer et al., 1991; Walaas and Fonnum, 1979; Zahm et al., 1985) and the VPm projects to the LH (Fig.1) (Groenewegen et al, 1993; Tripathi et al., 2013), the fact that both can modulate the neural activity of the LH could imply that the VPm is acting as a relay between the AcbSh and the VPm. Since VPm or LH lesions reduce feeding induced by muscimol in the AcbSh (Stratford and Wirtshafter, 2012), it is possible that VPm lesions could also prevent the AcbSh from inducing LH Fos expression. I address this question in chapter 3.

Given that the majority of the VPm's projections are GABAergic (Groenewegen et al., 1993), it is somewhat counterintuitive that excitation of this region using NMDA causes neuronal excitation anywhere in the brain. There are multiple non-mutually exclusive explanations for this phenomenon. For one, the VP contains populations of cells positive for vesicular glutamate transporter 2 (Geisler el at., 2007; Hur and Zaborszky, 2005), making it possible that activation of these glutamatergic neurons is responsible for the observed increases in Fos expression. Alternatively, one could propose that

GABAergic pallidal neurons synapse on hypothalamic inhibitory interneurons located in the LH (Oomura et al., 1975). However, it is unknown if the VPm projects to these population of hypothalamic inhibitory interneurons described by Oomura (Oomura et al., 1975). If they did, NMDA in the VPm would ultimately cause hypothalamic excitation by blocking inhibitory projections to the Fos positive cells. Additionally, the Fos expression in the LH induced by intra-VPm NMDA injections could be explained in part by the fact that the VP projects back to the AcbSh (Churchill and Kalivas, 1994). Indeed, my own preliminary analysis suggests that intra-VPm NMDA infusions induce Fos expression in the AcbSh. Moreover, induction of Fos expression between the AcbSh and the VP has been shown in the past using opioid agonists (Smith and Berridge, 2007). According to this interpretation, the LH would be modulated by a direct AcbSh to LH projection, itself modulated by a connection between the VPm and the AcbSh, and a direct VPm to LH projection.

b) The VPm and the PVN, DMH are functionally linked. In addition to the LH, I show here that the PVN and DMH are also activated by injections of bicuculline in the VPm. Substantial evidence indicates that these structures in are involved in the control of food intake. For example, injections of neuropeptide Y (NPY), a powerful central appetite booster (Valassi et al., 2008), into the PVN increase food and water intake (Stainley et al., 1985), while intra-PVN 5-HT injections have an anorectic effect (Leibowitz and Alexander, 1998). The DMH is interposed between the LH and the PVN (Bernardis and Bellinger, 1998) and has roles in multiple behaviors including feeding (Chou et al., 2003) as shown by the fact that DMH lesions induce hypophagia and hypodipsia

(Bernardis and Bellinger, 2003), and intra-DMH muscimol injections block selective fat intake induced by intra-Acb DAMGO injections (Will et al., 2003). These findings suggest that modulation of the PVN and the DMH by the VPm is involved in the feeding response observed after pharmacological pallidal excitation. The possible involvement of the PVN and DMH in the ingestive response to intra-VPm bicuculline or NMDA injections could be investigated by determining whether lesions or inactivation with muscimol of these structures alter feeding induced from pharmacological excitation of the VPm.

c) The excitation of the ARC and the VMH are independent of VPm excitation. My results indicate that the VMH and the ARC are unaffected by VPm excitation. Some studies indicate that intra-AcbSh muscimol injections induce higher ipsilateral Fos staining in the ARC (Baldo et al., 2004; Stratford, 2005), while others fail to see this effect with muscimol (Pulman et al., 2012). Thus, it is possible that the expression of Fos by ARC using these pharmacological manipulations is not a reliable effect. Furthermore, a close inspection of the VMH and specially the ARC data indicates a trend toward a bilateral increase of Fos staining. This bilateral signal could be produced from crossed projections to the ARC from the LH or the DMH. Given the role of the ARC as a nodal regulator of food intake that integrates inputs for multiple hypothalamic regions (Bouret, et al., 2004), it is possible that a bilateral signal could be observed even with unilateral injections as the ARC integrates signals from other hypothalamic areas such as the PVN and the DMH. For the exposed reasons, further experimentation is needed to determine the relative importance of the ARC in VPm-LH feeding circuit.

d) The orexin/hypocretin system is independent of VPm excitation. It is notable that the orexin/hypocretin system is not recruited by feeding induced by unilateral intra-VPm NMDA injections. It is possible that bilateral injections are required for intra-VP NMDA infusions to recruit the orexin/hypocretin system. It is also possible that these unilateral VPm injections activate orexin/hypocretin-expressing neurons bilaterally, a trend I observed in fig. 11. A bilateral effect caused by a unilateral injection would suggest an indirect VPm to LH projection is modulating the effect. A possible candidate is the nucleus of the tractus solitarius that normally inhibits LH orexin neurons when food reaches the gut (Cai et al., 2001) but could be itself inhibited by GABAergic projections from the VPm. Additionally, it is possible that VP feeding operates through other orexigenic peptides, like NPY. Future studies should attempt to demonstrate if these orexigenic populations are involved in pallidal feeding.

e) The MCH system is independent of VPm excitation. In addition to the orexin/hypocretin neurons, the other orexigenic hypothalamic neural population studied in this dissertation is the MCH expressing cells. As I have indicated, my results indicate that the hypothalamic MCH system seems to be independent of intra-VPm NMDA feeding. Electrophysiological techniques show that neurons expressing orexin/hypocretin or MCH are activated by hypoglycemia, a condition that elicits food intake in animals (Karnani and Burdakov, 2011). In contrast, acute hypoglycemic stress increases c-Fos mRNA expression in orexin/hypocretin but not in MCH neurons (Nishimura et al., 2014). It seems then that the activity of MCH neurons can be detected

in electrophysiological measures even when the same manipulation fails to show changes in c-Fos. Electrophysiological recordings also suggest that MCH neurons do not fire during awake states under normal physiological conditions (Hassani et al., 2009). In essence, MCH expressing neurons might not be active long enough to detect Fos protein expression due to the marked circadian variation of activity which is increased during the dark (Hassani et al., 2009). One way to improve this time resolution issue would be to deploy a combination of pharmacological and electrophysiological techniques to study the effects that pharmacological manipulations of the VPm have on the firing patterns of different hypothalamic populations implicated in the regulation of food intake. For the exposed reasons, further experiments are needed to determine the relationship between the VPm and MCH neurons.

f) Differences between VPm and AcbSh stimulated feeding. The differences in the hypothalamic patterns of Fos expression between manipulations that induce feeding in the AcbSh and the VPm indicate that these two feeding effects might be related but are not identical. Intra-VPm bicuculline injections tend to increase locomotor activity, a response not observed with intra-AcbSh muscimol injections (Stratford and Kelley, 1997; Stratford et al., 1999). Although it has been recently suggested that the increase in locomotor activity might be produced by excitation of the preoptic area and not the VP (Zahm et al., 2013). Indeed, our own laboratory has failed to observe increased locomotor activity after intra-VPm bicuculline injections (Covelo et al., 2014). Thus, the locomotor-activating effects of intra-VPm bicuculline injections might be related to the nearby preoptic and not the VP proper. Moreover, the current results indicate that

feeding elicited in rats fed ad libitum by injections of NMDA into the VPm is independent from orexin/hypocretin and MCH expressing neurons in the LH. In contrast, bilateral $GABA_A$ agonists injected into the AcbSh induce Fos expression in the orexin/hypocretin containing cells in the LH (Baldo et al., 2004, Zheng et al., 2003). It is possible that the direct projections from the AcbSh to the LH are responsible for recruiting orexin/hypocretin neurons while projections from the VPm activate different cells. Future studies should explore if other hypothalamic orexigenic peptides, besides orexin and MCH, are implicated in VPm stimulated feeding. Furthermore, intra-VPm bicuculline infusions, but not intra-AcbSh muscimol injections, selectively increase fat intake in a macronutrient selecting paradigm (chapter 4, Covelo et al., 2014). Taken together, these results suggest that both AcbSh and VPm stimulated feeding modulate the LH but via different patterns of hypothalamic Fos expression. It is plausible that the similarities account for their shared behavioral responses, namely the increase in food intake, while the differences explain their divergences, like the hyperactivity and the fat preference observed in VPm but not in AcbSh induced feeding.

g) Summary. My results indicate that feeding can be elicited by unilateral injections of NMDA in the VPm, adding glutamate to the list of neurotransmitters potentially implicated in the modulation of this structure. Additionally, my data demonstrate that the LH, PVN and DMH are involved in feeding induced by excitation of the VPm. I propose that the VPm stimulates food intake by modulating hypothalamic regions involved in the regulation of homeostatic food intake. Finally, I show that feeding induced by excitation of the VPm is independent of the orexin/ hypocretin and MCH systems, two neural

populations activated by energy deficits like hypoglycemia (Karnani and Burdakov, 2011). These results indicate that VPm stimulated feeding may be different from feeding induced by homeostatic energy deficits. Taken together, these results support the hypothesis that the VPm is a neural structure involved in the modulation of non-homeostatic feeding and that it recruits hypothalamic circuits involved in the regulation of food intake.

CHAPTER 3. INJECTIONS OF MUSCIMOL INTO THE SHELL OF THE NUCLEUS ACCUMBENS INCREASE FOS EXPRESSION IN THE LATERAL HYPOTHALAMUS INDEPENDENTLY OF THE INTEGRITY OF THE MEDIAL VENTRAL PALLIDUM

The lateral hypothalamus (LH) is heavily involved in the regulation of feeding and due to its direct connection with the shell of the nucleus accumbens (AcbSh) (Heimer et al., 1991) and indirect through the medial ventral pallidum (VPm) (Groenewegen et al, 1993), the LH is likely critical for feeding induced by stimulation of the AcbSh. This hypothesis is supported by the fact that LH lesions attenuate feeding induced by intra-AcbSh muscimol (a $GABA_A$ agonist) injections in satiated rats (Stratford and Wirtshafter, 2012). Additionally, the possibility of a functional connection between the AcbSh, the VPm and the LH is confirmed by studying the pattern of Fos expression induced by inhibiting the AcbSh using muscimol and by intra-VPm NMDA injections as discussed in Chapter 2. Unilateral injections of muscimol in the AcbSh induce ipsilateral Fos expression in the LH and in the VPm, as well as other brain regions (Pulman et al., 2012; Stratford, 2005). As a whole, these results support the existence of a functional AcbSh-VPm-LH circuit (Stratford, 2007; Stratford and Kelley, 1999; Stratford and Wirtshafter, 2012).

Despite the known connections between these brain regions, it is unclear how these three structures are connected to form a putative feeding circuit. As the AcbSh projects to the LH both directly and indirectly through the VPm (fig. 1), our goal here is to

distinguish the contributions of the AcbSh and the VPm to LH activation. By lesioning the VPm and then comparing the patterns of Fos immunoreactivity obtained by injections of muscimol in the AcbSh, we can distinguish between direct and indirect projections of the AcbSh to the LH. I hypothesize that Fos immunoreactivity in the LH will be lower ipsilateral to the VPm lesion when compared to non lesioned rats, suggesting that the VPm is a necessary relay station for AcbSh induced LH activation.

A. **Subjects, materials and methods**

Animals & surgery. Twenty rats were anesthetized using sodium pentobarbital (50 mg/kg), placed in a rat stereotaxic apparatus, and surgically implanted with double guide cannulae aimed 2 mm above the AcbSh in the following coordinates (in mm): AP: 1.6, ML: ±0.9, DV: -6.1. During the surgery procedure, ten rats received a unilateral lesion of the VPm using the fiber sparing excitotoxic agent ibotenic acid (10 μg/μl at a rate of 0.25 μl/min for a total injection of 5 μg), the remaining rats received a unilateral saline injection in the same location. The rats were allowed to recover for at least two weeks. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UIC Institutional Animal Care and Use Committee.

Injection protocol. After the recovery period, the rats were habituated to the injection procedure by exposing them to all the steps involved in the procedure minus the actual injection. Two days before the injection, the injectors were inserted but no infusions

were made. On injection day, the rats were gently restrained and two injectors extending 2 mm from the tip of the guide cannula were inserted to unilaterally inject the rats with the $GABA_A$ agonist muscimol (100 μg/0.5ul) into the AcbSh ipsilateral to the side of the VPm ibotenic or saline injections. Immediately after the injection, the rats were placed back in their home cages without food or water for 90 minutes.

Tissue harvest. After the 90 min rest period, rats were deeply anesthetized using sodium pentobarbital (150 mg/kg) and perfused transcardially with 50 ml of 0.15-M saline followed by 200 ml of 10% buffered formalin at pH 6.5, then 300 ml of 10% buffered formalin at pH 9.0. The brains were then extracted, placed in 10% buffered formalin at pH 9.0 and stored in PBS with 20% sucrose for at least 48 h.

Brain sectioning. The brains were frozen cut in 35μm serial coronal sections, stored in cryoprotectant and processed for Fos expression. Serial sections were taken through the LH and were analyzed at three AP levels: -1.6, -2.6 and -3.6.

NeuN labeling. The lesions were assessed using neuronal nuclear protein (NeuN) a marker of intact neurons. Sections were rinsed in PBS and incubated on a rotary shaker table for 24 h at 4 ◦ C in a monoclonal mouse anti-NeuN primary anti- body (diluted 1:20,000 with 0.01 M PBS containing 0.2% Triton X-100 and 4% normal horse serum; Oncogene Research Products, La Jolla, CA). The sections were rinsed in PBS and incubated in the biotinylated horse anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA, diluted 1:200 with PBS containing 4% normal horse serum) for 60 min

at room temperature. They were then rinsed in PBS (3 × 10 min), and incubated in the avidin–biotin complex solution (Vector) for 60 min. Following another series of rinses in PBS, the peroxidase was visualized by incubating the tissue for 5 min in a nickel-enhanced chromogen solution obtained from a Vector 3,3′ -diaminobenzidine tetrahydrochloride peroxidase substrate kit. The sections were mounted on chrome-alum coated slides, air-dried, cleared in xylene, and coverslipped with Permount mounting medium.

Fos labeling. Sections for Fos and Fos neuronal counting were processed using methods identical to those described in chapter 2, section B1.

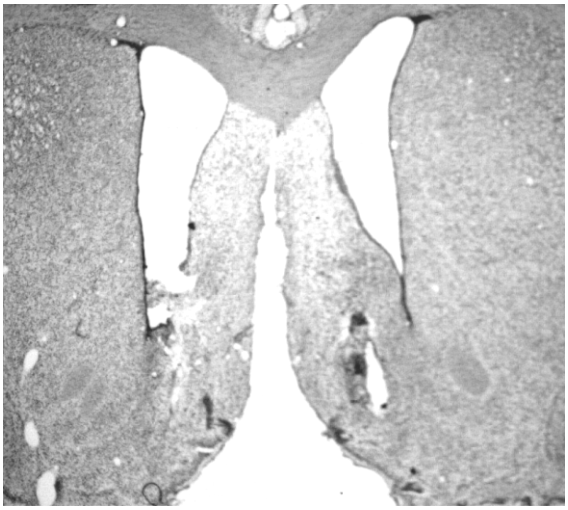

Figure 13. Photomicrograph of a cresyl violet stained section showing a typical cannula placement in the AcbSh.

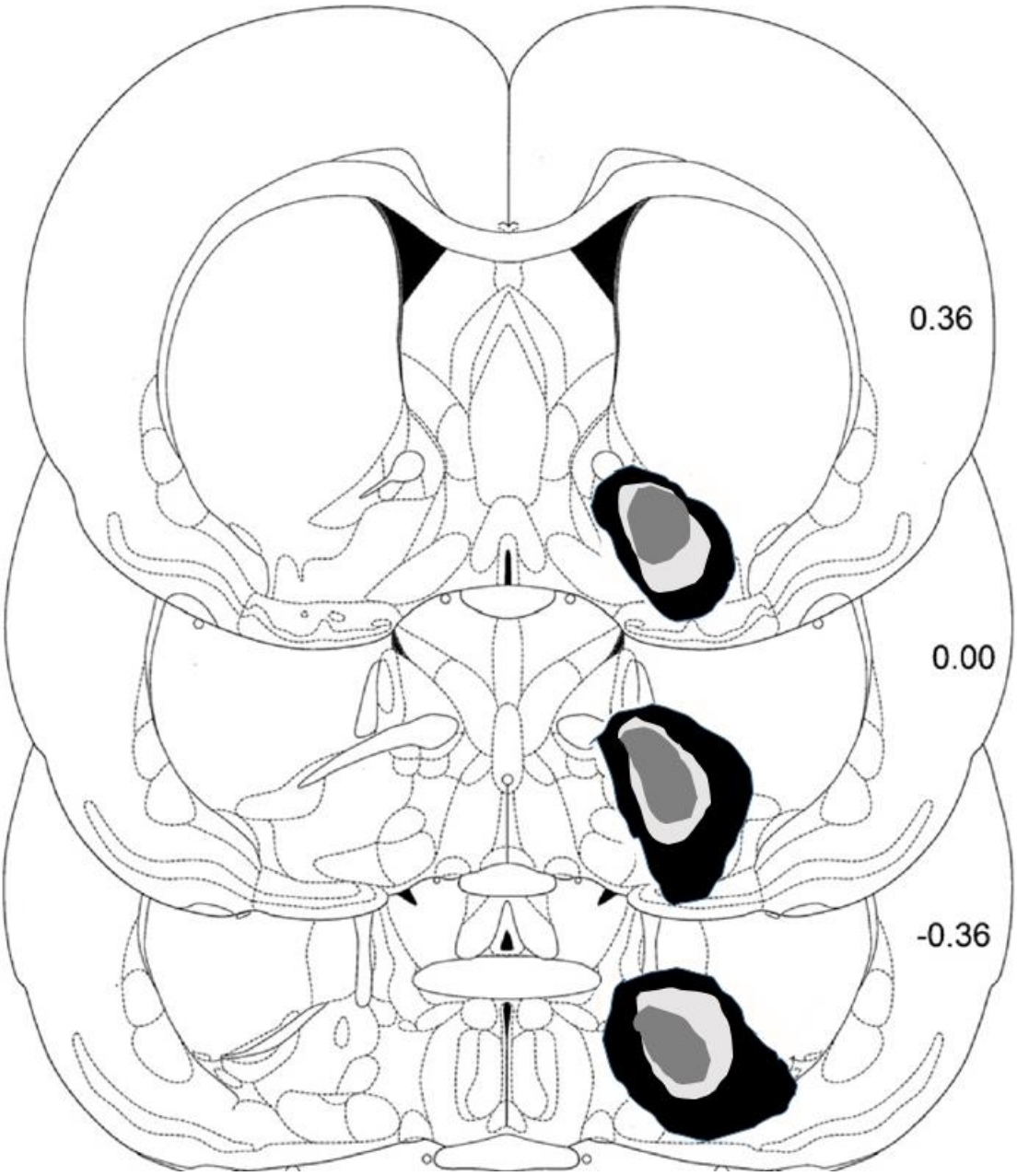

Figure 14. Illustrations of the smallest, average and largest lesions of the VPm, the numbers indicate distance from bregma (Modified from Stratford and Witshafter, 2012; Paxinos and Watson, 2007).

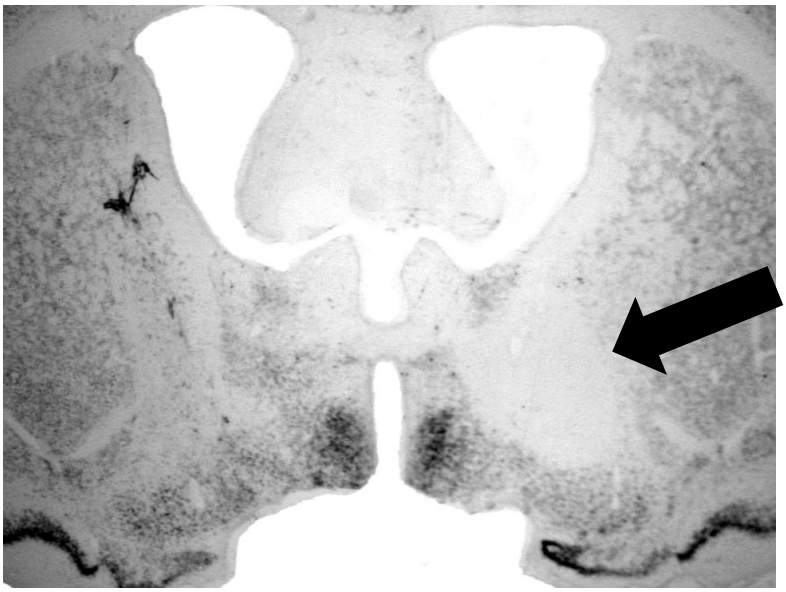

Figure 15. Photomicrograph of a section stained for NeuN through a typical excitotoxic lesion of the VPm (black arrow).

