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Characterizing the Role of GABA in the Paraventricular Thalamic Nucleus in the Control of Feeding and Body Weight

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ABSTRACT OF THE DISSERTATION

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The paraventricular nucleus of the thalamus (PVT) is a midline thalamic nucleus traditionally thought to play a role in nonspecific arousal given its extensive connections throughout the brain. However, there has been a recent emphasis on the role of the PVT in the control of feeding due to its associations with hypothalamic regions that integrate energy states and its projections to amygdala and ventral striatal regions implicated in reward and motivation. The purpose of the current series of experiments was to characterize the role of GABA in the PVT in relation to feeding. An introduction is presented in the first chapter summarizing what is currently known about how the PVT fits into the neurocircuitry governing feeding behavior, from an anatomical and functional point of view. Then, the following questions were addressed: 1) Do GABA receptor subtypes differentially mediate GABA-induced feeding in the PVT? 2) Are there

regional differences between the anterior (aPVT) and posterior (pPVT) subregions of the PVT? 3) Do GABA receptors in the PVT play a role in natural feeding? In Chapter 2, the PVT was inhibited by microinjections of the $GABA_A$ receptor agonist muscimol or the $GABA_B$ receptor agonist baclofen, and food intake was measured. I found that the agonist for both receptor subtypes produced dose-dependent increases in feeding in satiated rats. In Chapter 3, separate groups of rats received stereotaxic implantation of a cannula into either the aPVT or pPVT. While pPVT injections of either muscimol or baclofen elicited eating, the effect of muscimol was reduced while baclofen was ineffective when injected into the aPVT, demonstrating a regionally-specific effect. In Chapter 4, rats were fasted for 24 hr, which produced a large increase in intake upon refeeding. PVT injection of the $GABA_A$ receptor antagonist bicuculline suppressed this deprivation-induced feeding, suggesting that $GABA_A$ receptors in the PVT may mediate this form of natural feeding. Together, these studies suggest that the PVT, in particular its posterior division, is part of a specialized circuit governing feeding behavior.

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Chapter 1 - Introduction

Background

What controls food intake? The answer to that question is complex but also crucial to understanding the regulation of body weight and the processes that lead to obesity. An organism's ability to survive depends on maintaining a relatively constant internal environment in the presence of external factors that may disrupt this balance. For example, adrenalectomized rats will increase voluntary consumption of a salt solution in order to compensate for sodium deficiency, increasing their survival rate (Richter, 1936). Similarly, food intake is governed, at least in part, by the body's response to maintain energy homeostasis. Body weight is maintained when energy intake is balanced with energy expenditure, and food intake is a primary regulator of this balance. An example of this balance at play is given by the glucostatic mechanism of homeostasis, where low blood glucose, occurring spontaneously or produced by an injection of insulin, is sufficient to induce hunger and elicit feeding, thus normalizing blood glucose levels (Campfield & Smith, 1990; Langhans, 1996). However, it is clear that animals do not only eat under such deficits. Humans will eat of their own volition, influenced by factors such as time of day, availability of food and social situations. An imbalance in favor of energy intake relative to expenditure will lead to excess weight gain which, if left unchecked, can cause obesity.

Obesity is a serious health issue that affects over one-third (39.8%) of the US population (Centers for Disease Control and Prevention 2017). In addition, it is associated with increased mortality and has been linked to secondary health conditions including coronary heart disease, stroke, type 2 diabetes and several cancers (Borrell & Samuel, 2014). Obesity also puts a strain on the economy, with associated medical costs estimated to be around $315.8 billion in 2010 (Biener et al., 2017). Modern day overconsumption of readily-available, high-calorie fast foods and soft drinks is a major catalyst towards the increased prevalence of obesity, likely exacerbated by an increasingly sedentary lifestyle. Obesity arises due to a complex interaction between genetic, social and environmental factors; in turn, these factors together influence physiological brain mechanisms that control eating and body weight regulation. Despite being a major public health issue, obesity rates continue to rise. Part of this is due to an incomplete understanding of the central mechanisms involved. Continued research on the biological substrates underlying the control of food intake and body weight regulation would further our understanding of normal eating, as well as how dysregulation of these systems may lead to obesity. This understanding would also be an important step towards the development of potential treatments for disorders of eating behavior and body weight.

The hypothalamus has been a primary focus for research into the regulation of motivated behaviors, including feeding. Early evidence for a role in feeding came from studies showing that direct electrical stimulation of the lateral hypothalamus (LH)

elicited feeding in satiated animals; conversely, electrolytic lesions of the LH produced aphagia and long-term reduction in body weight (Winn et al., 1984; Wise, 1974). On the other hand, lesions of the ventromedial hypothalamus (VMH) produced hyperphagia and significant increases in body weight (Brobeck et al., 1943). These observations gave rise to the "dual-center" hypothesis, which postulated that the LH was a feeding center and the VMH a satiety center (Stellar, 1954). Over time, other studies showed that this was an oversimplification. For example, severing the connections between the LH and VMH, while leaving the regions intact, was sufficient to produce changes in appetite (Sclafani & Grossman, 1969). The current view is that there are discrete pathways which are interconnected to form a complex neural network, involving multiple brain sites that can exert control over feeding. Nevertheless, the hypothalamus remains a critical component within this circuitry. Neurons in the LH respond to the sight of food and to stimuli associated with the presentation of food or to the taste of food; the degree of change in firing rate in response to the latter was found to be inversely proportional to the hunger state of the animal (Mora et al., 1976; Rolls et al., 1980). The LH also contains cell bodies that produce hypocretin/orexin and melanin-concentrating hormone (MCH), two peptides involved in feeding that are found to originate almost exclusively in this region (Qu et al., 1996; Sakurai et al., 1998). Another region of the hypothalamus, the arcuate nucleus (ArcN), is sensitive to peripheral signals that are released in direct proportion to the amount of body fat. Specifically, insulin from pancreatic β cells and leptin from adipocytes can cross the blood-brain barrier and

interact with neurons in the ArcN (Banks, 2006). If the body senses there is sufficient fat present in the body, food intake is reduced. The ArcN also contains two cell types, expressing the neuropeptides agouti-related peptide (AgRP) or proopiomelanocortin (POMC), that have been shown to play important, and opposing, roles in food intake. AgRP neurons, which co-express the orexigenic neuropeptide Y (NPY), are activated by fasting but rapidly inhibited by the presentation of food (Y. Chen et al., 2015; T. M. Hahn et al., 1998). Using chemogenetic manipulation to activate or inhibit its activity, it was shown that AgRP neurons are both necessary and sufficient for feeding (Krashes et al., 2011). Thus, the hypothalamus can integrate extrinsic signals, including peripheral hormones and orexigenic/anorexigenic neurotransmitters and neuropeptides, to influence feeding behavior and energy metabolism.

Although the hypothalamus is important for controlling many aspects related to ingestive behavior, studies have shown that feeding control mechanisms are not exclusive to this region. This is most evident in the chronically decerebrate rat, in which a transection is made to isolate the forebrain from the caudal brainstem. Treatment with insulin, which lowers plasma glucose concentrations, increases sucrose intake in control rats as a compensatory response to low energy. Similarly, decerebrate rats increased their consumption of sucrose following an injection of insulin, demonstrating that the caudal brainstem was sufficient to sense metabolic deficiencies and respond with changes in feeding (Flynn & Grill, 1983). In another study, both intact and decerebrate rats were able to discriminate between palatable and bitter taste

substances, as determined by oral-facial response patterns (Grill & Norgren, 1978). Nevertheless, differences exist. Decerebrate rats do not eat spontaneously and must be tube-fed. In the taste reactivity test, control rats developed a taste aversion when a substance was paired with lithium chloride, but decerebrate rats did not. These studies demonstrate that some fundamental control mechanisms of ingestive behavior are restricted to the caudal brainstem, while others require an interaction with forebrain/hypothalamic structures. Among these other brain regions is the paraventricular nucleus of the thalamus which, as the focus of these studies, will be described in more detail.