B. Results

Placement verification. The placement of the cannulae was assessed using cresyl violet staining and the tips mapped into a rat brain atlas (Paxinos and Watson, 2007). Five rats were discarded because the location of the cannula tips was too ventral and their results are not included in this analysis. In the accepted rats, the AP ranged from 1.3 to 2.3 mm, a representative histological picture can be found in fig. 13. The lesion boundaries were mapped and the AcbSh injection sites were examined for placement with reference to the atlas of Paxinos and Watson (2007). Fig. 14 shows the smallest, average and largest excitotoxic lesion. Fig. 15 displays a typical VPm lesion. As in the previous experiment, for measuring purposes we divided the LH in perifornical LH and LH (fig. 4).

Increased Fos expression in the perifornical LH independently of the integrity of the VPm. A 2 X 2 X 3 (Drug X Lesion X Level) repeated-measures ANOVA was conducted to analyze the number of Fos positive neurons in three different perifornical regions (APs: -1.6, -2.6 and -3.6). The factor drug (vehicle or muscimol) was significant ($F_{(1,13)}$ =33.08, p<.001) indicating higher levels of Fos signal in the side ipsilateral to the muscimol injection in both VPm lesioned and sham lesioned rats (fig.16). The factor level was also significant ($F_{(2,12)}$ =9.73, p<.01) indicating higher levels of Fos signal caudally. No other factor or interaction was significant (p>0.05), suggesting that the VPm lesion had no effect on Fos expression in the perifornical LH.

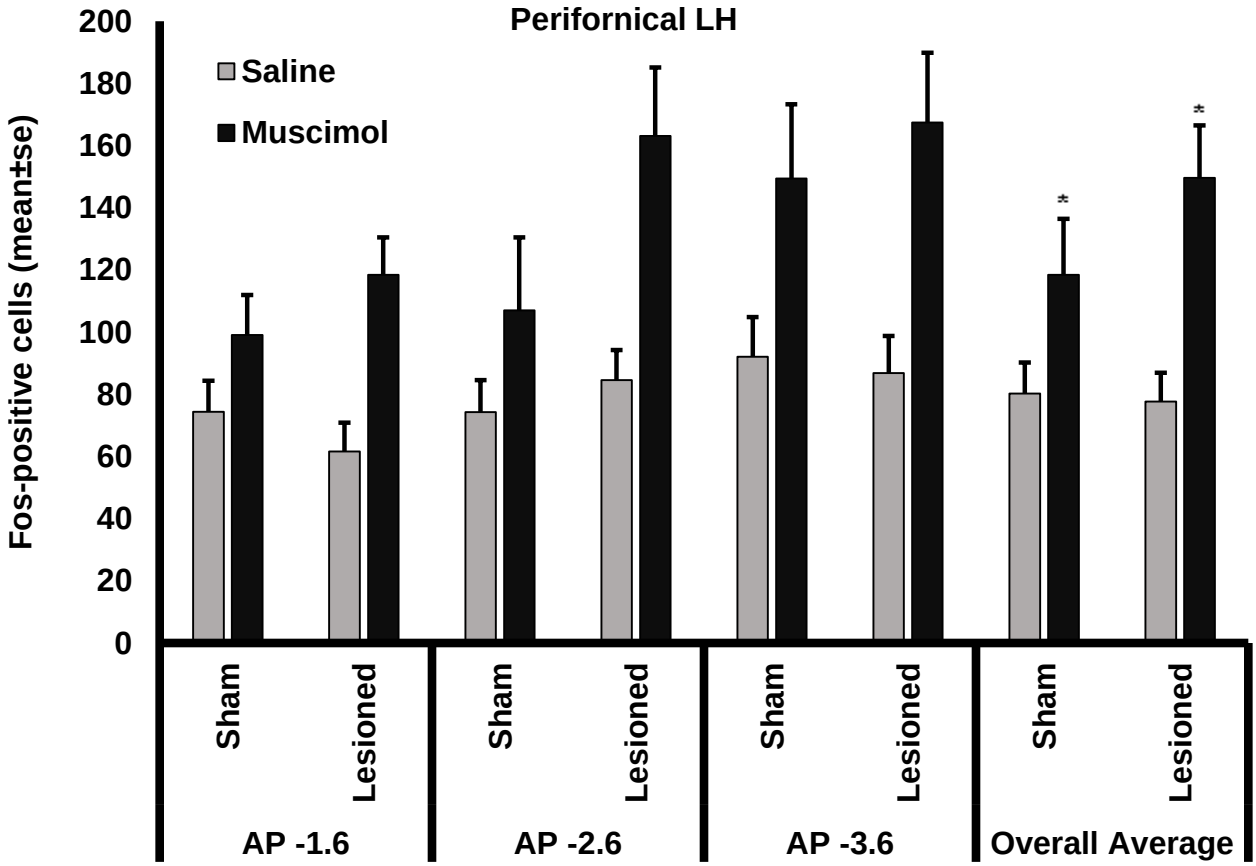

Figure 16. Number of Fos-positive neurons in the perifornical region of the LH measured in three different levels (abscissa). Both sham lesioned and VPm lesioned rats display a similar pattern of Fos signal: the side ipsilateral to the muscimol injection (100 μg/side) shows more Fos positive neurons than the vehicle injected side. *p < .05 vs. saline.

Increased Fos expression in the LH independently of the integrity of the VPm.

Additionally, a 2 X 2 X 3 (Drug X Lesion X Level) repeated-measures ANOVA was performed to analyze the number of Fos positive neurons in three LH AP levels: -1.6, -2.6 and -3.6. The factor drug (vehicle or muscimol) was significant ($F_{(1,13)}$ =39.24, p<.001) indicating higher levels of Fos signal in the side ipsilateral to the muscimol

injection in both VPm lesioned and sham lesioned rats. The factor level was also significant ($F_{(2,12)}$ =8.77, p<.01) indicating higher levels of Fos signal rostrally. The interaction between the factors drug and lesion was significant ($F_{(1,13)}$ =5.36, p<.05). Post-hoc comparisons using the Least Significant Difference test indicate higher levels of Fos signal in the side ipsilateral to the muscimol injection in the VPm lesioned rats compared with the sham lesioned ones (fig. 17). To analyze this increased Fos signal in the VPm lesioned rats further, a single factor (Group) 2 levels (sham lesion and VPm lesion) ANCOVA was conducted. I used ratios calculated by dividing the number of Fos cells in the muscimol condition by the number of Fos in the saline condition. In this analysis, the number of Fos signal under saline condition was used as a co-variate. The factor group was significant ($F_{(1,12)}$ =5.21, p<.05) indicating a higher ratio of muscimol by saline Fos in the rats with a VPm lesion than in the rats with a sham lesion, thus supporting the effect observed in the 2 X 2 X 3 ANOVA described above.

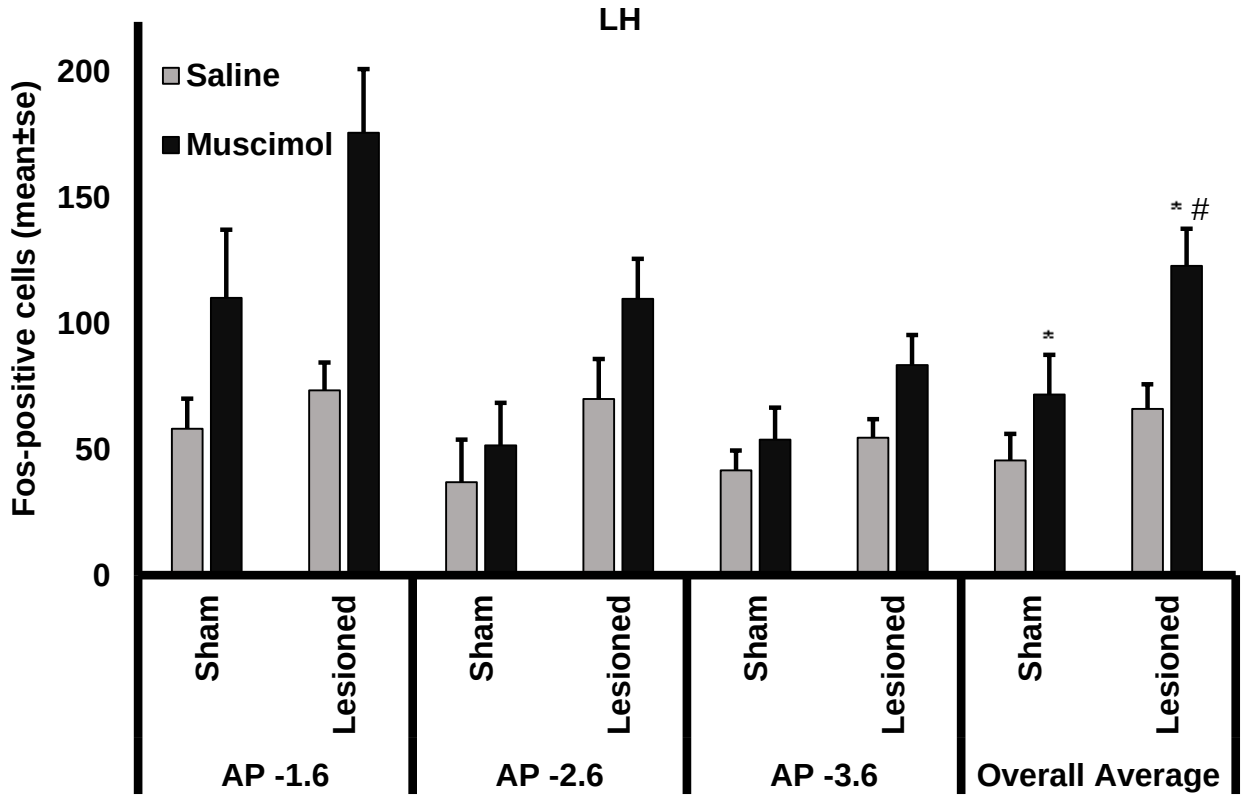

Figure 17. Number of Fos-positive neurons in the LH measured in three different levels (abscissa). Both sham lesioned and VPm lesioned rats display a similar pattern of Fos signal: the side ipsilateral to the muscimol injection (100 μg/side) shows more Fos positive neurons than the vehicle injected side. Additionally, the muscimol side of the lesioned rats displays significantly more Fos than the muscimol side of the sham lesioned rats. *p < .05 vs. saline. #p < .05 vs. muscimol.

C. Discussion

Our results confirm previous reports that bilateral or unilateral intra-AcbSh muscimol injections induced an increase in Fos expression in the LH (Stratford, 2005; Stratford and Kelley, 1999). We predicted that VPm lesions would prevent intra-AcbSh muscimol from causing increased Fos expression in the LH. In contrast to my hypothesis, I

observed an increase in Fos expression in the LH and perifornical LH independently of the integrity of the VPm. These results suggest that the VPm is not necessary for the AcbSh to modulate Fos expression in the LH.

The direct projection from the AcbSh to the LH (Heimer et al., 1991; Zahm and Brog, 1992), offers a possible mechanism for the observed Fos expression increase within the LH of VPm lesioned rats. The role of this direct connection has been understated in the past and it could be responsible for the increase in Fos expression in the LH observed here. If this interpretation is correct, this direct AcbSh to LH projection would allow the modulation of the LH bypassing the VPm.

Surprisingly, the VPm lesion increased LH Fos expression above what we observed in intact rats. The synergistic effect of the chronic destruction of GABAergic projections from the VPm to the lateral region of the LH in addition to the temporal blockade of GABAergic input to the LH caused by intra-AcbSh muscimol, could be responsible for this increased Fos in the LH. An interesting question is why this synergistic effect takes place in the LH but not in the perifornical LH, which also receives these pallidal GABAergic projections. It is possible that the density of projections from the VPm to the LH and perifornical LH is different and our results are the product of this discrepancy. Previous studies have indicated that VP projections to the LH present medial-lateral topography (Groenewegen et al., 1993; Haber et al., 1993; Tripathi et al., 2013; Zahm et al., 1985). Indeed, a recent, painstakingly detailed, single axon tracing study indicates that all of the eighty-seven axons traced innervate the LH, and that these innervations

tend to be confined to the medial LH (Tripathi et al., 2013). A complementary but alternative hypothesis is that the neural populations in the perifornical LH might habituate faster to the lack of GABAergic pallidal input than those in the LH, thus preventing a synergistic increase in Fos expression caused by the intra-AcbSh muscimol injections. Taken together, these results demonstrate a differential functional connectivity with a medial-lateral topography between the VPm and the perifornical LH and the LH.

The current results are compatible with the hypothesis that the AcbSh can modulate LH activity independently of the integrity of the VPm. This hypothesis has to be reconciled with the observed reductions in food intake after intra-AcbSh muscimol injections in VPm lesioned rats (Stratford and Wirtshafter, 2012). The first thing to take into consideration to explain this incongruence is the possibility of some fundamental difference between the VPm lesions induced here and the ones generated by Stratford and Wirtshafter (2012). Both studies were performed in the same laboratory, using the identical rat strains, of similar age, exposed to similar diets and with equivalent surgical procedures. Additionally, a comparative qualitative inspection of the VPm lesions generated by both studies indicates that the lesions are similar in size and extension. In consequence, we consider the lesions generated in this current study and the ones generated by Stratford and Wirtshafter (2012) analogous.

An alternative explanation for this incongruence is that the VPm to LH connection is still necessary to elicit feeding initiated from inhibition of the AcbSh because it mediates

some effect in the LH which is not apparent in terms of Fos expression. For example, activation of GABAergic projections originating in the VPm may inhibit anorexigenic neurons in the LH like those expressing Cocaine and amphetamine-regulated transcript (CART). CART is expressed by neurons in the LH among other hypothalamic regions (Koylu et al., 1997) and has anorectic effects when injected into the ventricles (Lambert et al., 1998). It is possible that the full expression of feeding following muscimol in the AcbSh might depend both on the stimulation of orexigenic cells in the LH (like those cells expressing orexin/hypocretin) and the inhibition of anorexigenic cells (like those expressing CART). In this case, the orexigenic/anorexigenic balance required to observe feeding would require the presence of an intact VPm relay. Another interpretation, compatible with the previous one, is that the VPm relays influences to the LH and/or the AcbSh from other pallidal afferents like the cortex or the ventral tegmental area. In the absence of this pallidal input into the LH and/or the AcbSh, the AcbSh can still modulate LH activity, as we have seen here, but cannot induce feeding as illustrated in Stratford and Wirtshafter (2012).

The data presented here indicate that the ability of AcbSh cells to turn on Fos in the LH is independent of a VPm relay. In chapter 2, section B3, I proposed that the LH could be modulated by a direct AcbSh to LH projection that would contain the influences of an indirect VPm to AcbSh to LH projection, and a direct VPm to LH one. The current results support this interpretation. Future experiments should explore the effects of AcbSh lesions on Fos expression in the LH and feeding induced by intra-VPm NMDA or bicuculline injections.

In conclusion, our results show that, in contrast with our hypothesis, intra-AcbSh muscimol injections increase Fos expression in the ipsilateral perifornical LH and whole LH independently of the integrity of the VPm. Additionally, we observed a dissociation between two regions of the LH in terms of their Fos expression. These results support the existence of medial-lateral differences in the VPm to LH projections and/or differences in the neural populations in these regions and is compatible with the interpretation that the AcbSh-VPm-LH feeding circuit is multidirectional.

CHAPTER 4. MANIPULATION OF GABA IN THE VENTRAL PALLIDUM, BUT NOT THE NUCLEUS ACCUMBENS, INDUCES INTENSE, PREFERENTIAL, FAT CONSUMPTION IN RATS

This chapter have been published as a scientific article in the journal "Behavioural Brain Research" (Covelo et al., 2014). It contains the contributions of all the participating authors and not only those made by the author of this dissertation. The use of this article in this dissertation is in concordance with the author scholarly rights according to the publisher's copyright policies (see appendix).

A. Introduction

When a variety of foods are available, animals must decide not only how much to eat, but also how to distribute their intake across the accessible items. One could imagine that the available foods could simply be ranked in an order of preference determined in part by innate factors and in part by past experiences, and that animals would always tend to consume the largest quantities of the most preferred items. Evidence against this type of "fixed hierarchy model" is, however, provided by observations that the relative preferences of subjects for different foods may vary depending on the internal state of the animal. For example, sodium depletion is associated with increased intake of salty foods (Schulkin, 1991), and extreme food deprivation may lead to increased intake of foods rich in fat (Welch et al., 1994).

Although much less effort has been devoted to food selection than to the control of total intake, substantial evidence suggests that a number of different brain systems may participate in mediating the relative preferences expressed by animals for different foods. For example, in animals given a choice between foods rich in fat and those rich in carbohydrates, systemic injections of opiates tend to preferentially increase the intake of fatty foods, whereas intraventricular injections of NPY tend to preferentially increase the intake of carbohydrates (Welch et al., 1994).

Some of the most convincing evidence of the role of the brain in mediating macronutrient selection, has been obtained following manipulations of the paraventricular nucleus of the hypothalamus (PVN). In this brain region, local injections of the peptides enkephalin and galanin induce a marked preference for fat (Kyrkouli et al., 1990; Naleid et al., 2007; Tempel et al., 1988), whereas activation of NPY or norepinephrine receptors preferentially increases carbohydrate intake (Leibowitz et al., 1985; Stanley et al., 1985). Injections of serotonin at this site also specifically decrease carbohydrate intake (Shor-Posner et al., 1986). Such results indicate that brain mechanisms are able to influence not only how much, but also what types of food will be eaten.

Brain mechanisms controlling feeding are not restricted to the hypothalamus, and in recent years it has been shown that the nucleus accumbens (Acb), a structure located in the basal telencephalon, also exerts a powerful influence on ingestive behavior.

Pronounced increases in food intake can be induced by injections of a variety of drugs into the Acb, especially its shell region (AcbSh), and these injections alter activity at a number of sites in the hypothalamus, including the PVN (Stratford, 2005; Stratford and Kelley, 1999). In the current context, it is important that some of these injections alter relative food preferences. For example, in animals given a choice of high-fat and high-carbohydrate diets, injections of the μ-opioid agonist D-Ala2,N,Me-Phe4,Gly-ol5-enkephalin (DAMGO) into the core of the Acb induce a strong preference for fat, irrespective of the baseline preferences of the animals (Zhang et al., 1998). In contrast, rats made hyperphagic by intra-AcbSh injections of the $GABA_A$ agonist muscimol into AcbSh show similar increases in both fat and carbohydrate intake (Basso and Kelley, 1999).

Although the circuit through which the Acb may mediate macronutrient intake has yet to be identified, it is known that projections from GABAergic medium spiny neurons in the Acb synapse on cells in the medial ventral pallidum (VPm) (Churchill et al., 1990; Nauta et al., 1978; Heimer et al., 1991; Zahm and Heimer, 1990), and that this structure in turn can influence the lateral hypothalamus (LH) and the PVN (Rivero-Covelo et al., 2013; Urstadt and Stanley, 2013). While the LH is well known as a brain region involved in the control of ingestion (Bernardis and Bellinger, 1996), the VPm also has a demonstrated role in the control of food intake. Injections of $GABA_A$ antagonists, glutamate agonists, or mu-opioid agonists into the VPm induce intense feeding in satiated rats (Rivero-Covelo et al., 2013; Stratford et al., 1999; Smith and Berridge, 2005; Stratford and Wirtshafter, 2013). Furthermore, VPm lesions reduce the feeding induced by muscimol

injections in the AcbSh through specific disruption of the AcbSh-VPm circuit (Stratford and Wirtshafter, 2012). It has been proposed that the Acb, VPm, and LH work together to form a functional circuit controlling some aspects of feeding behavior (Stratford, 2007; Stratford and Kelley, 1999; Stratford and Wirtshafter, 2012). While the ability of chemical stimulation of the VPm to induce intense hyperphagia is well documented, nothing is known about whether these procedures also alter the relative preference of rats for different dietary components in a fashion similar to that seen after intra-Acb injections of DAMGO.

In the current set of studies, we therefore investigated the effects of intra-VPm injections of the $GABA_A$ antagonist bicuculline on food selection in ad libitum fed rats consuming a diet containing independent sources of fat, carbohydrate and protein. We also examined deprivation-induced feeding in these same subjects in order to determine whether this manipulation produced a pattern of ingestion similar to that seen after bicuculline injections. Since certain aspects of our procedure differed from those employed by earlier investigators, we additionally examined macronutrient selection in subjects with injections of muscimol into the AcbSh.