PVT Anatomy

The paraventricular thalamic nucleus (PVT) has received recent attention as a structure involved in the modulation of motivated behaviors, including the regulation of appetite. It is the dorsal-most member of the midline thalamic nuclei, located just below the dorsal portion of the third ventricle and extending the anterior-posterior length of the thalamus. Although not morphologically distinct, the PVT has been divided into anterior and posterior subregions based on behavioral and anatomical studies. Neurons in the PVT are primarily excitatory, utilizing the excitatory amino acid glutamate as a neurotransmitter (Frassoni et al., 1997). Traditionally, the PVT and other midline thalamic nuclei were thought to function as relays that “nonspecifically” modulated behavior through a general effect on arousal. In recent years, it has been recognized

that individual members of the midline group innervate discrete cortical targets, and thus have the potential to regulate functionally segregated circuits controlling specific behaviors (Kirouac, 2015).

Inputs

The PVT receives projections from brainstem nuclei including the locus coeruleus and dorsal raphe nuclei, consistent with a role in general arousal (S. Chen & Su, 1990; Cornwall & Phillipson, 1988). It also receives a large input from nociceptive and visceral relay areas such as the periaqueductal gray and the nucleus of the solitary tract, as well as strong input from prefrontal cortical areas including the prelimbic, infralimbic and insular cortical areas. It has been suggested that these cortical inputs may integrate information about salient stimuli in order to guide the appropriate behavioral response. Indeed, neurons in the PVT are activated by both positive and negative stimuli (Dayas et al., 2008; Yasoshima et al., 2007). The PVT also receives forebrain input from areas of the hypothalamus, as determined by both retrograde and anterograde tract tracing studies (J. D. Hahn & Swanson, 2010; Kirouac et al., 2006). The abundance of connections from the hypothalamus is unique to the PVT among the thalamic nuclei. This input includes the LH, which sends a dense projection of orexinergic fibers to the PVT. Orexins, which excite PVT neurons, have been implicated in the regulation of sleep and arousal, food intake, reward and energy metabolism, functions that are mutually related (Ishibashi et al., 2005). Another hypothalamic area, the dorsomedial

hypothalamus, contains leptin-sensitive neurons which project to the PVT, suggesting that the PVT is part of a pathway mediating the effects of this hormone, including metabolism and energy homeostasis (Gautron et al., 2010). The PVT also has reciprocal connections from the suprachiasmatic nucleus, the major circadian pacemaker in the brain (Watts et al., 1987). Notably, the PVT is also characterized by dense innervation of peptidergic fibers originating from hypothalamic nuclei. These include the aforementioned orexin, the anorexinergic cocaine- and amphetamine-regulated transcript (CART), the satiety signal cholecystokinin, stress-related CRF and neuropeptide Y (Kirouac et al., 2005, 2006; Lee et al., 2015; Otake, 2005; Otake & Nakamura, 1995). Interestingly, it was found that PVT neurons projecting to the nucleus accumbens receive converging NPY/CART inputs, suggesting that the PVT integrates antagonistically-acting, feeding-related inputs to cortical reward circuit (Lee et al., 2015). The functional significance of this diversity within the PVT is yet to be fully explored, but clearly implicates the PVT in feeding, arousal, reward and energy balance. Together, the PVT appears to be active during periods of high arousal or in the context of salient stimuli, and in turn projects to brain areas that can influence multiple circuits that control behavior.

Outputs

The PVT projects strongly to limbic areas including the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (Li &

Kirouac, 2008). The NAc is traditionally associated with hedonic and appetitive behaviors. It receives dopaminergic innervation and has been implicated in the rewarding effects of drugs of abuse including cocaine, opiates and alcohol (Di Chiara & Imperato, 1985; Olds, 1982; Zito et al., 1985). The NAc can be subdivided into two distinct regions, the core and the shell (Zahm & Brog, 1992). The core connects to classic basal ganglia structures such as the ventral pallidum and substantia nigra, while the shell connects to limbic regions including the hypothalamus and ventral tegmental area. Thus, the core has been associated with motor systems and goal-directed actions, while the shell has been associated with incentive salience, or the "wanting" of stimuli (Day et al., 2011). The PVT may influence these behaviors through its connections to the NAc, as retrograde tracing has shown that the PVT projects to both the core and shell regions (T. M. Hahn et al., 1998). The PVT is also able to modulate dopamine (DA) release in the NAc, as demonstrated by close apposition between glutamatergic terminals from PVT neurons and dopamine axons projecting to the NAc. Additionally, electrical stimulation of the PVT increased DA levels in the NAc, an effect that appears to be independent of VTA DA neuron firing (Parsons et al., 2007). Thus, the PVT may mediate motivational behaviors such as reward and appetite through its connections with the NAc. Anterograde labeling studies have also reported dense innervation of the extended amygdala, including the BNST and central nucleus of the amygdala, regions that have been implicated in emotional functions such as fear, anxiety and reward (Li & Kirouac, 2008; Moga et al., 1995). These PVT fibers were found to overlap with CRF neurons in

the extended amygdala which are known to mediate anxiety (T. M. Hahn et al., 1998). As previously mentioned, the PVT receives one of the densest projections of orexin fibers, and it can also regulate stress through these inputs. Studies have also indicated that the PVT can simultaneously influence multiple subcortical targets by sending collaterals to additional areas, thus influencing behavior by a coordinated response (Dong et al., 2017).

Functional circuits other than feeding

Studies have shown how these various inputs and outputs put the PVT in position to influence a variety of motivated behaviors. As previously mentioned, the PVT and the SCN are reciprocally connected. Thus, the PVT may relay circadian information to other areas of the forebrain. Some of this activity involves non-photic signals. For example, food entrainment via a restricted feeding schedule produces food anticipatory activity, and this phenomenon is associated with an increase in c-Fos immunoreactivity in corticolimbic structures, including the PVT (Angeles-Castellanos et al., 2007; Nakahara et al., 2004). The rhythmic expression of the clock gene PER1 in the PVT was shifted in rats in concert with a daily palatable meal (Mendoza et al., 2005). PVT c-Fos expression was also increased by a light stimulus signaling delivery of a saccharin solution (Igelstrom et al., 2010; Nakahara et al., 2004). These studies show that the PVT is activated by signals predicting the availability or timing of food. Increasing evidence also points to the PVT as being part of the addiction circuitry, from the acute reinforcing

effects of certain drugs to relapse, given its connections to relevant brain regions including the prefrontal cortex, nucleus accumbens and amygdala. Fos immunoreactivity was measured in PVT neurons retrogradely labeled from the NAc in rats pretreated with amphetamine; PVT neurons were similarly found to be recruited by acute treatment with morphine, cocaine and THC (Allen et al., 2003; Deutch et al., 1998; Gutstein et al., 1998). Inactivation of the PVT has been shown to prevent cocaine-induced locomotor sensitization and the expression of cocaine conditioned place preference (Browning et al., 2014; Young & Deutch, 1998). Context-induced reinstatement for alcohol was abolished by excitotoxic lesions of the PVT (Hamlin et al., 2009). Rats trained to drink ethanol, or exposed to ethanol via oral gavage, showed increased c-Fos activation in the PVT (Barson et al., 2015). An acute injection of nicotine was also able to activate orexin neurons projecting to the PVT (Pasumarthi & Fadel, 2008). Retrograde tracers injected into the NAc combined with immunohistochemistry showed that PVT neurons projecting to the NAc overlapped with orexin and CART staining (Kirouac et al., 2006). Direct injection of orexin-A increased accumbal dopamine levels, an effect that appears to be independent of VTA involvement (Choi et al., 2012). Projections to the extended amygdala support a role for the PVT in behavioral responses associated with fear and anxiety. The PVT is activated by both psychological and physical stressors, including conditioned fear, footshock and air puff (Beck & Fibiger, 1995; Bubser & Deutch, 1999; Spencer et al., 2004). Activation of the PVT in depressive-like behavior was shown to involve transfer of information to the central amygdala (Zhu et al., 2011). Selective

inhibition of PVT neurons projecting to the central amygdala prevented fear conditioning (Penzo et al., 2015). Together, these findings suggest that the PVT may integrate emotionally associated information from multiple forebrain areas and relay that information to limbic areas in order to carry out the appropriate behavioral response.