B. Subjects, materials and methods

Animals & diet. The subjects were 15 male Sprague–Dawley rats (Charles River), weighing between 290 and 350 g at the time of surgery. The rats were housed

individually in plastic cages (L 43 X W 22 X H 21 cm) with wire floors on a 12-h light: 12-h dark cycle at a constant room temperature (~21°C). Water and food were available ad libitum*,* except as noted below. For the period before surgery and for the first week after surgery, food was provided in the form of standard lab chow (Harlan Teklad), after which time animals were switched for the remainder of the experiments to a nutritionally-complete diet consisting of three separate macronutrients components, described below. Throughout the experiments, rats were handled and weighed on a daily basis. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UIC Institutional Animal Care and Use Committee.

Surgery. Surgery was performed using standard, aseptic, flat-skull stereotaxic techniques under sodium pentobarbital (60 mg/kg) anesthesia. In 8 experimental subjects, bilateral 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA), aimed so as to terminate 2.0 mm dorsal to the VPm, were implanted at coordinates of AP: -0.2, LM: ±1.8, DV: −6.7. In the remaining subjects, bilateral 22-gauge cannulae, aimed so as to terminate 2.0 mm dorsal to the AcbSh, were implanted at coordinates of AP: 1.6, LM: ±0.8, DV: −6.1. The guide cannulae were held in place using stainless steel screws and denture lining material and a stainless steel obturator was inserted into the lumen of each cannula to help maintain patency. After surgery, the rats received an injection of the analgesic carprofen (5 mg/kg, sc) and the antibiotic cefazolin (60 mg/kg). Each rat was allowed to recover for at least 7 days before the start of behavioral testing.

Diet composition. The rats were allowed to select their food from three separate sources of pure macronutrients (protein, carbohydrate, and fat) presented simultaneously in glass jars with dimensions of approximately 62 X 60 mm (diameter X height) which were placed in the corners of the animals' home cages. The detailed composition of each of the dietary components is presented in Table 1; all the ingredients were obtained from Harlan Laboratories (Madison, WI) except for sucrose (Domino Foods, Chicago, IL), lard (Armour, Peoria, IL) and dibasic calcium phosphate (Sigma- Aldrich, St. Louis, MO). The dietary components were similar to those used in previous studies by other authors (Leinowitz et al., 1985; Welch et al., 1994) except that we used custom modified versions of the AIN-93 vitamin and mineral mixes in which cellulose was substituted for sucrose. Thus our protein and fat components contained no sugar, whereas the sucrose concentrations in the mixtures used by earlier workers have been as high as 2%.

Testing protocol & injections. Placement of the food jars containing various components was changed daily to prevent the development of position preferences. Subjects were given two weeks to adapt to the multicomponent diet before the start of drug injections. On the last three days of the adaptation period, 24-h intakes of each of the three dietary components were determined, correcting for spillage. During the intracerebral injections, the rats were restrained gently, the obturators removed, and 28-gauge injection cannulae, extending 2.0 mm beyond the ventral tip of the guide, were inserted into each guide cannula. In order to acclimate the animals to the injection procedure, all subjects were given a "sham injection," in which the injectors were inserted but no injection made, two days prior to the first actual injection. Injections were made in a volume of

0.5 µl at a rate of 0.33 µl/min using motor-driven microsyringe pumps connected to the injection cannulae with fluid- filled polyethylene tubing. After the infusion, the injection cannulae were left in place for an additional 60 s in order to minimize leakage up the cannula track. The obturators were then replaced and the rats were returned to their home cages, which contained freshly weighed sources of macronutrients. Intake was assessed after 60 or 120 min, as detailed below, and subsequently corrected for spillage. During this period video cameras were located above the animals' cages to record their behavior during the test sessions. The recordings were later examined to determine which of the three macronutrient components was first sampled by the subjects. Additionally, locomotor activity following bicuculline or the matched saline injections was assessed by dividing the animals' cages into four quadrants and manually counting the number of times that animals crossed from one quadrant to another.

Two weeks following placement on the macronutrient diets the responses to injections of saline and bicuculline were examined in a counterbalanced fashion. Half of the subjects were given bilateral injections of bicuculline (100 ng/side) into the VPm and the remainder sterile saline. After the injections, the rats were returned immediately to their home cages where intake of the three macronutrient diets was assessed for 60 min. Three days later, subjects received the converse injection and intake was again assessed. After another 3 day break in testing, half of the subjects were deprived of all food for 24 hr after which the three macronutrient components were returned, and intake measured over a 60 min period. In the remaining ad libitum fed animals, the

foods were briefly removed from animals' cages and then returned, after which intakes were again measured 60 min later. Finally, three days later, animals were tested under the opposite condition.

Table I. Composition of dietary components (g/kg).

Ingredient	Protein	Fat	Carbohydrate
Casein, VFT	920.45	0	0
L-Cystine	13.8	0	0
[a]AIN-93-vitamin mix	15	15	15
[a]AIN-93 mineral mix (W/O Ca, P)	20	20	20
Calcium carbonate, anhydrous	27	5.4	5.4
Calcium phosphate, dibasic, anhydrous	0	30.2	30.2
Choline bitartrate	3.75	3.75	3.75
Lard	0	925.65	0
Sucrose	0	0	365.65
Dextrin	0	0	280
Corn starch	0	0	280
kcal/g	3.4	8.5	3.6

[a] Cellulose was substituted for the sucrose normally found in these mixes.

Tissue harvest & brain sectioning. When behavioral testing was completed, each of the rats was deeply anesthetized using sodium pentobarbital (150 mg/kg) and perfused transcardially with 50 mL of a 0.15 M saline solution followed immediately by 500 ml of a 10% buffered formalin solution at pH 7.3. The brains were removed and stored in the

formalin solution for at least 7 days. They were then frozen and 50 µm-thick coronal sections were taken throughout the extent of the VPm or the AcbSh and stained with cresyl violet. The locations of cannula tips were plotted on atlas sections (Paxinos and Watson, 2007) matched as closely as possible to the level of the placements.

AcbSh rats. Rats with AcbSh cannulae were tested using the same basic approach as described above. Following the two week adaptation period, 60 min intakes were measured following counterbalanced bilateral injections of muscimol (100 ng/side) or the sterile saline vehicle.

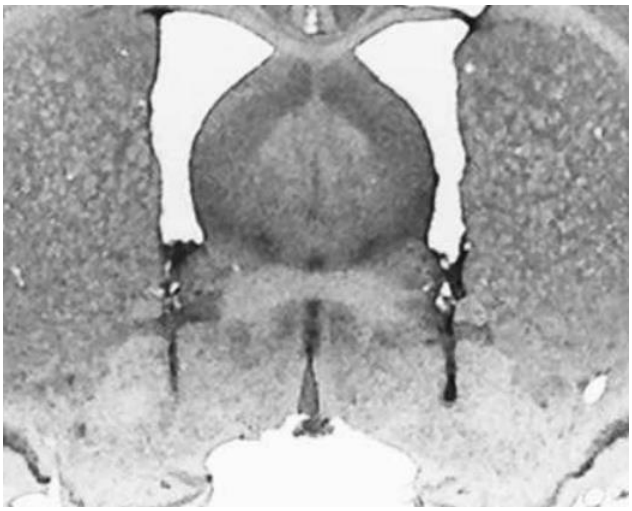

Figure 18. Cresyl violet stained section showing a typical cannula placement in the VPm.

C. Results

Statistical analysis. Data for food intake expressed as either grams or calories were analyzed by two way repeated measures analyses of variance (ANOVAs). When significant interaction effects were observed, the source of these was investigated using the Fisher LSD test. Correlations and locomotor activity were evaluated using t tests. A criterion of $p<.05$ was adopted for all tests.

VPm placement verification. In one animal, the injector tips terminated just below the ventral border of the VPm; data from this subject was not included in the analysis, although its behavioral results were similar to those seen in the remaining animals. In all of the other subjects, injector tips terminated within the boundaries of the VPm. No correlation could be observed between behavioral responses and location of the cannula tips within the VPm. Fig. 18 is a photomicrograph of a coronal brain section demonstrating a typical bilateral VPm injection site.

Intra-VPm bicuculline potentiated fat intake. As can be seen on fig. 19, injections of bicuculline into the VPm produced a large increase in total 60 min intakes in the macronutrient selection paradigm. Whether measured in terms of grams or calories consumed (left and right panels of fig. 19, respectively), fat intake was potentiated to a much greater extent than intakes of the other macronutrients. Examination of grams eaten by means of a 2 X 3 (bicuculline X macronutrient) repeated-measures ANOVA

indicated a significant effect of bicuculline ($F = 24.8$, $p < .002$), of nutrient ($F = 20.1$, $p < .001$) and of the bicuculline X nutrient interaction ($F(2,12) = 20.5$, $p < .001$). *Post hoc* contrasts indicated that bicuculline significantly increased intakes of fat ($p < .005$) and carbohydrate ($p < .05$), but not protein, although a small trend was seen in that case as well. The increase in fat intake was significantly larger than the increase in carbohydrate intake ($p < .005$). Similar results were observed for calories consumed where there was a significant effect of bicuculline ($F(1,6) = 23.1$, $p < .003$), macronutrient ($F(2,12) = 21.38$, $p < .001$) and of the bicuculline X macronutrient interaction ($F(2,12) = 21.3$, $p < .001$). *Post hoc* tests again indicated that the increases in fat and carbohydrate were both significant ($p < .005$ and $p < .05$ respectively) and that the increase in fat calories was larger than that in carbohydrate calories ($p < .005$). Examination of the video recordings indicated that fat was the first macronutrient consumed by all 7 subjects after bicuculline injections; in contrast only 2 of the seven subjects consumed fat first after saline injections. These distributions differ significantly using the Fisher exact probability test ($p < .01$). Locomotor activity, measured as quadrants entered over the test period, tended to be higher after bicuculline than saline (55.2 ± 6.5 vs. 44.4 ± 12.2), but this difference was not significant ($p > .20$).

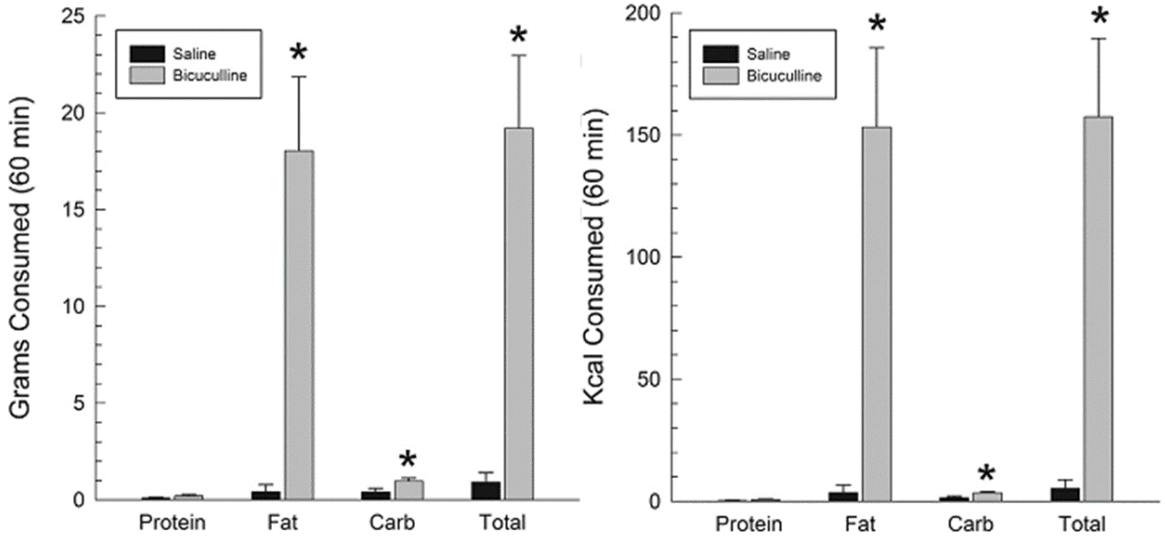

Figure 19. Grams (left panel) and calories (right panel) consumed following injections of bicuculline (100 ng/side) or saline into the VPm. *p < .05 vs. control.

24-h ad libitum feeding does not differ in fat or carbohydrate intake. Given the strong preference for fat seen following bicuculline injections, we examined whether nutrient preferences after this drug were related to baseline preferences. Fig. 20 shows mean proportional intakes of each of the three macronutrients averaged over the three days before the start of testing (left panel). In contrast to the dramatic fat preference shown following bicuculline injections (middle panel), animals during 24-h ad libitum feeding periods did not significantly differ in their fat and carbohydrate intake, and actually tended to consume more carbohydrates than fat. The difference in the proportionate consumption of fat under bicuculline and deprivation conditions was significant ($F(1,6)=70.1$, $p<.0001$). The left panel of fig. 21 shows that baseline 24-h fat intake was significantly correlated with the increase in fat intake produced by bicuculline ($p<.05$). It

should be noted, however, that even rats that ate little or no fat under free feeding conditions still showed large increases in fat intake after bicuculline and that even subjects who, under baseline condition consumed less than 10% of their calories from fat, consumed 80-98% of their calories from fat after bicuculline (fig. 21, right panel).

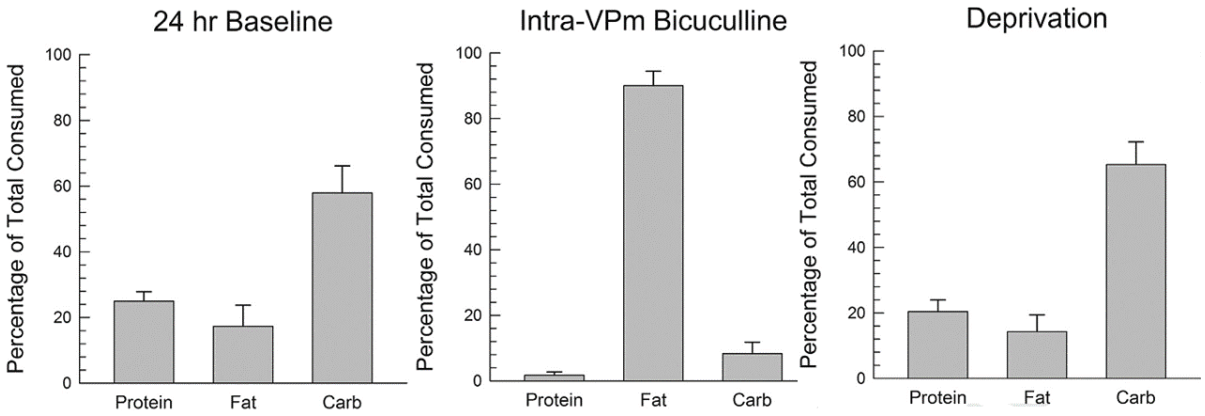

Figure 20. Percentages of the total number of grams consumed which were taken from the protein, fat, and carbohydrate dietary components under several conditions in subjects with VPm cannulae. The left panel shows baseline percentages averaged across the three days before the start of intracranial injections. The middle panel shows percentages based on intakes in the 60 min following bicuculline injections, and the right panel percentages based on intakes in the 60 min period following the return of food after overnight food deprivation.

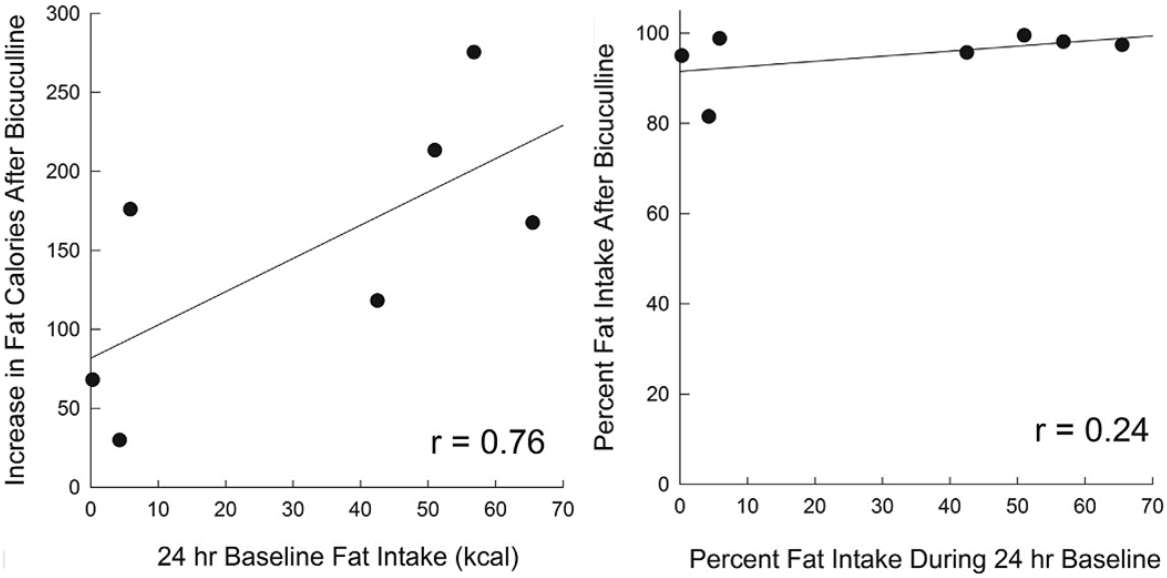

Figure 21. *Left panel*: Scatter plot and least squares regression line showing the relation between mean baseline caloric fat intake (abscissa) and the increase in absolute caloric intake of fat after bicuculline injections into the VPm, versus saline injections (ordinate). *Right panel*: Scatter plot and least squares regression line showing the relation between the percent of caloric intake which consisted of fat during the 3 day baseline period (abscissa) and in the 60 min following bicuculline injections (ordinate). Pearson correlation coefficients are indicated on the figures.

Food deprivation for 24-h increases carbohydrate and protein intake. One rat became sick before completing the deprivation tests, so the data presented below represent the remaining six subjects. The left panel of fig. 22 displays 60 min macronutrient intake following food deprivation or under ad libitum fed conditions. Analysis of the data for grams consumed indicated a significant effect of deprivation (F)=73.3, $p<.001$),

p<.001), macronutrient (F(2,10)=7.2, p<.001) and of the deprivation X macronutrient interaction (F(2,10)=3.2, p<.002). *Post hoc* comparisons indicated that intakes of protein and carbohydrate were increased significantly (p<.05), while a non-significant trend in the same direction was seen with respect to fat intake (p<.10). Furthermore the increase in carbohydrate intake was significantly larger than the increase in fat intake (p<.05). Analogous results in each case were obtained with respect to caloric intake (right panel of fig. 22). The proportional intakes of the three macronutrients are shown in the right panel of fig. 20, which demonstrates that deprivation did not produce a pattern of fat preference similar to that seen after bicuculline. Subjects consumed a significantly smaller proportion of their total food from fat after deprivation than they did after bicuculline infusions (F(1,5)=108.2, p<.001). Video recordings of the deprivation sessions were available for five animals of which only two sampled fat first.

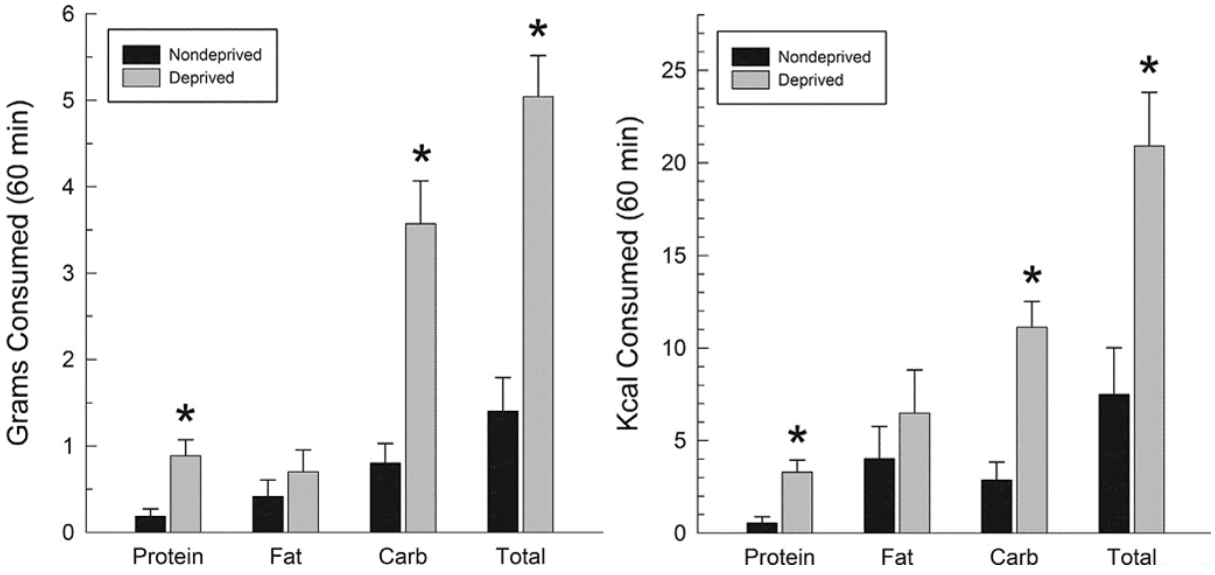

Figure 22. Grams (left panel) and calories (right panel) consumed in 60 min period following 24-h food deprivation or a nondeprived control condition. *p < .05 vs. control.