Role in feeding

There is also increasing support for the PVT in the control of feeding or feeding-related reward behavior. Lesions of the PVT, or inactivation of the PVT with the $GABA_A$ receptor agonist muscimol, increased food intake (Bhatnagar & Dallman, 1999; Stratford & Wirtshafter, 2013). PVT neurons are activated by refeeding following a period of deprivation or in the presence of cues signaling palatable food (Choi et al., 2010; Timofeeva & Richard, 2001), and this in part involves orexin signaling, as OX1R knockdown attenuated consumption of a high-fat diet (Choi et al., 2012). Numerous studies have begun to elucidate the functional circuits and neuropeptides involved in PVT regulation of appetite. Activation of glucagon-like peptide-1 (GLP-1) receptors in the PVT reduced food intake. GLP-1 innervation arises from preproglucagon neurons in the NTS, and these neurons are activated by food intake. Finally, activation of GLP-1R in the PVT reduced excitation of PVT to NAc-projecting neurons (Ong et al., 2017). Optogenetic activation of a population of glucose-sensitive, glutamatergic neurons within the PVT that project to the NAc increased operant responses for a sucrose solution (Labouebe et

al., 2016). Circuit mapping also found the PVT to be intermediate in the pathway of AgRP neurons to the insular cortex, which is required for mediating behavior in response to anticipatory cues (Livneh et al., 2017). Specifically, mice were trained to lick in response to a visual cue predicting delivery of a palatable food reward. Behavioral responses to a food cue were abolished by satiation but could be restored by selective activation of AgRP neurons. It was discovered that AgRP neurons project indirectly to the insular cortex by synapsing onto and inhibiting PVT neurons that project to the basolateral amygdala (Livneh et al., 2017). Furthermore, optogenetic stimulation of a specific subpopulation of AgRP to PVT projection neurons was sufficient to induce feeding (Betley et al., 2013). Thus, the PVT appears to be anatomically situated to integrate multiple signals that can influence feeding.

Feeding: GABA and glutamate

Gamma-aminobutyric acid (GABA) and glutamate, the most abundant inhibitory and excitatory neurotransmitters, respectively, in the mammalian brain, have been shown to regulate feeding. For example, injection of glutamate or glutamate receptor agonists, or a $GABA_A$ receptor antagonist, into the LH elicits food intake in satiated rats (Stanley et al., 1993; Turenius et al., 2009). A microdialysis study showed that, in food-deprived rats, initiation of a meal is accompanied by a rise in LH glutamate levels which then progressively falls to below baseline levels over the course of the meal; conversely, GABA levels increase and continue to rise until they peak during the last third of the

meal (Rada et al., 2003). Inhibition of neurons in the AcbSh, via a GABA agonist or blockade of AMPA receptors, also elicits intense feeding in satiated rats (Stratford et al., 1998). These studies support the idea that changes in GABA signaling within specific brain regions can influence food intake. Although the PVT is largely devoid of interneurons, it displays a high density of GABA-immunoreactive fibers and boutons, suggesting that GABAergic input from other brain sites strongly influences PVT neuron activity. Therefore, it is likely that GABA plays a direct role in PVT-mediated feeding. Recent evidence supports this idea. It was discovered that some of this input originates from the zona incerta (ZI), as optogenetic stimulation of ZI GABA neurons projecting to the PVT induced rapid, binge-like eating while intermittent stimulation over a 2 week period increased body weight gain (Zhang & van den Pol, 2017). Direct injection of the $GABA_A$ agonist muscimol into the PVT, but not the adjacent mediodorsal nuclei, also increased food intake (Stratford & Wirtshafter, 2013). GABA can act on two receptor subtypes, ionotropic $GABA_A$ receptors and G-protein-coupled metabotropic $GABA_B$ receptors. A specific role for PVT $GABA_B$ receptors in the control of feeding has not been directly addressed. These receptors are widely expressed in the thalamus, including the PVT, and thus deserve further attention for a potential role in modulating feeding (Margeta-Mitrovic et al., 1999).

Overall, my proposed studies will further characterize GABA-mediated feeding in the PVT. The following questions will be addressed: 1) Are GABA receptor subtypes A and B both involved in GABA-induced feeding in the PVT? 2) Do the anterior and

posterior subregions of the PVT differentially regulate feeding? And 3) Do GABA receptors in the PVT play a role in natural feeding? These experiments will be conducted using adult male Sprague-Dawley rats and require the implantation of a guide cannula to allow injection of GABA agonists or antagonists directly into the PVT. Together, the proposed studies will shed additional light not only on how GABA in the PVT is involved in the central regulation of food intake, but also how the PVT fits into the neurocircuitry governing a complex behavior such as feeding.

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Chapter 2: The role of GABA receptor subtypes in feeding induced by GABA in the PVT

Abstract

The paraventricular thalamus (PVT) is a midline thalamic nucleus uniquely situated to integrate brainstem/hypothalamic inputs providing homeostatic and visceral information, which projects to limbic areas such as the amygdala and nucleus accumbens, areas known to modulate behaviors related to motivation, feeding and reward. The current study aimed to characterize the involvement of PVT GABA receptors in feeding behavior. To accomplish this, adult male Sprague-Dawley rats were stereotaxically implanted with a guide cannula aimed at the PVT to allow direct infusion of drugs into the target region. I found that injection of the $GABA_A$ agonist muscimol into the PVT elicited statistically significant feeding in satiated rats, consistent with previous findings demonstrating a feeding-stimulatory effect after activation of this receptor subtype. More specifically, the 300 ng dose of muscimol elicited intake of 10.0 g of food 60 min postinjection. Similarly, the $GABA_B$ agonist baclofen produced a consistent and dose-dependent feeding response, with rats consuming 4.0 g following the highest 100 ng dose. These findings are consistent with the notion that the PVT serves an important role in the regulation of feeding and highlight the involvement of both $GABA_A$ and $GABA_B$ receptors.

Introduction

In trying to better understand the brain circuitry underlying the control of food intake, research has looked beyond well-characterized areas such as the hypothalamus. The paraventricular nucleus of the thalamus (PVT) has emerged as one such candidate. The PVT receives inputs from brainstem and hypothalamic regions and in turn projects to limbic areas including the nucleus accumbens and amygdala. Therefore, the PVT is anatomically situated in a unique position to influence many aspects of behavior, including feeding. Several functional studies link the PVT to food intake. For example, PVT c-Fos expression increases before expected meal availability and in contexts signaling palatable foods (Nakahara et al., 2004). In addition, PVT lesions increase food intake and body weight (Bhatnagar & Dallman, 1999). Recent studies have supported the PVT as a discrete region that can evoke feeding. Optogenetic stimulation of a specific subpopulation of hypothalamic arcuate nucleus AgRP to PVT projection neurons was sufficient to induce feeding (Betley et al., 2013). Conversely, activation of glucagon-like peptide-1 (GLP-1) receptors in the PVT reduced food intake (Ong et al., 2017).

Glutamate and gamma-Aminobutyric Acid (GABA) are the primary excitatory and inhibitory amino acid neurotransmitters, respectively, in the mammalian brain. Prior research in our laboratory has examined the actions of these neurotransmitters in the lateral hypothalamus (LH) and their effects on feeding. Direct injection of glutamate or its NMDA or AMPA/kainate receptor agonists, or a $GABA_A$ receptor antagonist, elicited

feeding in rats (Stanley et al., 1993; Turenius et al., 2009). Moreover, in vivo microdialysis demonstrated that the onset of a meal coincided with an increase in lateral hypothalamic glutamate levels in food-deprived rats which returned to baseline following meal termination (Rada et al., 2003). These experiments show that the balance of GABA and glutamate may be an important regulator of feeding, and that changes in neuronal activity in discrete brain regions mediated by these neurotransmitter systems is capable of directly influencing food intake.