AcbSh placement verification. In two animals, the injector tracts passed out the ventral surface of the brain; in both of these subjects, drug injections had little or no effect on ingestive behavior. In the remaining subjects, injector tips terminated in the ventral portion of the medial AcbSh. Fig. 23 is a photomicrograph of a coronal brain section demonstrating a typical bilateral AcbSh injection site.

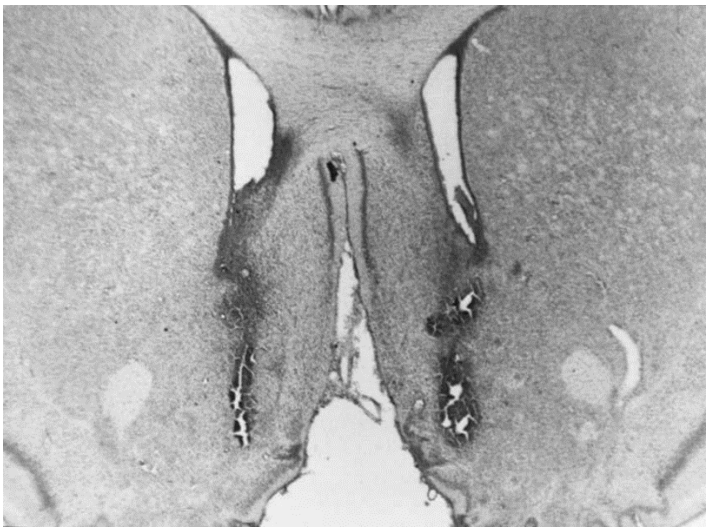

Figure 23. Photomicrograph of a cresyl violet stained section showing a typical cannula placement in the AcbSh.

Intra-AcbSh muscimol potentiated overall intake. Fig. 24 displays macronutrient intakes following injections of saline or muscimol into the AcbSh. Muscimol significantly increased overall intakes; when intakes were measured as grams eaten, the effect on carbohydrates tended to be larger than that on fat (left panel), whereas the opposite tendency was seen when the data were expressed as calories consumed (right panel). Analysis of grams eaten indicated a significant overall effect of muscimol ($F = 17.2$, $p < .02$) and macronutrient ($F(2,8) = 8.5$, $p < .01$) but the muscimol X macronutrient interaction was not significant ($p > .05$). Analysis of caloric intakes indicated a significant effect of muscimol ($F(1,4) = 29.4$, $p < .01$), macronutrient ($F(2,8) = 6.7$, $p < .02$) and of the muscimol X macronutrient interaction ($F(2,8) = 5.2$, $p < .05$). *Post hoc* contrasts in this case indicated that muscimol significantly increased caloric intake of fat ($p < .05$) and

tended to increase carbohydrate intake (p<.06), but had no effect on protein intake (F<1). The muscimol-induced increases in caloric intake of fat and carbohydrate did not differ statistically (F<1). The right panel of fig. 25 illustrates intakes following muscimol, expressed as a percent of total number of grams consumed, and shows that the carbohydrates tended to make up a larger proportion of total intake than did fats, although these differences were not significant (F<1). As was the case in the animals with VPm cannulae, subjects in the current experiment tended overall to consume more carbohydrate than fat under baseline conditions (fig. 25, left panel).

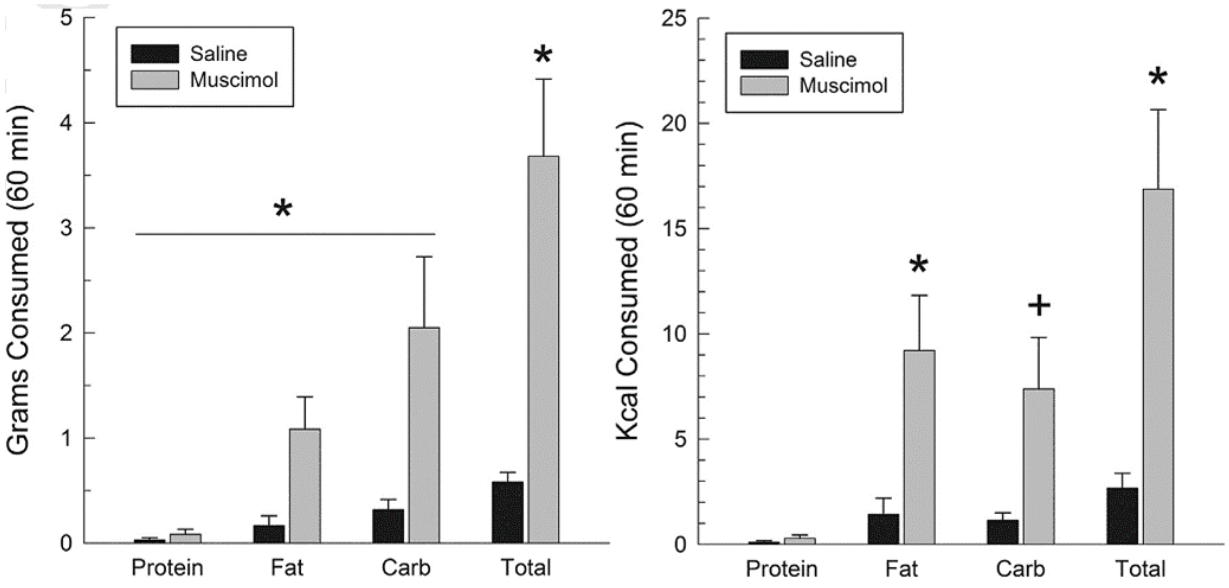

Figure 24. Grams (left panel) and calories (right panel) consumed following injections of saline or muscimol (100 ng/side) into the AcbSh. *p < .05, + p < .06.

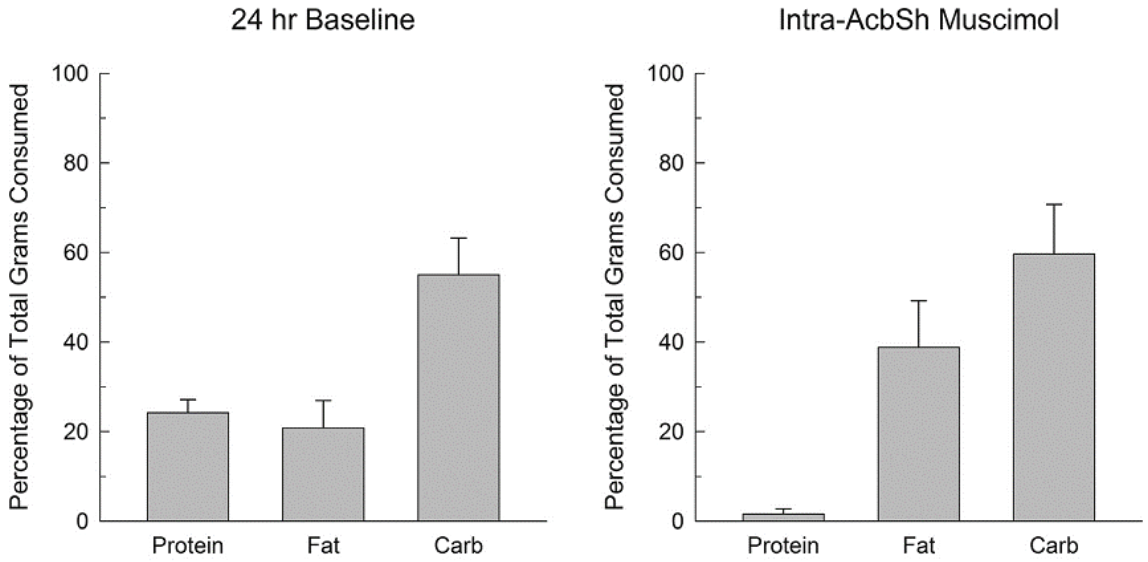

Figure 25. Percentages of the total number of grams consumed that were taken from the protein, fat, and carbohydrate dietary components under several conditions in subjects with AcbSh cannulae. The left panel shows the distribution of baseline percentages averaged across the three days prior to the start of injections; the right panel shows percentages after muscimol (100 ng/side) injections in the AcbSh.

D. Discussion

Our results confirm previous reports that blocking $GABA_A$ receptors in the VPm increases food intake (Rivero-Covelo et al., 2013; Shimura et al., 2006; Smith and Berridge, 2005; Stratford et al., 1999; Stratford and Wirtshafter, 2013). As the Acb sends a strong GABAergic projection to the VPm (Walaas and Fonnum, 1979; Zahm et al., 1985), it is likely that this effect results from a disruption of inhibitory influences of the Acb on the VPm, but further studies will be required to unequivocally establish this conclusion.

Additionally, our findings show that in animals offered a choice between three sources of individual macronutrients, this hyperphagia manifests itself as a selective and dramatic increase in the amount of fat consumed. To the best of our knowledge, this is the first time that pronounced alterations in fat preference in a choice situation have been observed following central manipulations of a nonpeptide neurotransmitter system. It is striking that results similar to those obtained here, although not quite as pronounced, have also been observed after injections of the mu-opioid agonist DAMGO into the nucleus accumbens core (AcbC) (Zhang et al., 1998). Given that there are dense, reciprocal interconnections between the Acb and the VPm (Churchill et al., 1990; Heimer et al., 1991; Nauta et al., 1978; Zahm and Heimer, 1990), it is likely that there is some relation between the influences of these two regions on fat intake. Further studies will, however, be necessary to determine the nature of this relationship, as a number of circuits consistent with these results are theoretically possible.

All of the subjects in the present study showed a strong preference for fat following bicuculline injections into the VPm. Although fat intake after bicuculline was correlated with baseline intake, even animals who spontaneously consumed only tiny amounts of lard obtained 80-98% of their calories from this substance following bicuculline injections. These findings demonstrate that blocking GABA transmission in the VPm does not simply increase the inclination of animals to select those foods preferred under baseline conditions. Similar results have been reported following injections of DAMGO into the Acb (Zhang et al., 1998). It seems likely, therefore, that the selectivity of these manipulations on fat intake has little to do with the intrinsic palatability of the lard.

Indeed, there is little in the data obtained from this group of subjects to suggest that the lard component is an especially palatable or generally preferred substance in untreated animals. The notion that effects on fat intake can be accounted for by a special role of Acb opioids in the intake of palatable or “rewarding” foods, appears to have arisen less from empirical data than from a prior belief that the Acb is a component of a “reward system.” In contrast, the current and previous results (Zhang et al., 1998) suggest that, in macronutrient selection situations, the VPm and Acb are able to reorder the ingestive preferences of subjects in a way that has little to do with their preferences in the absence of experimental treatments. It is possible that Acb-VPm circuitry selectively influences preferences for substances with the sensory characteristics (textures, taste or odors) of lipids or for foods with higher caloric densities, an attribute which subjects could have learned about during dietary adaptation period.

Analysis of video recordings showed that all of the bicuculline treated animals, regardless of their baseline preferences or the specific location of the food jars, initially oriented themselves toward the jar containing lard and ingested the fat before either of the other macronutrients, a pattern not seen following food deprivation. Observations of this type have not, to our knowledge, been made in prior macronutrient selection studies. These observations suggest that the injections may not only alter fat intake, *per se,* but may also alter the incentive properties of the fat component in such a way as to direct the animal’s behavior towards it, even before any oral contact has been made. The effects of the injections on fat intake therefore cannot be entirely the result of factors which arise during ingestion of the diet and one could speculate that the

injections may induce a form of “fat craving,” which might be analogous to other forms of food cravings which have been described in the literature (Avena et al., 2008; Weingarten and Elston, 1990). It should be stressed, however, that the orexigenic effects of intra-VPm bicuculline are not restricted to high-fat foods. In the current study, a small but significant increase in carbohydrate intake was also seen, and in previous studies, we have observed substantial increases in the intakes of relatively low-fat diets such as 6% fat lab chow (Stratford and Kelley, 1999) or 3.8% fat Bioserve pellets (Stratford and Wirtshafter, 2013) when these were the only foodstuffs offered.

The feeding seen after bicuculline injections into the VPm was fundamentally different from that we observed after 24 hours of food deprivation in terms of both magnitude and macronutrient profile. Both conditions increased total caloric intake during the 60 min test, but after intra-VPm bicuculline, the rats consumed approximately 7.5 times the number of calories that they did following overnight deprivation. In terms of macronutrient profile, intake after 24-h food deprivation was characterized by significant increases in carbohydrate and protein intake with a smaller effect on fat consumption, a pattern very different from the highly selective fat intake seen following bicuculline. These results indicate that, under comparable conditions, these injections do not generate a state resembling that induced by 24-h food deprivation. It is, of course, possible that larger effects on fat intake might be produced by longer periods of deprivation. It should also be stressed that our lard component contained no sugar, whereas the high fat components studied by many earlier workers contained sucrose in concentrations as high as 2% e.g., (Welch et al., 1994; Basso and Kelley, 1999; Zhang

and Kelley, 2000), a difference which might tend to promote fat intake under a variety of conditions.

Information about the central mechanisms underlying food preferences is so limited that it is not currently possible to identify the pathways through which the effects of intra-VPm bicuculline are exerted. These effects may be mediated through GABAergic neurons in the VPm, but cells utilizing glutamate and enkephalin as transmitters are also found in this nucleus (Geisler et al., 2007; Hur and Zaborszky, 2005; Kalivas et al., 1993) and might also be involved. Evidence indicates that the lateral hypothalamus plays a role in mediating some of the ingestive effects of VPm activation (Rivero-Covelo et al., 2013; Stratford and Wirtshafter, 2013), although only limited data exist suggesting that this region is able to influence food choice (Leibowitz et al., 1986; Morganstern et al., 2010). It is interesting, however, that acute injections of NMDA into the VPm are able to induce strong Fos expression in the paraventricular hypothalamic nucleus (PVN) (Rivero-Covelo et al., 2013), the brain region most closely associated with elicited alterations in macronutrient selection, demonstrating a functional link between the two structures. The possibility that the large preferential increase in fat intake we observed is due to VPm influences on PVN cells is consistent with a report that intra-PVN injections of a NPY antagonist suppress intake of high-fat chow induced by DAMGO injections in the Acb, a region sharing dense reciprocal connections with the VPm (Zheng et al., 2010). Since only a single food source was examined in that study, however, it is impossible to determine whether this effect is specifically related to the fat content of the diet, or simply reflects a general suppression of feeding. Intra- ventricular

injections of NPY antagonists, for example, are also able to suppress the intake of low-fat chow induced by injections of muscimol in the AcbSh (Stratford and Wirtshafter, 2004). Although most work suggests that acute manipulations of the NPY system in the PVN selectively influence carbohydrate intake (Leibowitz et al., 1985; Stanley et al., 1985), chronic intra-PVN administration of NPY significantly increases both carbohydrate and fat intake (Stanley et al., 1989) and manipulations of other neuropeptide systems there do selectively alter fat intake (Kyrkouli et al., 1990; Naleid et al., 2007; Tempel et al., 1988). Further studies will be necessary to determine the extent and nature of the involvement of the LH and PVN in the effects on feeding and macronutrient selection observed after manipulations of the VPm.

It has been reported previously that the vigorous feeding induced by injections of the $GABA_A$ agonist muscimol in the accumbens shell is associated with roughly equivalent increases in the caloric consumption fat and carbohydrate (Basso and Kelley, 1999). This result is surprising, as some evidence suggests that the ingestive effects of muscimol at this site may be mediated in part through projections to the VPm (Stratford and Wirtshafter, 2012), a structure which, as the current results show, is capable of preferentially increasing fat intake. The methodology used in the current study, however, differed from that of previous studies in several potentially important details including the greater length of time we allowed for adaptation to the diet and the absence of utilizable carbohydrates in our fat mixture. It seemed possible, therefore, that the observed differences in fat selectivity between our current VPm results and earlier experiments with muscimol in the AcbSh might result from these discrepancies.

In order to investigate this possibility, we examined the effects of intra-AcbSh muscimol under conditions identical to those used in the first experiment on the VPm. Our data suggest that methodological differences are not responsible for these varying outcomes as intra-AcbSh muscimol produced roughly equivalent increases in fat and carbohydrate intake under conditions identical to those in which bicuculline in the VPm selectively affected fat consumption. The current study suggests that in a macronutrient selection test, the nature of the feeding induced by disinhibition of the VPm more closely resembles that produced by DAMGO in the AcbC than that seen after GABA agonists in the AcbSh. Further work will be needed to determine if this pattern holds in other test situations, and to identify the circuitry underlying these resemblances.

In summary, our results indicate that blockade of GABA receptors in the VPm induces a dramatic increase in fat intake and preference and also preferentially directs animals' approach behavior towards fat. This effect has a different profile from those observed after food deprivation or injections of GABA or into the ventral AcbSh. The magnitude and preferential nature of VPm-induced fat intake suggests that this structure is important in the regulation of both food intake and macronutrient selection and that circuitry involving the Acb and the VPm may play a major role in controlling fat intake.

CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

Most animals living in the wild consume food strictly for survival, and their feeding behaviors follow homeostatic mechanisms. Unlike most other animals, humans, laboratory rats and pets engage in non-homeostatic as well as homeostatic feeding. Non-homeostatic feeding is postulated to lead to brain deregulation and disease (Berthoud, 2007; Neel, 1962; Ravussin and Bogardus, 2000; Speakman, 2008; Woods et al., 2004). In this dissertation, I propose that the VPm is a critical brain region that modulates non-homeostatic feeding. Glutamatergic manipulations of the VPm induce feeding in rats fed ad libitum and additionally, modulate activity in hypothalamic areas implicated in the regulation of feeding such as the LH, DMH and PVN. Moreover, these pharmacological manipulations of the VPm which induce feeding are independent of the orexin/hypocretin and MCH expressing neurons. This lack of orexin/hypocretin and MCH involvement suggests that feeding induced in satiated rats by excitation of the VPm does not operate via the traditional hypothalamic mechanisms implicated in the regulation of homeostatic feeding. Furthermore, behavioral experiments indicate an additional difference between feeding induced by excitation of the VPm and homeostatic feeding such as fasting. GABAergic manipulations of the VPm induce a preferential increase of fat intake in rats fed ad libitum while 24-h food deprivation does not. These results also suggest that the VPm might have a role in the regulation of fat intake. Finally, my experiments suggest that GABAergic manipulations of the AcbSh can modulate LH activity independently of the integrity of the VPm.

Together, I propose the following circuit (fig. 26) in which glutamatergic projections from the prefrontal cortex (PFC) can activate the VPm. Activation of the VPm can induce feeding through a direct LH projection that recruits the DMH and the PVN. Fig. 26 predicts that an indirect GABAergic VPm-AcbSh-LH projection might be necessary to observe feeding in satiated animals by excitation the VPm. Functionally, the PFC acts as a source of non-homeostatic feeding influences (Berthoud, 2006) and the LH-DMH-PVN as regulators of homeostatic feeding (Harrold et al., 2012). Ideally positioned between these feeding pathways sits the VPm and the AcbSh, two brain areas that can then relay non-homeostatic influences to the homeostatic regulators.

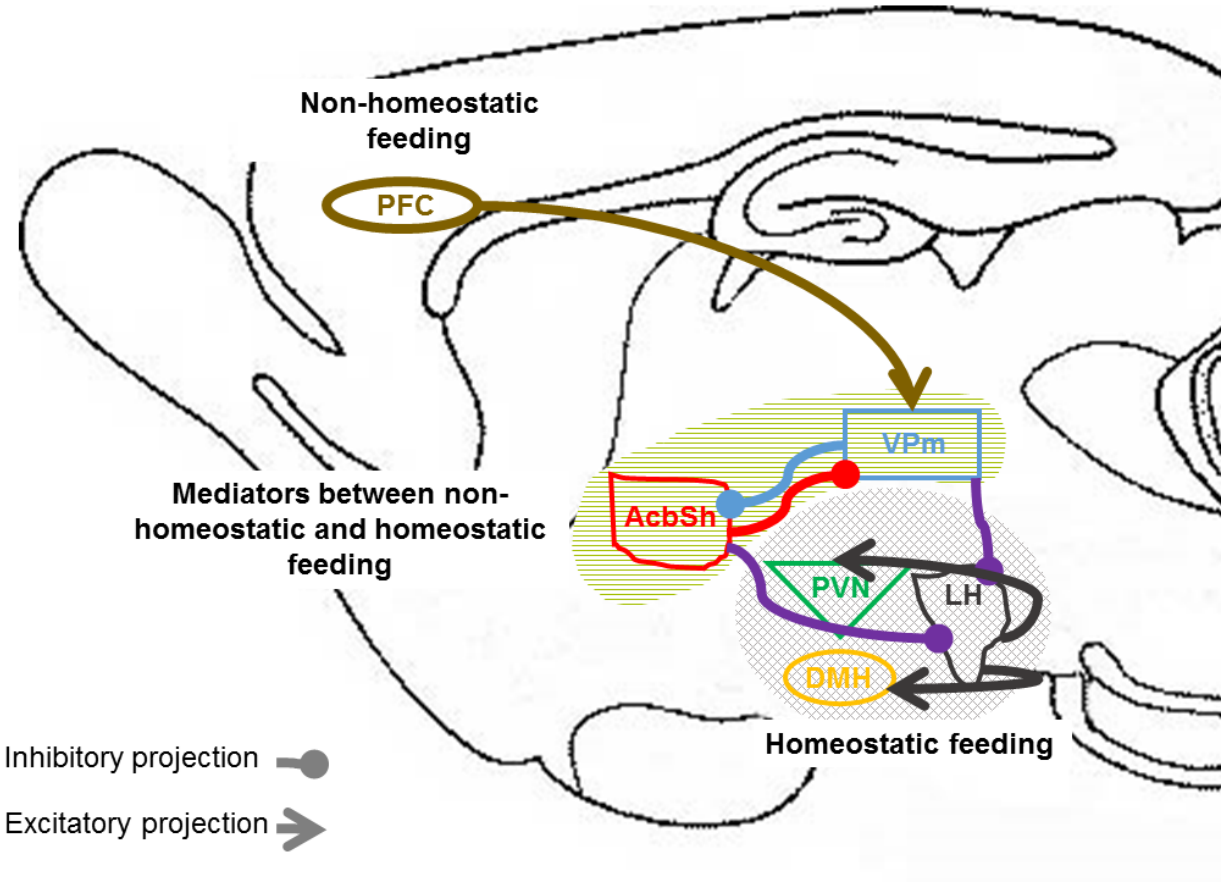

Figure 26. *Updated AcbSh-VPm-LH feeding circuit*. Glutamatergic projections from the PFC activate the VPm. From the VPm a direct GABAergic projection modulates the LH, the DMH and the PVN. Also from the VP emerges an indirect GABAergic projection to the LH through the AcbSh. The PFC is a source of non-homeostatic feeding drives. The LH-DMH-PVN are regulators of homeostatic feeding. The VPm and the AcbSh constitute brain areas that relay non-homeostatic influences to the hypothalamic homeostatic regulators. The AcbSh and the VPm are interconected (blue and red arrows). The purple arrows indicate projections to the LH that are modulated by this AcbSh-VPm reciprocal conection. The integrity of this reciprocal AcbSh-VPm connection is necessary to observe feeding elicited by pharmacological manipulation of the AcbSh or the VPm.

Glutamatergic inputs into the VPm can elicit food consumption in rats fed ad libitum (chapter 2). One of the glutamatergic projections to the VPm is the PFC (Hur and Zaborszky, 2005; Maurice et al.,1997; Sesack et al., 1989; Vives and Mogenson, 1985).

Here we observed increased food intake in satiated rats after intra-VPm NMDA injections (chapter 2). This result suggests the possibility that glutamatergic afferents to the VPm like the PFC might play a role in the regulation of food intake by the VPm. Reduced frontal grey matter has been linked with increased adiposity (Willette and Dimitrios, 2014). This frontal reduction in combination with the fact that in most developed countries the initiation of feeding is an executive decision (Berthoud, 2006), makes likely that alterations of fronto-pallidal projections might be responsible for increased food consumption in some human conditions like obesity.

Pharmacological excitation of the VPm may induce feeding by altering normal hypothalamic control. The VPm modulates activity in the LH as well as the PVN and DMH, three hypothalamic structures implicated in the regulation of homeostatic feeding (Harrold et al, 2012). At the same time, neurotransmitters implicated in homeostatic feeding like orexin/hypocretin and MCH (Parker and Bloom, 2012) seem to be unaffected by pharmacological excitation of the VPm. Moreover, my results do not indicate a clear effect of excitation of the VPm on the VMH or ARC. These results suggest that VPm modulation of hypothalamic structures do not completely mimic homeostatic feeding. Our macronutrient preference data further supports the hypothesis that feeding induced in satiated rats by exciting the VPm is not identical to feeding induced by fasting, a form of homeostatic feeding. Both GABAergic manipulations of the VPm and 24-h food deprivation increase food intake, but feeding induced by intra-VPm bicuculline injections preferentially increases fat intake while feeding induced by deprivation does not. Taken together, these results indicate that excitation of the VPm

modulates some but not all hypothalamic regions implicated in food intake, these differences could be responsible for the differences in macronutrient preference between feeding induced by pharmacological excitation of the VPm in rats fed ad libitum and homeostatic feeding (e.g. fasting). Further work should study the role of hypothalamic regions involved in the regulation of food intake in feeding induced by excitation of the VPm.

Lesion studies can offer additional information about the role of the hypothalamus in feeding induced by manipulation of the VPm. For example, unilateral LH lesions attenuate feeding induced by ipsilateral intra-VPm bicuculline injections (Stratford & Wirtshafter, 2013). This result suggests that the integrity of the LH is relevant to observe feeding after excitation of the VPm in ad lib fed rats. Given that the VPm can modulate the PVN and the DMH as well as the LH (chapter 2), lesion studies should be conducted studying the effects that PVN and DMH lesions have on feeding induced by pharmacological excitation of the VPm. Conversely, the ARC and the VMH seem to be unaffected by pharmacological manipulations of the VPm. Again, lesion studies could be useful to study the role of the ARC and the VMH in feeding. Based on Fos labeling data (chapter 2), I would predict that PVN and DMH lesions would attenuate feeding induced by excitation of the VPm in a fashion similar to that observed after lesions of the LH (Stratford & Wirtshafter, 2013). On the other hand, VMH and ARC lesions should have little to no effect on feeding induced by intra-VPm bicuculline or NMDA injections.

Lesioning the AcbSh could potentially attenuate feeding induced by excitation of the VPm in satiated rats. As diagramed in fig. 26, I would predict that lesions of the AcbSh would prevent feeding induced by excitation of the VPm. This would occur because the AcbSh lesion would block modulation of the LH through the VPm-AcbSh-LH route. Indeed, the AcbSh can modulate LH function independently of the integrity of the VPm (chapter 3). Furthermore, preliminary data indicates that intra-VPm NMDA injections induce Fos in the AcbSh. Future lesion experiments would help elucidate the possible multidirectional nature of the AcbSh-VPm-LH feeding circuit.

Excitotoxic lesion studies have limitations. Hypothalamic regions contain multiple peptides implicated in feeding (Parker and Bloom, 2012) and it is technically difficult to target these specific neural populations using excitotoxic compounds. To overcome this limitation, the study of the AcbSh-VPm-LH feeding circuitry would benefit enormously by moving beyond lesion studies to focusing on key, loss-of-function genetic models. Indeed, by moving from rats to mice there are multiple knockout (KO) strains available which can be used to answer specific hypothesis. For example, if feeding can be observed in mice after pharmacological manipulations of the AcbSh or the VPm in satiated mice, a new line of research could be generated by studying the specific role of key peptides (e.g. orexin/hypocretin, MCH and NPY) for which KO mice are available (Bannon et al., 2000; Mochizuki et al., 2004; Roy et al., 2005). My results would indicate that orexin/hypocretin and MCH KO mice would display increased feeding responses after activation of the VPm, indicating that feeding induced by excitation of the VPm is independent from these two peptides. On the other hand, given that orexin/hypocretin

neurons have been implicated in intra-AcbSh muscimol feeding (Baldo et al., 2004, Zheng et al., 2003), orexin/hypocretin KO mice should show attenuated feeding induced by intra-AcbSh muscimol injections compared with wild-type controls. Furthermore, since intra-ventricular injections of NPY antagonists suppress food intake elicited by injections of muscimol in the AcbSh (Stratford and Wirtshafter, 2004), it is likely that NPY KO mice would show a similar suppression of VPm feeding. Studies with NPY KO mice would also offer insight into the role of this peptide in feeding induced by excitation of the VPm in satiated animals.

One of the most unexpected results presented here was the dramatic and specific increase in fat intake induced by GABAergic manipulations of the VPm. Future experiments using KO mice and additional lesion experiments could offer more information about the underlying mechanisms of this increased fat preference. The PVN would be a relevant target region for lesion studies. Indeed, the data presented in this dissertation (chapter 2) indicate that excitation of the VPm can modulate activity in the PVN. The PVN has also been implicated in macronutrient selection: injections of the peptides enkephalin and galanin into this hypothalamic nucleus induce a marked preference for fat (Kyrkouli et al., 1990; Naleid et al., 2007; Tempel et al., 1988), whereas activation of NPY or norepinephrine receptors in the PVN preferentially increases carbohydrate intake (Leibowitz et al., 1985; Stanley et al., 1985). It is possible that excitotoxic PVN lesions would attenuate fat and possibly carbohydrate intake induced by excitation of the VPm but not protein, which was marginally increased by intra-VPm bicuculline injections. In terms of KO experiments, transthyretin KO mice

display increased NPY expression as well as increased carbohydrate preference (Nunes et al., 2006). It would be interesting to see if these transthyretin KO would display an attenuation of fat preference after excitation of the VPm. Results from these experiments would help clarify the role of different hypothalamic regions and peptides in feeding induced by excitation of the VPm and the novel change in fat preference presented here.

Preliminary data indicates that the preferential increase in lard intake induced by manipulations of the VPm is generalizable to other fat sources. In follow-up studies, we have seen increases in corn oil preference after intra-VPm bicuculline injections. Further investigation should compare fats from different sources as well as unsaturated versus saturated fats. These studies would help identify the element or elements present in lipids that drive the preference for fat observed after intra-VPm bicuculline injections. Additional, our preliminary data indicate that intra-VPm bicuculline injections also increase fat intake in naïve rats, suggesting that the lard overconsumption observed after excitation of the VPm does not depended on learned post-ingestive effects. Current experiments are focused on exploring the role of texture in fat overconsumption induced by intra-VPm bicuculline injections.

One of the main limitations of macronutrient studies is that most animals including humans consume food stuffs containing mixed macronutrients. Hence, the results presented in chapter 4 have limited translational potential. This limitation could be addressed by studying how intra-VPm bicuculline injections affect the preference for

mixed diets containing different concentrations of fat, carbohydrates and protein. The results present in this dissertation would indicate that excitation of the VPm would increase the consumption of food stuff with high fat.

The results presented in this dissertation are relevant to the study of food intake and human disorders characterized by dysregulation of food consumption. Unfortunately, translation from the bench to the clinic is problematic. The procedures presented here are extremely invasive and technologies like target delivery of drugs to specific brain regions would need to be first developed before these kind of studies could be safely performed in humans. However, neuroimaging studies could be conducted to measure different aspect of brain metabolism in patients affected by disorders like obesity, binge eating, bulimia and anorexia nervosa. It is possible that some of these patients present dysregulation in areas normally ignored by clinicians like the VP. Indeed, presentation of pictures of high-caloric food to obese women induced greater activation of the VP than controls (Stoeckel et al., 2008). Furthermore, volume alterations of the pallidum have been described in young women affected by anorexia nervosa (Fuglset et al., 2014). These individuals could benefit from treatments designed to modulate dysregulation of the basal ganglia, such as deep brain stimulation (DBS) used to treat Parkinson's disease. DBS has been shown to be an effective treatment for Parkinson's (Halpern et al., 2007) and has been proposed as a potential treatment for obesity (Halpern et al., 2011) and anorexia nervosa (Oudijn et al., 2013; Wu et al., 2013). Based on the data presented in this dissertation, the VPm could be a potential target for the treatment of obesity and eating disorders.

REFERENCES

Albin, H. J., & Berendse, H. W. (1990). Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat. *The Journal of comparative neurology*, *294*(4), 607–622. doi:10.1002/cne.902940408

Albin, R. L., Makowiec, R. L., Hollingsworth, Z. R., Dure IV, L. S., Penney, J. B., & Young, A. B. (1992). Excitatory amino acid binding sites in the basal ganglia of the rat: A quantitative autoradiographic study. *Neuroscience*, *46*(1), 35–48. doi:10.1016/0306-4522(92)90006-N

Anand, B. K., & Brobeck, J. R. (1951). Hypothalamic Control of Food Intake in Rats and Cats. *The Yale Journal of Biology and Medicine*, *24*(2), 123–140.

Aou, S., Takaki, A., Karádi, Z., Hori, T., Nishino, H., & Oomura, Y. (1991). Functional heterogeneity of the monkey lateral hypothalamus in the control of feeding. *Brain Research Bulletin*, *27*(3–4), 451–455. doi:10.1016/0361-9230(91)90141-6

Appelhans, B. M. (2009). Neurobehavioral Inhibition of Reward-driven Feeding: Implications for Dieting and Obesity. *Obesity*, *17*(4), 640–647. doi:10.1038/oby.2008.638

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience & Biobehavioral Reviews*, *32*(1), 20–39. doi:10.1016/j.neubiorev.2007.04.019

Bahjaoui-Bouhaddi, M., Fellmann, D., Griffond, B., & Bugnon, C. (1994). Insulin treatment stimulates the rat melanin-concentrating hormone-producing neurons. *Neuropeptides*, *27*(4), 251–258. doi:10.1016/0143-4179(94)90006-X

Baldo, B. A., Gual-Bonilla, L., Sijapati, K., Daniel, R. A., Landry, C. F., & Kelley, A. E. (2004). Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. *European Journal of Neuroscience*, *19*(2), 376–386. doi:10.1111/j.1460-9568.2004.03093.x

Bannon, A. W., Seda, J., Carmouche, M., Francis, J. M., Norman, M. H., Karbon, B., & McCaleb, M. L. (2000). Behavioral characterization of neuropeptide Y knockout mice. *Brain Research*, *868*(1), 79–87. doi:10.1016/S0006-8993(00)02285-X

Basso, A. M., & Kelley, A. E. (1999). Feeding induced by GABA(A) receptor stimulation within the nucleus accumbens shell: regional mapping and characterization of macronutrient and taste preference. *Behavioral neuroscience*, *113*(2), 324–336.

Bell, K., Churchill, L., & Kalivas, P. W. (1995). GABAergic projection from the ventral pallidum and globus pallidus to the subthalamic nucleus. *Synapse (New York, N.Y.)*, *20*(1), 10–18. doi:10.1002/syn.890200103

Bernard, C. (1974). *Lectures on the phenomena of life common to animals and plants*. Thomas.

Bernardis, L. L., & Bellinger, L. L. (1993). The lateral hypothalamic area revisited: Neuroanatomy, body weight regulation, neuroendocrinology and metabolism. *Neuroscience & Biobehavioral Reviews*, *17*(2), 141–193. doi:10.1016/S0149-7634(05)80149-6

Bernardis, L. L., & Bellinger, L. L. (1996). The lateral hypothalamic area revisited: Ingestive behavior. *Neuroscience & Biobehavioral Reviews*, *20*(2), 189–287. doi:10.1016/0149-7634(95)00015-1

Bernardis, L. L., & Bellinger, L. L. (1998). The Dorsomedial Hypothalamic Nucleus Revisited: 1998 Update. *Experimental Biology and Medicine*, *218*(4), 284–306. doi:10.3181/00379727-218-44296

Bellinger, L. L., & Bernardis, L. L. (2002). The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation: Lessons learned from lesioning studies. *Physiology & Behavior*, *76*(3), 431–442. doi:10.1016/S0031-9384(02)00756-4

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience & Biobehavioral Reviews*, *24*(2), 173–198. doi:10.1016/S0149-7634(99)00072-X

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. doi:10.1016/S0166-2236(03)00233-9

Berridge, C. W., Stratford, T. L., Foote, S. L., & Kelley, A. E. (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse (New York, N.Y.)*, *27*(3), 230–241. doi:10.1002/(SICI)1098-2396(199711)27:3<230::AID-SYN8>3.0.CO;2-E

Berridge, K. C., & Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behavioral Neuroscience*, *105*(1), 3–14.

Berthoud, H.-R. (2007). Interactions between the “cognitive” and “metabolic” brain in the control of food intake. *Physiology & Behavior*, *91*(5), 486–498. doi:10.1016/j.physbeh.2006.12.016

Berthoud, H.-R. (2006). Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity (Silver Spring, Md.)*, *14 Suppl 5*, 197S–200S. doi:10.1038/oby.2006.308

Berthoud, H.-R., & Münzberg, H. (2011). The lateral hypothalamus as integrator of metabolic and environmental needs: From electrical self-stimulation to opto-genetics. *Physiology & Behavior*, *104*(1), 29–39. doi:10.1016/j.physbeh.2011.04.051

Bevan, M. D., Clarke, N. P., & Bolam, J. P. (1997). Synaptic Integration of Functionally Diverse Pallidal Information in the Entopeduncular Nucleus and Subthalamic Nucleus in the Rat. *The Journal of Neuroscience*, *17*(1), 308–324.

Bevan, M. D., Smith, A. D., & Bolam, J. P. (1996). The substantia nigra as a site of synaptic integration of functionally diverse information arising from the ventral pallidum and the globus pallidus in the rat. *Neuroscience*, *75*(1), 5–12.

Bonaz, B., De Giorgio, R., & Tache´, Y. (1993). Peripheral bombesin induces c-fos protein in the rat brain. *Brain Research*, *600*(2), 353–357. doi:10.1016/0006-8993(93)91397-B

Bouret, S. G., Draper, S. J., & Simerly, R. B. (2004). Formation of Projection Pathways from the Arcuate Nucleus of the Hypothalamus to Hypothalamic Regions Implicated in the Neural Control of Feeding Behavior in Mice. *The Journal of Neuroscience*, *24*(11), 2797–2805. doi:10.1523/JNEUROSCI.5369-03.2004

Boyle, P. C., & Keesey, R. E. (1975). Chronically reduced body weight in rats sustaining lesions of the lateral hypothalamus and maintained on palatable diets and drinking solutions. *Journal of Comparative and Physiological Psychology*, *88*(1), 218–223.

Boyson, S. J., McGonigle, P., & Molinoff, P. B. (1986). Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *The Journal of neuroscience*, *6*(11), 3177–3188.

Briski, K., & Gillen, E. (2001). Differential distribution of Fos expression within the male rat preoptic area and hypothalamus in response to physical vs. psychological stress. *Brain Research Bulletin*, *55*(3), 401–408. doi:10.1016/S0361-9230(01)00532-9

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *The Journal of comparative neurology*, *338*(2), 255–278. doi:10.1002/cne.903380209

Burt, J., Alberto, C. O., Parsons, M. P., & Hirasawa, M. (2011). Local network regulation of orexin neurons in the lateral hypothalamus. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *301*(3), R572–R580. doi:10.1152/ajpregu.00674.2010

Cai, X. J., Evans, M. L., Lister, C. A., Leslie, R. A., Arch, J. R. S., Wilson, S., & Williams, G. (2001). Hypoglycemia Activates Orexin Neurons and Selectively Increases Hypothalamic Orexin-B Levels Responses Inhibited by Feeding and Possibly Mediated by the Nucleus of the Solitary Tract. *Diabetes*, *50*(1), 105–112. doi:10.2337/diabetes.50.1.105

Canteras, N. S., Shammah-Lagnado, S. J., Silva, B. A., & Ricardo, J. A. (1990). Afferent connections of the subthalamic nucleus: a combined retrograde and anterograde horseradish peroxidase study in the rat. *Brain Research*, *513*(1), 43–59. doi:10.1016/0006-8993(90)91087-W

Carelli, R. M., Ijames, S. G., & Crumling, A. J. (2000). Evidence That Separate Neural Circuits in the Nucleus Accumbens Encode Cocaine Versus “Natural” (Water and Food) Reward. *The Journal of Neuroscience*, *20*(11), 4255–4266.

Carlezon Jr., W. A., & Thomas, M. J. (2009). Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology*, *56, Supplement 1*(0), 122–132. doi:10.1016/j.neuropharm.2008.06.075

Cason, A. M., Smith, R. J., Tahsili-Fahadan, P., Moorman, D. E., Sartor, G. C., & Aston-Jones, G. (2010). Role of orexin/hypocretin in reward-seeking and addiction: Implications for obesity. *Physiology & Behavior*, *100*(5), 419–428. doi:10.1016/j.physbeh.2010.03.009

Chastrette, N., Pfaff, D. W., & Gibbs, R. B. (1991). Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Research*, *563*(1–2), 339–344. doi:10.1016/0006-8993(91)91559-J

Chang, H. T., Tian, Q., & Herron, P. (1995). GABAergic axons in the ventral forebrain of the rat: An electron microscopic study. *Neuroscience*, *68*(1), 207–220. doi:10.1016/0306-4522(95)00109-V

Chang, J.-Y., Zhang, L., Janak, P. H., & Woodward, D. J. (1997). Neuronal responses in prefrontal cortex and nucleus accumbens during heroin self-administration in freely moving rats. *Brain Research*, *754*(1–2), 12–20. doi:10.1016/S0006-8993(97)00012-7

Charles, J. R., Duva, M. A., Ramirez, G. L., Lara, R. L., Yang, C. R., & Stanley, B. G. (2013). Activation of lateral hypothalamic mGlu1 and mGlu5 receptors elicits feeding in rats. *Neuropharmacology*. doi:10.1016/j.neuropharm.2013.10.033

Chou, T. C., Scammell, T. E., Gooley, J. J., Gaus, S. E., Saper, C. B., & Lu, J. (2003). Critical Role of Dorsomedial Hypothalamic Nucleus in a Wide Range of Behavioral Circadian Rhythms. *The Journal of Neuroscience*, *23*(33), 10691–10702.

Christie, M. J., Summers, R. J., Stephenson, J. A., Cook, C. J., & Beart, P. M. (1987). Excitatory amino acid projections to the nucleus accumbens septi in the rat: A retrograde transport study utilizingd[3H]aspartate and [3H]GABA. *Neuroscience*, *22*(2), 425–439. doi:10.1016/0306-4522(87)90345-9

Chrobak, J. J., & Napier, T. C. (1993). Opioid and GABA modulation of accumbens-evoked ventral pallidal activity. *Journal of neural transmission. General section*, *93*(2), 123–143.

Churchill, L., Dilts, R. P., & Kalivas, P. W. (1990). Changes in γ-aminobutyric acid, μ-opioid and neurotensin receptors in the accumbens-pallidal projection after discrete quinolinic acid lesions in the nucleus accumbens. *Brain Research*, *511*(1), 41–54. doi:10.1016/0006-8993(90)90223-X

Churchill, L., & Kalivas, P. W. (1994). A topographically organized gamma-aminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. *The Journal of Comparative Neurology*, *345*(4), 579–595. doi:10.1002/cne.903450408

Churchill, L., Zahm, D. S., & Kalivas, P. W. (1996). The mediodorsal nucleus of the thalamus in rats—I. Forebrain gabaergic innervation. *Neuroscience*, *70*(1), 93–102. doi:10.1016/0306-4522(95)00351-I

Clegg, D. J., Air, E. L., Woods, S. C., & Seeley, R. J. (2002). Eating Elicited by Orexin-A, But Not Melanin-Concentrating Hormone, Is Opioid Mediated. *Endocrinology*, *143*(8), 2995–3000. doi:10.1210/en.143.8.2995

Compan, V., Daszuta, A., Salin, P., Sebben, M., Bockaert, J., & Dumuis, A. (1996). Lesion study of the distribution of serotonin 5-HT4 receptors in rat basal ganglia and hippocampus. *The European journal of neuroscience*, *8*(12), 2591–2598.

Corbett, S. W., & Keesey, R. E. (1980). Digestibility in male rats with lateral hypothalamic lesions. *Physiology & Behavior*, *24*(6), 1165–1168. doi:10.1016/0031-9384(80)90065-7

Corwin, R. L., & Hajnal, A. (2005). Too much of a good thing: Neurobiology of non-homeostatic eating and drug abuse. *Physiology & Behavior*, *86*(1–2), 5–8. doi:10.1016/j.physbeh.2005.06.021

Covelo, I. R., Wirtshafter, D., & Stratford, T. R. (2012). GABAA and dopamine receptors in the nucleus accumbens shell differentially influence performance of a water-reinforced progressive ratio task. *Pharmacology Biochemistry and Behavior*, *101*(1), 57–61. doi:10.1016/j.pbb.2011.11.015

Covelo, I. R., Patel, Z. I., Luviano, J. A., Stratford, T. R., & Wirtshafter, D. (2014). Manipulation of GABA in the ventral pallidum, but not the nucleus accumbens, induces intense, preferential, fat consumption in rats. *Behavioural Brain Research*, *270*, 316–325. doi:10.1016/j.bbr.2014.05.032

Da Silva, A. A., Marino-Neto, J., & Paschoalini, M. A. (2003). Feeding induced by microinjections of NMDA and AMPA–kainate receptor antagonists into ventral striatal and ventral pallidal areas of the pigeon. *Brain Research*, *966*(1), 76–83. doi:10.1016/S0006-8993(02)04196-3

Davis, J. D. (1989). The Microstructure of Ingestive Behavior. *Annals of the New York Academy of Sciences*, *575*(1), 106–121. doi:10.1111/j.1749-6632.1989.tb53236.x

Davis, J. D. (1998). A model for the control of ingestion - 20 years later. *Progress in Psychobiology and Physiological Psychology, Vol 17*, *17*, 127–173.

Delfs, J. M., Zhu, Y., Druhan, J. P., & Aston-Jones, G. S. (1998). Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat. *Brain Research*, *806*(2), 127–140. doi:10.1016/S0006-8993(98)00672-6

Delgado, J. M. R., & Anand, B. K. (1953). Increase of food intake induced by electrical stimulation of the lateral hypothalamus. *The American Journal of Physiology*, *172*(1), 162–168.

Dragunow, M., & Faull, R. (1989). The use of c-fos as a metabolic marker in neuronal pathway tracing. *Journal of Neuroscience Methods*, *29*(3), 261–265. doi:10.1016/0165-0270(89)90150-7

Elmquist, J. K., Ahima, R. S., Elias, C. F., Flier, J. S., & Saper, C. B. (1998). Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proceedings of the National Academy of Sciences*, *95*(2), 741–746.

Farrar, A. M., Font, L., Pereira, M., Mingote, S., Bunce, J. G., Chrobak, J. J., & Salamone, J. D. (2008). Forebrain circuitry involved in effort-related choice: Injections of the GABAA agonist muscimol into ventral pallidum alter response allocation in food-seeking behavior. *Neuroscience*, *152*(2), 321–330. doi:10.1016/j.neuroscience.2007.12.034

Flier, J. S. (2004). Obesity Wars: Molecular Progress Confronts an Expanding Epidemic. *Cell*, *116*(2), 337–350. doi:10.1016/S0092-8674(03)01081-X

Fuglset, T. S., Endestad, T., Landrø, N. I., & Rø, O. (2014). Brain structure alterations associated with weight changes in young females with anorexia nervosa: a case series. Neurocase. doi:10.1080/13554794.2013.878728

Fuller TA, Russchen FT, Price JL. 1987. Sources of presumptive glutamergic/aspartergic afferents to the rat ventral striatopallidal region. *J Comp Neurol* 258:317–338.

Gaykema, R. P., & Zaborszky, L. (1996). Direct catecholaminergic-cholinergic interactions in the basal forebrain. II. Substantia nigra-ventral tegmental area projections to cholinergic neurons. *The Journal of comparative neurology*, *374*(4), 555–577. doi:10.1002/(SICI)1096-9861(19961028)374:4<555::AID-CNE6>3.0.CO;2-0

Geisler, S., Derst, C., Veh, R. W., & Zahm, D. S. (2007). Glutamatergic Afferents of the Ventral Tegmental Area in the Rat. *The Journal of Neuroscience*, *27*(21), 5730–5743. doi:10.1523/JNEUROSCI.0012-07.2007

Groenewegen, H. J., & Berendse, H. W. (1990). Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat. *The Journal of Comparative Neurology*, *294*(4), 607–622. doi:10.1002/cne.902940408

Groenewegen, H. J., Berendse, H. W., & Haber, S. N. (1993). Organization of the output of the ventral striatopallidal system in the rat: Ventral pallidal efferents. *Neuroscience*, *57*(1), 113–142. doi:10.1016/0306-4522(93)90115-V

Groenewegen, H. J., & Russchen, F. T. (1984). Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. *The Journal of comparative neurology*, *223*(3), 347–367. doi:10.1002/cne.902230303

Haber, S. N., Groenewegen, H. J., Grove, E. A., & Nauta, W. J. (1985). Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. *The Journal of comparative neurology*, *235*(3), 322–335. doi:10.1002/cne.902350304

Haber, S. N., & Knutson, B. (2010). The Reward Circuit: Linking Primate Anatomy and Human Imaging. *Neuropsychopharmacology*, *35*(1), 4–26. doi:10.1038/npp.2009.129

Haber, S. N., Lynd-Balta, E., & Mitchell, S. J. (1993). The organization of the descending ventral pallidal projections in the monkey. *The Journal of comparative neurology*, *329*(1), 111–128. doi:10.1002/cne.903290108

Hakan, R. L., Berg, G. I., & Henriksen, S. J. (1992). Electrophysiological evidence for reciprocal connectivity between the nucleus accumbens septi and ventral pallidal region. *Brain Research*, *581*(2), 344–350. doi:10.1016/0006-8993(92)90730-W

Halpern, C., Hurtig, H., Jaggi, J., Grossman, M., Won, M., & Baltuch, G. (2007). Deep brain stimulation in neurologic disorders. *Parkinsonism & Related Disorders*, *13*(1), 1–16. doi:10.1016/j.parkreldis.2006.03.001

Halpern, C. H., Torres, N., Hurtig, H. I., Wolf, J. A., Stephen, J., Oh, M. Y., Baltuch, G. H. (2011). Expanding applications of deep brain stimulation: a potential therapeutic role in obesity and addiction management. *Acta Neurochirurgica*, *153*(12), 2293–2306. doi:10.1007/s00701-011-1166-3

Hanson, E. S., & Dallman, M. F. (1995). Neuropeptide Y (NPY) may integrate responses of hypothalamic feeding systems and the hypothalamo-pituitary-adrenal axis. *Journal of Neuroendocrinology*, *7*(4), 273–279.

Harrold, J. A., Dovey, T. M., Blundell, J. E., & Halford, J. C. G. (2012). CNS regulation of appetite. *Neuropharmacology*, *63*(1), 3–17. doi:10.1016/j.neuropharm.2012.01.007

Hassani, O. K., Lee, M. G., & Jones, B. E. (2009). Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep–wake cycle. *Proceedings of the National Academy of Sciences*, *106*(7), 2418–2422. doi:10.1073/pnas.0811400106

Heimer, L., & Wilson, R. D. (1975). The subcortical projections of the allocortex: similarities in the neural associations of the hippocampus, the piriform cortex, and the neocortex. In *Golgi Centennial Symposium Proceedings, Raven Press, New York* (pp. 177–193).

Heimer, L., Zahm, D. S., Churchill, L., Kalivas, P. W., & Wohltmann, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, *41*(1), 89–125.

Hernandez, G., Hamdani, S., Rajabi, H., Conover, K., Stewart, J., Arvanitogiannis, A., & Shizgal, P. (2006). Prolonged rewarding stimulation of the rat medial forebrain bundle: Neurochemical and behavioral consequences. *Behavioral Neuroscience*, *120*(4), 888–904. doi:10.1037/0735-7044.120.4.888

Hetherington, A. W., & Ranson, S. W. (1940). Hypothalamic lesions and adiposity in the rat. *The Anatomical Record*, *78*(2), 149–172. doi:10.1002/ar.1090780203

Hettes, S. R., Gonzaga, W. J., Heyming, T. W., Nguyen, J. K., Perez, S., & Stanley, B. G. (2010). Stimulation of lateral hypothalamic AMPA receptors may induce feeding in rats. *Brain Research*, *1346*(0), 112–120. doi:10.1016/j.brainres.2010.05.008

Hiroi, N., & White, N. M. (1993). The ventral pallidum area is involved in the acquisition but not expression of the amphetamine conditioned place preference. *Neuroscience Letters*, *156*(1–2), 9–12. doi:10.1016/0304-3940(93)90426-L

Horst, G. J. T., De Boer, P., Luiten, P. G. M., & Van Willigen, J. D. (1989). Ascending projections from the solitary tract nucleus to the hypothalamus. A Phaseolus vulgaris lectin tracing study in the rat. *Neuroscience*, *31*(3), 785–797. doi:10.1016/0306-4522(89)90441-7

Hubner, C. B., & Koob, G. F. (1990). The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. *Brain Research*, *508*(1), 20–29. doi:10.1016/0006-8993(90)91112-T

Hughes, R. N. (1965). Food deprivation and locomotor exploration in the white rat. *Animal Behaviour*, *13*(1), 30–32. doi:10.1016/0003-3472(65)90068-0

Hur, E. E., & Zaborszky, L. (2005). Vglut2 afferents to the medial prefrontal and primary somatosensory cortices: A combined retrograde tracing in situ hybridization. *The Journal of Comparative Neurology*, *483*(3), 351–373. doi:10.1002/cne.20444

Inui, A., Asakawa, A., Bowers, C. Y., Mantovani, G., Laviano, A., Meguid, M. M., & Fujimiya, M. (2004). Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. *The FASEB Journal*, *18*(3), 439–456. doi:10.1096/fj.03-0641rev

Janak, P. H., Chang, J.-Y., & Woodward, D. J. (1999). Neuronal spike activity in the nucleus accumbens of behaving rats during ethanol self-administration. *Brain Research*, *817*(1–2), 172–184. doi:10.1016/S0006-8993(98)01245-1

Johnson, P. I., Parente, M. A., & Stellar, J. R. (1996). NMDA-induced lesions of the nucleus accumbens or the ventral pallidum increase the rewarding efficacy of food to deprived rats. *Brain Research*, *722*(1–2), 109–117. doi:10.1016/0006-8993(96)00202-8

Kalivas, P. W., Churchill, L., & Klitenick, M. A. (1993). GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience*, *57*(4), 1047–1060. doi:10.1016/0306-4522(93)90048-K

Karnani, M., & Burdakov, D. (2011). Multiple hypothalamic circuits sense and regulate glucose levels. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *300*(1), R47–R55. doi:10.1152/ajpregu.00527.2010

Keesey, R. E., & Boyle, P. C. (1973). Effects of quinine adulteration upon body weight of LH-lesioned and intact male rats. *Journal of Comparative and Physiological Psychology*, *84*(1), 38–46. doi:10.1037/h0035016

Kelley, A. E., Bakshi, V. P., Haber, S. N., Steininger, T. L., Will, M. J., & Zhang, M. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiology & Behavior*, *76*(3), 365–377. doi:10.1016/S0031-9384(02)00751-5

Kelley, A. E., & Swanson, C. J. (1997). Feeding induced by blockade of AMPA and kainate receptors within the ventral striatum: a microinfusion mapping study. *Behavioural Brain Research*, *89*(1–2), 107–113. doi:10.1016/S0166-4328(97)00054-5

Klitenick, M. A., DeWitte, P., & Kalivas, P. W. (1992). Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. *The Journal of neuroscience*, *12*(7), 2623–2632.

Kokkotou, E. G., Tritos, N. A., Mastaitis, J. W., Slieker, L., & Maratos-Flier, E. (2001). Melanin-Concentrating Hormone Receptor Is a Target of Leptin Action in the Mouse Brain. *Endocrinology*, *142*(2), 680–686. doi:10.1210/en.142.2.680

Koylu, E. O., Couceyro, P. R., Lambert, P. D., Ling, N. C., DeSouza, E. B., & Kuhar, M. J. (1997). Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland. *Journal of Neuroendocrinology*, *9*(11), 823–833.

Kretschmer, B. D. (2000). Functional aspects of the ventral pallidum. *Amino Acids*, *19*(1), 201–210. doi:10.1007/s007260070050

Kyrkouli, S. E., Stanley, B. G., Hutchinson, R., Seirafi, R. D., & Leibowitz, S. F. (1990). Peptide-amine interactions in the hypothalamic paraventricular nucleus: analysis of galanin and neuropeptide Y in relation to feeding. *Brain Research*, *521*(1–2), 185–191. doi:10.1016/0006-8993(90)91541-N

Lambert, K. (2011). *A Tale of 2 Rodents*. Retrieved July 1, 2014, from http://www.scientificamerican.com/article/a-tale-of-two-rodents

Lambert, P. D., Couceyro, P. R., McGirr, K. M., Dall Vechia, S. E., Smith, Y., & Kuhar, M. J. (1998). CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse (New York, N.Y.)*, *29*(4), 293–298. doi:10.1002/(SICI)1098-2396(199808)29:4<293::AID-SYN1>3.0.CO;2-0

Lawrence, C. B., Snape, A. C., Baudoin, F. M.-H., & Luckman, S. M. (2002). Acute Central Ghrelin and GH Secretagogues Induce Feeding and Activate Brain Appetite Centers. *Endocrinology*, *143*(1), 155–162. doi:10.1210/en.143.1.155

Leibowitz, S. F., & Alexander, J. T. (1998). Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biological Psychiatry*, *44*(9), 851–864. doi:10.1016/S0006-3223(98)00186-3

Leibowitz, S. F., Shor-Posner, G., Maclow, C., & Grinker, J. A. (1986). Amphetamine: effects on meal patterns and macronutrient selection. *Brain Research Bulletin*, *17*(5), 681–689.

Leibowitz, S. F., Weiss, G. F., Yee, F., & Tretter, J. B. (1985). Noradrenergic innervation of the paraventricular nucleus: specific role in control of carbohydrate ingestion. *Brain Research Bulletin*, *14*(6), 561–567.

Leibowitz, S. F., Weiss, G. F., & Suh, J. S. (1990). Medial hypothalamic nuclei mediate serotonin's inhibitory effect on feeding behavior. *Pharmacology Biochemistry and Behavior*, *37*(4), 735–742. doi:10.1016/0091-3057(90)90556-W

Levine, A. S., & Billington, C. J. (2004). Opioids as agents of reward-related feeding: a consideration of the evidence. *Physiology & Behavior*, *82*(1), 57–61. doi:10.1016/j.physbeh.2004.04.032

Levine, A. S., Weldon, D. T., Grace, M., Cleary, J. P., & Billington, C. J. (1995). Naloxone blocks that portion of feeding driven by sweet taste in food-restricted rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *268*(1), R248–R252.

Li, L., Fulton, J. D., & Yeomans, J. S. (1999). Effects of bilateral electrical stimulation of the ventral pallidum on acoustic startle. *Brain Research*, *836*(1–2), 164–172. doi:10.1016/S0006-8993(99)01651-0

Loriaux, A. L., Roitman, J. D., & Roitman, M. F. (2011). Nucleus accumbens shell, but not core, tracks motivational value of salt. *Journal of Neurophysiology*, *106*(3), 1537–1544. doi:10.1152/jn.00153.2011

Lu, J., & Greco, M. A. (2006). Sleep circuitry and the hypnotic mechanism of GABAA drugs. *Journal of clinical sleep medicine: JCSM: official publication of the American Academy of Sleep Medicine*, *2*(2), S19–26.

Lutter, M., & Nestler, E. J. (2009). Homeostatic and Hedonic Signals Interact in the Regulation of Food Intake. Journal of Nutrition, 139(3), 629–632. doi:10.3945/jn.108.097618

Maldonado-Irizarry, C. S., Swanson, C. J., & Kelley, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *The Journal of Neuroscience*, *15*(10), 6779–6788.

Margules, D. L., & Olds, J. (1962). Identical “Feeding” and “Rewarding” Systems in the Lateral Hypothalamus of Rats. *Science*, *135*(3501), 374–375. doi:10.2307/1709351

Maslowski, R. J., & Napier, T. C. (1991). Dopamine D1 and D2 receptor agonists induce opposite changes in the firing rate of ventral pallidal neurons. *European journal of pharmacology*, *200*(1), 103–112.

Maurice, N., Deniau, J. ., Menetrey, A., Glowinski, J., & Thierry, A. (1997). Position of the ventral pallidum in the rat prefrontal cortex–basal ganglia circuit. *Neuroscience*, *80*(2), 523–534. doi:10.1016/S0306-4522(97)00002-X

Meredith, G. E. (1999). The Synaptic Framework for Chemical Signaling in Nucleus Accumbens. *Annals of the New York Academy of Sciences*, *877*(1), 140–156. doi:10.1111/j.1749-6632.1999.tb09266.x

Meredith, G. E., Agolia, R., Arts, M. P. M., Groenewegen, H. J., & Zahm, D. S. (1992). Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. *Neuroscience*, *50*(1), 149–162. doi:10.1016/0306-4522(92)90389-J

Meredith, G. E., Pennartz, C. M., & Groenewegen, H. J. (1993). The cellular framework for chemical signalling in the nucleus accumbens. *Progress in Brain Research*, *99*, 3–24.

McAlonan, G. M., Robbins, T. W., & Everitt, B. J. (1993). Effects of medial dorsal thalamic and ventral pallidal lesions on the acquisition of a conditioned place preference: Further evidence for the involvement of the ventral striatopallidal system in reward-related processes. *Neuroscience*, *52*(3), 605–620. doi:10.1016/0306-4522(93)90410-H

Mitrovic, I., & Napier, T. C. (1995). Electrophysiological Demonstration of Mu, Delta and Kappa Opioid Receptors in the Ventral Pallidum. *Journal of Pharmacology and Experimental Therapeutics*, *272*(3), 1260–1270.

Mochizuki, T., Crocker, A., McCormack, S., Yanagisawa, M., Sakurai, T., & Scammell, T. E. (2004). Behavioral State Instability in Orexin Knock-Out Mice. *The Journal of Neuroscience*, *24*(28), 6291–6300. doi:10.1523/JNEUROSCI.0586-04.2004

Mogenson, G. J., Ciriello, J., Garland, J., & Wu, M. (1987). Ventral pallidum projections to mediodorsal nucleus of the thalamus: an anatomical and electrophysiological investigation in the rat. *Brain Research*, *404*(1–2), 221–230. doi:10.1016/0006-8993(87)91373-4

Morganstern, I., Chang, G.-Q., Karatayev, O., & Leibowitz, S. F. (2010). Increased orexin and melanin-concentrating hormone expression in the perifornical lateral hypothalamus of rats prone to overconsuming a fat-rich diet. *Pharmacology Biochemistry and Behavior*, *96*(4), 413–422. doi:10.1016/j.pbb.2010.06.013

Moriguchi, T., Sakurai, T., Nambu, T., Yanagisawa, M., & Goto, K. (1999). Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. *Neuroscience Letters*, *264*(1–3), 101–104. doi:10.1016/S0304-3940(99)00177-9

Morris, B. J. (1989). Neuronal localisation of neuropeptide Y gene expression in rat brain. *The Journal of comparative neurology*, *290*(3), 358–368. doi:10.1002/cne.902900305

Nagase, H., Nakajima, A., Sekihara, H., York, D. A., & Bray, G. A. (2002). Regulation of feeding behavior, gastric emptying, and sympathetic nerve activity to interscapular brown adipose tissue by galanin and enterostatin: the involvement of vagal--central nervous system interactions. *Journal of Gastroenterology*, *37*(11), 118.

Naleid, A. M., Grace, M. K., Chimukangara, M., Billington, C. J., & Levine, A. S. (2007). Paraventricular opioids alter intake of high-fat but not high-sucrose diet depending on diet preference in a binge model of feeding. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *293*(1), R99–R105. doi:10.1152/ajpregu.00675.2006

Napier, T. C., & Potter, P. E. (1989). Dopamine in the rat ventral pallidum/substantia innominata: biochemical and electrophysiological studies. *Neuropharmacology*, *28*(7), 757–760.

Nauta, W. J. H., & Mehler, W. R. (1966). Projections of the lentiform nucleus in the monkey. *Brain Research*, *1*(1), 3–42. doi:10.1016/0006-8993(66)90103-X

Nauta, W. J. H., Smith, G. P., Faull, R. L. M., & Domesick, V. B. (1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience*, *3*(4–5), 385–401. doi:10.1016/0306-4522(78)90041-6

Neel, J. V. (1962). Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? *American Journal of Human Genetics*, *14*, 353–362.

Nicola, S. M., Yun, I. A., Wakabayashi, K. T., & Fields, H. L. (2004). Firing of Nucleus Accumbens Neurons During the Consummatory Phase of a Discriminative Stimulus Task Depends on Previous Reward Predictive Cues. *Journal of Neurophysiology*, *91*(4), 1866–1882. doi:10.1152/jn.00658.2003

Niimi, M., Sato, M., Tamaki, M., Wada, Y., Takahara, J., & Kawanishi, K. (1995). Induction of Fos protein in the rat hypothalamus elicited by insulin-induced hypoglycemia. *Neuroscience Research*, *23*(4), 361–364. doi:10.1016/0168-0102(95)00965-V

Nishimura, Y., Mabuchi, K., Taguchi, S., Ikeda, S., Aida, E., Negishi, H., & Takamata, A. (2014). Involvement of orexin-A neurons but not melanin-concentrating hormone neurons in the short-term regulation of food intake in rats. *The Journal of Physiological Sciences*, *64*(3), 203–211. doi:10.1007/s12576-014-0312-0

Nunes, A. F., Saraiva, M. J., & Sousa, M. M. (2005). Transthyretin knockouts are a new mouse model for increased neuropeptide Y. *The FASEB Journal*. doi:10.1096/fj.05-4106fje

Obici, S. (2009). Molecular Targets for Obesity Therapy in the Brain. *Endocrinology*, *150*(6), 2512–2517. doi:10.1210/en.2009-0409

O'Donnell, P., Lavín, A., Enquist, L. W., Grace, A. A., & Card, J. P. (1997). Interconnected Parallel Circuits between Rat Nucleus Accumbens and Thalamus Revealed by Retrograde Transynaptic Transport of Pseudorabies Virus. *The Journal of Neuroscience*, *17*(6), 2143–2167.

Olney, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science (New York, N.Y.)*, *164*(3880), 719–721.

Ono, T., Sasaki, K., Nishino, H., Fukuda, M., & Shibata, R. (1986). Feeding and diurnal related activity of lateral hypothalamic neurons in freely behaving rats. *Brain Research*, *373*(1–2), 92–102. doi:10.1016/0006-8993(86)90319-7

Oomura, Y., Nakamura, T., & Manchanda, S. K. (1975). Excitatory and inhibitory effects of globus pallidus and substantia nigra on the lateral hypothalamic activity in the rat. *Pharmacology, Biochemistry, and Behavior*, *3*(1 Suppl), 23–36.

Oudijn, M. S., Storosum, J. G., Nelis, E., & Denys, D. (2013). Is deep brain stimulation a treatment option for anorexia nervosa? BMC Psychiatry, 13(1), 277. doi:10.1186/1471-244X-13-277

Page, K. J., Sirinathsinghji, D. J. S., & Everitt, B. J. (1995). AMPA-induced Lesions of the Basal Forebrain Differentially Affect Cholinergic and Non-cholinergic Neurons: Lesion Assessment Using Quantitative In Situ Hybridization Histochemistry. *European Journal of Neuroscience*, *7*(5), 1012–1021. doi:10.1111/j.1460-9568.1995.tb01089.x

Panagis, G., Miliaressis, E., Anagnostakis, Y., & Spyraki, C. (1995). Ventral pallidum self-stimulation: a moveable electrode mapping study. *Behavioural Brain Research*, *68*(2), 165–172. doi:10.1016/0166-4328(94)00169-G

Panagis, G., Nomikos, G., Miliaressis, E., Chergui, K., Kastellakis, A., Svensson, T., & Spyraki, C. (1997). Ventral pallidum self-stimulation induces stimulus dependent increase in c-fos expression in reward-related brain regions. *Neuroscience*, *77*(1), 175–186. doi:10.1016/S0306-4522(96)00471-X

Parent, A., Paré, D., Smith, Y., & Steriade, M. (1988). Basal forebrain cholinergic and noncholinergic projections to the thalamus and brainstem in cats and monkeys. *The Journal of comparative neurology*, *277*(2), 281–301. doi:10.1002/cne.902770209

Parker, J. A., & Bloom, S. R. (2012). Hypothalamic neuropeptides and the regulation of appetite. *Neuropharmacology*, *63*(1), 18–30. doi:10.1016/j.neuropharm.2012.02.004

Paxinos, G., & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates*. Academic Press.

Peoples, L. L., & West, M. O. (1996). Phasic Firing of Single Neurons in the Rat Nucleus Accumbens Correlated with the Timing of Intravenous Cocaine Self-Administration. *The Journal of Neuroscience*, *16*(10), 3459–3473.

Peyron, C., Petit, J.-M., Rampon, C., Jouvet, M., & Luppi, P.-H. (1997). Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience*, *82*(2), 443–468. doi:10.1016/S0306-4522(97)00268-6

Peyron, C., Tighe, D. K., Pol, A. N. van den, Lecea, L. de, Heller, H. C., Sutcliffe, J. G., & Kilduff, T. S. (1998). Neurons Containing Hypocretin (Orexin) Project to Multiple Neuronal Systems. *The Journal of Neuroscience*, *18*(23), 9996–10015.

Pulman, K. G. T., Somerville, E. M., & Clifton, P. G. (2012). Intra-Accumbens Baclofen, But Not Muscimol, Increases Second Order Instrumental Responding for Food Reward in Rats. *PLoS ONE*, *7*(7), e40057. doi:10.1371/journal.pone.0040057

Quaade, F., Yærnet, K., & Larsson, S. (1974). Stereotaxic stimulation and electrocoagulation of the lateral hypothalamus in obese humans. *Acta Neurochirurgica*, *30*(1-2), 111–117. doi:10.1007/BF01405759

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.

Ravussin, E., & Bogardus, C. (2000). Energy balance and weight regulation: genetics versus environment. *British Journal of Nutrition*, *83*(Supplement S1), S17–S20. doi:10.1017/S0007114500000908

Reynolds, S. M., & Berridge, K. C. (2001). Fear and Feeding in the Nucleus Accumbens Shell: Rostrocaudal Segregation of GABA-Elicited Defensive Behavior Versus Eating Behavior. *The Journal of Neuroscience*, *21*(9), 3261–3270.

Reynolds, S. M., & Berridge, K. C. (2002). Positive and Negative Motivation in Nucleus Accumbens Shell: Bivalent Rostrocaudal Gradients for GABA-Elicited Eating, Taste "Liking"/"Disliking" Reactions, Place Preference/Avoidance, and Fear. *The Journal of Neuroscience*, *22*(16), 7308–7320.

Rivero-Covelo, I., Salija, I., Wirtshafter, D., & Stratford, T. R., (2012). Accumbens shell and medial ventral pallidum increase ingestive behavior but not palatability: a microstructural study. New Orleans, LA: *Society for Neuroscience Annual Meeting.*

Rivero-Covelo I, Wirtshafter D, Stratford TR., (2013). Unilateral activation of the medial ventral pallidum using NMDA induces feeding and Fos expression in the lateral hypothalamus. San Diego, CA*: Society for Neuroscience Annual Meeting*.

Roberts, K. A., Krebs, L. T., Kramár, E., Shaffer, M. J., Harding, J. W., & Wright, J. W. (1995). Autoradiographic identification of brain angiotensin IV binding sites and differential c-Fos expression following intracerebroventricular injection of angiotensin II and IV in rats. *Brain Research*, *682*(1–2), 13–21. doi:10.1016/0006-8993(95)00289-3

Roitman, M. F., Wheeler, R. A., & Carelli, R. M. (2005). Nucleus Accumbens Neurons Are Innately Tuned for Rewarding and Aversive Taste Stimuli, Encode Their Predictors, and Are Linked to Motor Output. *Neuron*, *45*(4), 587–597. doi:10.1016/j.neuron.2004.12.055

Roitman, M. F., Wheeler, R. A., Wightman, R. M., & Carelli, R. M. (2008). Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. *Nature Neuroscience*, *11*(12), 1376–1377. doi:10.1038/nn.2219

Roy, M., David, N. K., Danao, J. V., Baribault, H., Tian, H., & Giorgetti, M. (2005). Genetic Inactivation of Melanin-Concentrating Hormone Receptor Subtype 1 (MCHR1) in Mice Exerts Anxiolytic-Like Behavioral Effects. *Neuropsychopharmacology*, *31*(1), 112–120. doi:10.1038/sj.npp.1300805

Ryabinin, A. E., Wang, Y.-M., Bachtell, R. K., Kinney, A. E., Grubb, M. C., & Mark, G. P. (2000). Cocaine- and alcohol-mediated expression of inducible transcription factors is blocked by pentobarbital anesthesia. *Brain Research*, *877*(2), 251–261. doi:10.1016/S0006-8993(00)02681-0

Sagar, S. M., Sharp, F. R., & Curran, T. (1988). Expression of c-fos Protein in Brain: Metabolic Mapping at the Cellular Level. *Science*, *240*(4857), 1328–1331.

Saito, Y., Cheng, M., Leslie, F. M., & Civelli, O. (2001). Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. *The Journal of Comparative Neurology*, *435*(1), 26–40. doi:10.1002/cne.1191

Salamone, J. D., Correa, M., Farrar, A., & Mingote, S. M. (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology*, *191*(3), 461–482. doi:10.1007/s00213-006-0668-9

Saper, C. B., Chou, T. C., & Elmquist, J. K. (2002). The Need to Feed: Homeostatic and Hedonic Control of Eating. *Neuron*, *36*(2), 199–211. doi:10.1016/S0896-6273(02)00969-8

Saper, C. B., Swanson, L. W., & Cowan, W. M. (1979). An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. *The Journal of comparative neurology*, *183*(4), 689–706. doi:10.1002/cne.901830402

Schulkin, J. (1991). *Sodium Hunger: The Search for a Salty Taste*. Cambridge University Press.

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *The Journal of comparative neurology*, *290*(2), 213–242. doi:10.1002/cne.902900205

Sesack, S. R., & Grace, A. A. (2010). Cortico-Basal Ganglia Reward Network: Microcircuitry. *Neuropsychopharmacology*, *35*(1), 27–47. doi:10.1038/npp.2009.93

Shimura, T., Imaoka, H., & Yamamoto, T. (2006). Neurochemical modulation of ingestive behavior in the ventral pallidum. *European Journal of Neuroscience*, *23*(6), 1596–1604. doi:10.1111/j.1460-9568.2006.04689.x

Shor-Posner, G., Grinker, J. A., Marinescu, C., Brown, O., & Leibowitz, S. F. (1986). Hypothalamic serotonin in the control of meal patterns and macronutrient selection. *Brain Research Bulletin*, *17*(5), 663–671.

Silveira, M. C. L., Sandner, G., & Graeff, F. G. (1993). Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. *Behavioural Brain Research*, *56*(1), 115–118. doi:10.1016/0166-4328(93)90028-O

Simerly,R. B. (1995). The Rat Nervous System second edition. *Academic Press Inc*. Anatomical Substrates of Hypothalamic Integration; pp. 353–376.

Simmons, W. K., Rapuano, K. M., Ingeholm, J. E., Avery, J., Kallman, S., Hall, K. D., & Martin, A. (n.d.). The ventral pallidum and orbitofrontal cortex support food pleasantness inferences. *Brain Structure and Function*, 1–11. doi:10.1007/s00429-013-0511-0

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behavioural Brain Research*, *196*(2), 155–167. doi:10.1016/j.bbr.2008.09.038

Smith, K. S., & Berridge, K. C. (2005). The Ventral Pallidum and Hedonic Reward: Neurochemical Maps of Sucrose “Liking” and Food Intake. *The Journal of Neuroscience*, *25*(38), 8637–8649. doi:10.1523/JNEUROSCI.1902-05.2005

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(7), 1594–1605. doi:10.1523/JNEUROSCI.4205-06.2007

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behavioural Brain Research*, *196*(2), 155–167. doi:10.1016/j.bbr.2008.09.038

Speakman, J. R. (2008). Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the “drifty gene” hypothesis. *International Journal of Obesity*, *32*(11), 1611–1617. doi:10.1038/ijo.2008.161

Stanley, B. G., Anderson, K. C., Grayson, M. H., & Leibowitz, S. F. (1989). Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. *Physiology & Behavior*, *46*(2), 173–177. doi:10.1016/0031-9384(89)90251-5

Stanley, B. G., Chin, A. S., & Leibowitz, S. F. (1985). Feeding and drinking elicited by central injection of neuropeptide Y: Evidence for a hypothalamic site(s) of action. *Brain Research Bulletin*, *14*(6), 521–524. doi:10.1016/0361-9230(85)90100-5

Stanley, B. G., Magdalin, W., Seirafi, A., Thomas, W. J., & Leibowitz, S. F. (1993). The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). *Brain Research*, *604*(1–2), 304–317. doi:10.1016/0006-8993(93)90382-W

Stanley, B. G., Urstadt, K. R., Charles, J. R., & Kee, T. (2011). Glutamate and GABA in lateral hypothalamic mechanisms controlling food intake. *Physiology & Behavior*, *104*(1), 40–46. doi:10.1016/j.physbeh.2011.04.046

Stellar, E. (1954). The physiology of motivation. *Psychological Review*, *61*(1), 5–22. doi:10.1037/h0060347

Stoeckel, L. E., Weller, R. E., Cook III, E. W., Twieg, D. B., Knowlton, R. C., & Cox, J. E. (2008). Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *NeuroImage*, *41*(2), 636–647. doi:10.1016/j.neuroimage.2008.02.031

Stratford, T. R. (2005). Activation of feeding-related neural circuitry after unilateral injections of muscimol into the nucleus accumbens shell. *Brain Research*, *1048*(1-2), 241–250. doi:10.1016/j.brainres.2005.05.002

Stratford, T. R. (2007). Chapter 3 - The Nucleus Accumbens Shell as a Model of Integrative Subcortical Forebrain Systems Regulating Food Intake. In T. C. Kirkham & S. J. Cooper

(Eds.), *Appetite and Body Weight* (pp. 27–65). Burlington: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123706331500037

Stratford, T. R., & Kelley, A. E. (1997). GABA in the Nucleus Accumbens Shell Participates in the Central Regulation of Feeding Behavior. *The Journal of Neuroscience*, *17*(11), 4434–4440.

Stratford, T. R., & Kelley, A. E. (1999). Evidence of a Functional Relationship between the Nucleus Accumbens Shell and Lateral Hypothalamus Subserving the Control of Feeding Behavior. *The Journal of Neuroscience*, *19*(24), 11040–11048.

Stratford, T. R., Kelley, A. E., & Simansky, K. J. (1999). Blockade of GABAA receptors in the medial ventral pallidum elicits feeding in satiated rats. *Brain Research*, *825*(1-2), 199–203.

Stratford, T. R., Swanson, C. J., & Kelley, A. (1998). Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behavioural Brain Research*, *93*(1–2), 43–50. doi:10.1016/S0166-4328(97)00140-X

Stratford, T. R., & Wirtshafter, D. (1990). Ascending dopaminergic projections from the dorsal raphe nucleus in the rat. *Brain Research*, *511*(1), 173–176. doi:10.1016/0006-8993(90)90239-8

Stratford, T. R., & Wirtshafter, D. (2004). NPY mediates the feeding elicited by muscimol injections into the nucleus accumbens shell. *Neuroreport*, *15*(17), 2673–2676.

Stratford, T. R., & Wirtshafter, D. (2012). Evidence that the nucleus accumbens shell, ventral pallidum, and lateral hypothalamus are components of a lateralized feeding circuit. *Behavioural Brain Research*, *226*(2), 548–554. doi:10.1016/j.bbr.2011.10.014

Stratford, T. R., & Wirtshafter, D. (2013). Lateral hypothalamic involvement in feeding elicited from the ventral pallidum. *European Journal of Neuroscience*, *37*(4), 648–653. doi:10.1111/ejn.12077

Stroebe, W., Papies, E. K., & Aarts, H. (2008). From Homeostatic to Hedonic Theories of Eating: Self-Regulatory Failure in Food-Rich Environments. Applied Psychology, 57(s1), 172–193. doi:10.1111/j.1464-0597.2008.00360.x

Taha, S. A., Katsuura, Y., Noorvash, D., Seroussi, A., & Fields, H. L. (2009). Convergent, not serial, striatal and pallidal circuits regulate opioid-induced food intake. *Neuroscience*, *161*(3), 718–733. doi:10.1016/j.neuroscience.2009.03.057

Tempel, D. L., Leibowitz, K. J., & Leibowitz, S. F. (1988). Effects of PVN galanin on macronutrient selection. *Peptides*, *9*(2), 309–314. doi:10.1016/0196-9781(88)90265-3

Tindell, A. J., Smith, K. S., Peciña, S., Berridge, K. C., & Aldridge, J. W. (2006). Ventral Pallidum Firing Codes Hedonic Reward: When a Bad Taste Turns Good. *Journal of Neurophysiology*, *96*(5), 2399–2409. doi:10.1152/jn.00576.2006

Tripathi, A., Prensa, L., & Mengual, E. (2013). Axonal branching patterns of ventral pallidal neurons in the rat. *Brain Structure and Function*, *218*(5), 1133–1157. doi:10.1007/s00429-012-0451-0

Turner, M., Gray, T., Mickiewicz, A., & Napier, T. (2008). Fos expression following activation of the ventral pallidum in normal rats and in a model of Parkinson's Disease: implications for limbic system and basal ganglia interactions. *Brain Structure and Function*, *213*(1), 197–213. doi:10.1007/s00429-008-0190-4

Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M. B., Meeran, K., Bloom, S. R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *, Published online: 04 January 1996; | doi:10.1038/379069a0*, *379*(6560), 69–72. doi:10.1038/379069a0

Urstadt, K. R., Coop, S. H., Banuelos, B. D., & Stanley, B. G. (2013). Behaviorally specific versus non-specific suppression of accumbens shell-mediated feeding by ipsilateral versus bilateral inhibition of the lateral hypothalamus. *Behavioural Brain Research*, *257*, 230–241. doi:10.1016/j.bbr.2013.09.048

Urstadt K, Stanley BG. (2013) Anatomical evidence that the ventral pallidum interconnects the accumbens shell and the lateral hypothalamus. San Diego, CA: *Society for Neuroscience Annual Meeting*.

Valassi, E., Scacchi, M., & Cavagnini, F. (2008). Neuroendocrine control of food intake. *Nutrition, Metabolism and Cardiovascular Diseases*, *18*(2), 158–168. doi:10.1016/j.numecd.2007.06.004

Vertes, R. P. (1991). A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *The Journal of comparative neurology*, *313*(4), 643–668. doi:10.1002/cne.903130409

Vilberg, T. R., & Keesey, R. E. (1990). Ventromedial hypothalamic lesions abolish compensatory reduction in energy expenditure to weight loss. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *258*(2), R476–R480.

Vives, F., & Mogenson, G. J. (1985). Electrophysiological evidence that the mediodorsal nucleus of the thalamus is a relay between the ventral pallidum and the medial prefrontal cortex in the rat. *Brain Research*, *344*(2), 329–337. doi:10.1016/0006-8993(85)90811-X

Volkow, N. D., & Wise, R. A. (2005). How can drug addiction help us understand obesity? *Nature Neuroscience*, *8*(5), 555–560. doi:10.1038/nn1452

Walaas, I., & Fonnum, F. (1979). The distribution and origin of glutamate decarboxylase and choline acetyltransferase in ventral pallidum and other basal forebrain regions. *Brain Research*, *177*(2), 325–336. doi:10.1016/0006-8993(79)90783-2

Weingarten, H. P., & Elston, D. (1990). The phenomenology of food cravings. *Appetite*, *15*(3), 231–246.

Welch, C. C., Grace, M. K., Billington, C. J., & Levine, A. S. (1994). Preference and diet type affect macronutrient selection after morphine, NPY, norepinephrine, and deprivation. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *266*(2), R426–R433.

Will, M. J., Franzblau, E. B., & Kelley, A. E. (2003). Nucleus Accumbens μ-Opioids Regulate Intake of a High-Fat Diet via Activation of a Distributed Brain Network. *The Journal of Neuroscience*, *23*(7), 2882–2888.

Willette, A. A., & Kapogiannis, D. (2014). Does the brain shrink as the waist expands? *Ageing Research Reviews*. doi:10.1016/j.arr.2014.03.007

Wirtshafter, D., Covelo, I. R., Salija, I., & Stratford, T. R. (2012). Effects of Muscimol in the Nucleus Accumbens Shell on Salt Appetite and Sucrose Intake: A Microstructural Study With a Comment on the Sensitization of Salt Intake. *Behavioral Neuroscience*. doi:http://dx.doi.org/10.1037/a0029641

Wirtshafter, D., & Stratford, T. R. (2010). Evidence for motivational effects elicited by activation of GABA-A or dopamine receptors in the nucleus accumbens shell. *Pharmacology Biochemistry and Behavior*, *96*(3), 342–346. doi:10.1016/j.pbb.2010.06.004

Woods, S. C., D'Alessio, D. A., Tso, P., Rushing, P. A., Clegg, D. J., Benoit, S. C., Seeley, R. J. (2004). Consumption of a high-fat diet alters the homeostatic regulation of energy balance. Physiology & Behavior, 83(4), 573–578. doi:10.1016/j.physbeh.2004.07.026

Wu, H., Van Dyck-Lippens, P. J., Santegoeds, R., van Kuyck, K., Gabriëls, L., Lin, G., … Nuttin, B. (2013). Deep-Brain Stimulation for Anorexia Nervosa. World Neurosurgery, 80(3–4), S29.e1–S29.e10. doi:10.1016/j.wneu.2012.06.039

Yang, C. R., & Mogenson, G. J. (1985). An electrophysiological study of the neural projections from the hippocampus to the ventral pallidum and the subpallidal areas by way of the nucleus accumbens. *Neuroscience*, *15*(4), 1015–1024. doi:10.1016/0306-4522(85)90250-7

Yang, S.-C., Shieh, K.-R., & Li, H.-Y. (2005). Cocaine- and amphetamine-regulated transcript in the nucleus accumbens participates in the regulation of feeding behavior in rats. *Neuroscience*, *133*(3), 841–851. doi:10.1016/j.neuroscience.2005.03.023

Yeomans, J. S., & Pollard, B. A. (1993). Amygdala efferents mediating electrically evoked startle-like responses and fear potentiation of acoustic startle. *Behavioral neuroscience*, *107*(4), 596–610.

Yokum, S., Ng, J., & Stice, E. (2011). Attentional Bias to Food Images Associated With Elevated Weight and Future Weight Gain: An fMRI Study. *Obesity*, *19*(9), 1775–1783. doi:10.1038/oby.2011.168

Záborszky, L., Alheid, G. F., Beinfeld, M. C., Eiden, L. E., Heimer, L., & Palkovits, M. (1985). Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. Neuroscience, 14(2), 427–453.

Záborszky, L., Léránth, C., & Heimer, L. (1984). Ultrastructural evidence of amygdalofugal axons terminating on cholinergic cells of the rostral forebrain. *Neuroscience Letters*, *52*(3), 219–225. doi:10.1016/0304-3940(84)90165-4

Zahm, D. S. (1989). The ventral striatopallidal parts of the basal ganglia in the rat—II. Compartmentation of ventral pallidal efferents. *Neuroscience*, *30*(1), 33–50. doi:10.1016/0306-4522(89)90351-5

Zahm, D. S., & Brog, J. S. (1992). On the significance of subterritories in the “accumbens” part of the rat ventral striatum. *Neuroscience*, *50*(4), 751–767. doi:10.1016/0306-4522(92)90202-D

Zahm, D. S., & Heimer, L. (1990). Two transpallidal pathways originating in the rat nucleus accumbens. *The Journal of comparative neurology*, *302*(3), 437–446. doi:10.1002/cne.903020302

Zahm, D. S., Schwartz, Z. M., Lavezzi, H. N., Yetnikoff, L., & Parsley, K. P. (2013). Comparison of the locomotor-activating effects of bicuculline infusions into the preoptic area and ventral pallidum. *Brain Structure and Function*, 1–16. doi:10.1007/s00429-013-0514-x

Zahm, D. S., Williams, E., & Wohltmann, C. (1996). Ventral striatopallidothalamic projection: IV. Relative involvements of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. *The Journal of comparative neurology*, *364*(2), 340–362. doi:10.1002/(SICI)1096-9861(19960108)364:2<340::AID-CNE11>3.0.CO;2-T

Zahm, D. S., Zaborszky, L., Alones, V. E., & Heimer, L. (1985). Evidence for the coexistence of glutamate decarboxylase and Met-enkephalin immunoreactivities in axon terminals of rat ventral pallidum. *Brain Research*, *325*(1–2), 317–321. doi:10.1016/0006-8993(85)90331-2

Zhang, M., Gosnell, B. A., & Kelley, A. E. (1998). Intake of High-Fat Food Is Selectively Enhanced by MuOpioid Receptor Stimulation within the Nucleus Accumbens. *Journal of Pharmacology and Experimental Therapeutics*, *285*(2), 908–914.

Zhang, M., & Kelley, A. E. (2000). Enhanced intake of high-fat food following striatal mu-opioid stimulation: microinjection mapping and fos expression. *Neuroscience*, *99*(2), 267–277.

Zheng, H., & Berthoud, H.-R. (2007). Eating for pleasure or calories. *Current Opinion in Pharmacology*, *7*(6), 607–612. doi:10.1016/j.coph.2007.10.011

Zheng, H., Corkern, M., Stoyanova, I., Patterson, L. M., Tian, R., & Berthoud, H.-R. (2003). Appetite-inducing accumbens manipulation activates hypothalamic orexin neurons and inhibits POMC neurons. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *284*(6), R1436–R1444. doi:10.1152/ajpregu.00781.2002

Zheng, H., Patterson, L. M., Phifer, C. B., & Berthoud, H.-R. (2005). Brain stem melanocortinergic modulation of meal size and identification of hypothalamic POMC projections. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *289*(1), R247–R258. doi:10.1152/ajpregu.00869.2004

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APPENDIX B

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

Office of Animal Care and
Institutional Biosafety Committees (MC 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

August 22, 2013

Robert David Wirtshafter
Psychology
M/C 285

Dear Dr. Wirtshafter:

The protocol indicated below was reviewed at a convened ACC meeting in accordance with the Animal Care Policies of the University of Illinois at Chicago on **6/18/2013**. *The protocol was not initiated until final clarifications were reviewed and approved on* **8/13/2013.** *The protocol is approved for a period of* ***3*** years with annual continuation.

Title of Application: Macronutrient Studies of Accumbal and Pallidal Feeding

ACC Number: 13-099

Initial Approval Period: 8/13/2013 to 6/18/2014

Current Funding: ***Currently protocol NOT matched to specific funding source. Modification will need to be submitted prior to Just in time or acceptance of award to match protocol to external funding source. All animal work proposed in the funding application must be covered by an approved protocol. UIC is the only performance site currently approved for this protocol.***

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare (OLAW), NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the funding proposal are matched to this ACC protocol.**

In addition, all investigators are responsible for ensuring compliance with all federal and institutional policies and regulations related to use of animals under this protocol and the funding sources listed on this protocol. Please use OLAW's "*What Investigators Need to Know about the Use of Animals*" (http://grants.nih.gov/grants/olaw/InvestigatorsNeed2Know.pdf) as a reference guide. Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM/*ss*
cc: BRL, ACC File, Thomas Stratford

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Office of Animal Care and
Institutional Biosafety Committees (MC 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

February 28, 2013

Thomas Stratford
Psychology
M/C 285

Dear Dr. Stratford:

The protocol indicated below was reviewed at a convened ACC meeting in accordance with the Animal Care Policies of the University of Illinois at Chicago on **2/19/2013**. *The protocol was not initiated until final clarifications were reviewed and approved on* **2/28/2013**. *The protocol is approved for a period of* ***3*** years with annual continuation.

Title of Application: Nucleus Accumbens-Mediated Feeding: Output Pathways

ACC Number: 13-013

Initial Approval Period: 2/28/2013 to 2/19/2014

Current Funding: ***Currently protocol NOT matched to specific funding source. Modification will need to be submitted prior to Just in time or acceptance of award to match protocol to external funding source. All animal work proposed in the funding application must be covered by an approved protocol. UIC is the only performance site currently approved for this protocol.***

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare (OLAW), NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the funding proposal are matched to this ACC protocol.**

In addition, all investigators are responsible for ensuring compliance with all federal and institutional policies and regulations related to use of animals under this protocol and the funding sources listed on this protocol. Please use OLAW's "*What Investigators Need to Know about the Use of Animals*" (http://grants.nih.gov/grants/olaw/InvestigatorsNeed2Know.pdf) as a reference guide. Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM/*ss*
cc: BRL, ACC File, Robert David Wirtshafter

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VITA

University of Illinois at Chicago
Graduate Program in Neuroscience

Ignacio Rivero Covelo

Education

University of Illinois at Chicago 2014
Ph.D. in Neuroscience

Universitat de Barcelona, Catalunya, Spain 2008
Postgraduate Course in Clinical Psychopathology

Universidade de Santiago de Compostela, Galiza, Spain 2006
DEA (Master's degree) in Neuroscience

Universidade de Santiago de Compostela, Galiza, Spain 2004
Licenciatura (Bachelor's degree) in Psychology

Awards & Honors

Sole UIC's representative for the SfN Chicago chapter meeting. 2014

Graduate Program in Neuroscience student presenter award. 2013
Department of Psychology travel award. 2012

Graduate College student presenter award. 2012
Pre-doctoral fellowship, "Neuroscience of Mental Health" 2011- 2012
(T32MH067631 – PI: Mark Rasenick).
Graduate Program in Neuroscience Fellowship. 2009-2010

Research Experience

Drs. Wirtshafter & Stratford Lab, Chicago, IL Spring 2010 – Present
Graduate Student. Studying the role of the accumbens shell and the ventral pallidum in the control of motivated behaviors using behavioral techniques, intracranial drug injections, and immunohistochemical anatomic explorations.

Dr. Guidotti Lab, Chicago, IL Spring 2007 – Summer 2009
Visiting Research Specialist in Health Science. Involved in the behavioral and metabolic characterization of mice deficit of omega 3 fatty acids. Used animal models to study the role of reelin on schizophrenia and the effect of aging and psychopathology on cerebellar Purkinje cells.

Dr. Caruncho Lab, Santiago de Compostela, Galiza, Spain Winter 2006-2007
Advanced Post-Master Training. Trained in neurobiology techniques including: immunohistochemistry and confocal microscopy.

Dr. Díaz Lab, Santiago de Compostela, Galiza, Spain 2003-2006
Master Student. Performed psychophysiological studies examining the effect of aging on cortical potentials related with hand movement and verbal articulation preparation.

Publications

Covelo I.R., Wirtshafter D., & Stratford T.R. (*In preparation*). Increased feeding and hypothalamic activation after unilateral injections of NMDA into the medial ventral pallidum.

Covelo, I.R., Patel, Z.I., Luviano, J.A., Stratford, T.R., & Wirtshafter, D. (2014). Manipulation of GABA in the ventral pallidum, but not the nucleus accumbens, induces intense, preferential, fat consumption in rats. *Behavioural Brain Research, 270*, 316–325.

Wirtshafter D., **Covelo I.R.**, Salija, I., & Stratford T.R. (2012). Effects of muscimol in the nucleus accumbens shell on salt appetite and sucrose intake: a microstructural study with a comment on the sensitization of salt intake. *Behavioral Neuroscience.* Oct;126(5):699-709

Covelo I.R., Wirtshafter D., & Stratford T.R. (2012). GABAA and dopamine receptors in the nucleus accumbens shell differentially influence performance of a water-reinforced progressive ratio task. *Pharmacolgy, Biochemistry and Behavior*. Mar;101(1):57-61

Maloku E., **Covelo I.R**., Hanbauer I., Guidotti A., Kadriu B., Hu Q., Davis J.M., & Costa E.(2010). Lower number of cerebellar Purkinje neurons in psychosis is associated with reducedreelin expression. *Proceedings of the National Academy of Sciences*. Mar 2;107(9):4407-11

Hanbauer I., **Rivero-Covelo I**., Maloku E., Baca A., Hu Q., Hibbeln J.R., & Davis J.M. (2009). The Decrease of n-3 Fatty Acid Energy Percentage in an Equicaloric Diet Fed to B6C3Fe Mice for Three Generations Elicits Obesity. *Cardiovascular Psychiatry and Neurology*. 2009, Article ID 867041

Research Abstracts

Covelo I., Wirtshafter D., & Stratford T.R. Bicuculline injections into the the medial ventral pallidum alter macronutrient selection in rats. *22nd Annual Meeting of the Society for the Study of Ingestive Behavior in Seattle, WA from July 29 - August 2, 2014*

Covelo I.R., Wirtshafter D., & Stratford T.R. Increased feeding and hypothalamic activation after unilateral injections of NMDA into the medial ventral pallidum. *43rd Annual Meeting of Society for Neuroscience, San Diego, November 9-13, 2013.*

Covelo I., Salija I., Wirtshafter D., & Stratford T.R. Accumbens shell and medial ventral pallidum increase ingestive behavior but not palatability: a microstructural study. *42nd Annual Meeting of Society for Neuroscience, New Orleans, October 12-16, 2012.*

Covelo I., Wirtshafter D., & Stratford T.R. Inactivation of the Median Raphe nucleus but not the Accumbens Shell increases breaking points on a water reinforced progressive ratio task *41st Annual Meeting of Society for Neuroscience, Washington, DC, November 12-16, 2011.*

Maloku E., **Covelo I.**, Hanbauer I., Guidotti A., Davis J.M., & Costa E. Reduced cerebellar Purkinje cell (CPC) count is associated with reelin downregulation in schizophrenia (SZ) and bipolar (BP) disorder patients. *39th Annual Meeting of Society for Neuroscience, Chicago, October 15-21, 2009.*

Covelo I., Maloku E., Davis J., Hibbeln J.R., Costa E., Guidotti A., & Hanbauer I. Alterations of behavioral reward seeking in heterozygous reeler mice and effects of n-3 fatty acid-deficient diet (n-3FADD). *38th Annual Meeting of Society for Neuroscience, Washington, DC, November 15-19, 2008.*

Maloku E., **Covelo I.**, Guidotti A., Costa E., & Hanbauer I. Studies on age-related cerebellar Purkinje cell loss and changes in open field motor activity in wild type (WT) and heterozygous reeler mice (HRM). *38th Annual Meeting of Society for Neuroscience, Washington, DC, November 15-19, 2008*

Hanbauer I., **Covelo I.**, Maloku E., Hibbeln J.R., Costa E., & Davis J. The effect of long-term n-3 fatty acid deficiency on physiology and behavior of male mice. *38th Annual Meeting of Society for Neuroscience, Washington, DC, November 15-19, 2008*

Covelo I., Lindín M., & Díaz F. Age effect on movement-related cortical potentials in a famous people face-naming task. *5th Congress of the Spanish Society of Psychophysiology (SEPF), Granada, Spain, September 28-30, 2006.*

Teaching Experience

As an instructor:

School of the Art Institute of Chicago

SCIENCE 3519-001 Neuroscience and the mind (fall 2013 & spring 2014)

As the instructor of "Neuroscience and the mind" my goal is to provide art students with enough scientific knowledge to understand essential concepts in neuroscience. In this class, we explore the form, function and dysfunction of the brain.

University of Illinois at Chicago

BIOS 386 Advanced topics in modern neuroscience (spring 2013 & 2014)

"Advanced topics in modern neuroscience" was designed by graduate neuroscience students as it is intended for undergraduate neuroscience majors. In this class we use research articles as a tool to discuss how scientific knowledge is created and how to analyze it critically. I was one of the three graduate students that designed the course (structure & syllabus) and I have taught two sessions of two hours each about neuroscience of sexual behavior.

As a teaching assistant

BIOS 483 Neuroanatomy (spring 2011, 2013 & 2014)

"Neuroanatomy" is taught by Dr. Wirtshafter, my graduate advisor, and I have collaborated as the teaching assistant. Neuroanatomy is a challenging subject to study and relies heavily on laboratory practice with specimens to understand the structure of the nervous system. In this class, I was involved in the preparation of the laboratory sessions and assisted the students as they progressed from macroscopical to microscopical samples of the brain.

PSCH 363 Laboratory in behavioral neuroscience (spring 2013)

The goal of this class is to provide a hands-on understanding of how scientific "facts" fill textbooks in physiological psychology or behavioral neuroscience. In this class the students get to conduct experiments using both non-human animal subjects and humans. As a TA, my role was to set the labs and help the students during the experiments as well as grade papers.

PSCH 351 Laboratory in perception (fall 2010 & fall 2012)

In this class the students completed four computer based lab experiments and learned how to write a scientific report using APA style guidelines. As a TA, my role was to set the labs and help the students during the experiments as well as grade papers.