A direct role for the PVT in the control of food intake was suggested by evidence that muscimol, a $GABA_A$ receptor agonist, dose-dependently increased food intake in non-deprived rats (Stratford & Wirtshafter, 2013). The PVT also contains a high density of $GABA_B$ receptors (Margeta-Mitrovic et al., 1999). Notably, the role of these receptors in the PVT in food intake remains largely unexplored. Whereas $GABA_A$ receptors are ionotropic receptors coupled to chloride ion channels to mediate fast postsynaptic inhibition, $GABA_B$ receptors are metabotropic receptors negatively coupled to adenylyl cyclase through an inhibitory G protein. Activation of $GABA_B$ receptors results in various signaling mechanisms, including stimulation of K^+ conductance and inhibition of Ca^{2+} conductance. $GABA_B$ receptors are also found on pre-synaptic terminals and can thus mediate neurotransmitter release (Bowery, 1993). Given the distinct mechanisms by which $GABA_B$ receptors mediate synaptic signaling, their differential involvement in influencing feeding behavior deserves attention.

Accordingly, feeding behavior was observed in satiated rats following microinjection of various GABA agonists and antagonists into the PVT. The experiment involves central implantation of a guide cannula that allows for delivery of microliter volumes of drugs into discrete brain regions, to study how pharmacological manipulation of that specific area influences a behavior in question. We first sought to duplicate previous findings using our own experimental paradigm by testing the $GABA_A$ receptor agonist, muscimol, to determine if stimulation of $GABA_A$ receptors within the PVT is sufficient to induce feeding. Then, to clarify the role of PVT $GABA_B$ receptors in mediating feeding, baclofen, a selective $GABA_B$ receptor agonist, was injected into the PVT. To test receptor specificity and the involvement of local GABA receptors for $GABA_A$-mediated feeding, rats were pretreated with receptor-selective antagonists in order to determine if the feeding response can be blocked.

Materials and Methods

Subjects

Adult male Sprague-Dawley rats, weighing 350-500 g at the time of surgery, were used throughout. They were originally purchased from Charles River (Boston, MA), and were born and raised in our colony at UCR. Rats were housed individually in wire mesh cages with food and water available *ad libitum,* unless otherwise stated. They were kept in a temperature- and humidity-controlled room with standard 12 hr

light/dark cycle, with lights on at 9 AM. All experiments were carried out during the light phase. All procedures were in accordance with the Institutional Animal Care and Use Committee (IACUC) of The University of California, Riverside (Riverside, CA).

Stereotaxic surgery

Rats under pentobarbital anesthesia (50 mg/kg body weight, i.p.) were stereotaxically implanted with an 18 mm, 26-gauge stainless steel guide cannula aimed to terminate 1 mm above the PVT. The coordinates were as follows: 0.0 mm lateral, 3.0 mm posterior to bregma and 4.4 mm ventral to the surface of the skull, with the incisor bar set 3.3 mm below the ear bars. Coordinates are based on the rat brain atlas of Paxinos and Watson (2007). Cannulas were held in place using cold-cure dental acrylic secured to the skull with stainless steel screws, and kept clear with a 33-gauge stainless steel obturator. Rats were allowed to recover for several days and handled during this time to acclimate them to the testing procedure.

Experimental procedure and measurement of food intake

At least 3 days before testing, standard food pellets were replaced with a fresh mash diet consisting of 500 g Purina rat chow powder, 400 g sugar and 354 mL evaporated milk. This diet was used as it is readily consumed and is easy to measure without spillage. On the day of testing, fresh food was provided 60 min before injections to ensure satiation, unless otherwise noted. Drugs were given in a volume of 0.3 µL per injection through 33-gauge stainless steel injectors protruding 1 mm beyond the base of

the cannula, in order to reach the target area. Injectors were left in place for an additional 10 seconds in order to minimize leakage up the cannula tract. When possible, a within subjects design was used, with all subjects tested in all conditions in counterbalanced order, with 48 h between injections. The drugs used were the $GABA_A$ receptor agonist muscimol, the $GABA_B$ receptor agonist baclofen, the $GABA_A$ receptor antagonist bicuculline methbromide and the $GABA_B$ receptor antagonist saclofen. All drugs were purchased from Sigma Aldrich. They were dissolved in artificial cerebrospinal fluid containing 147 mM Na^+, 154 mM Cl^-, 3 mM K^+, 1.2 mM Ca^{2+} and 0.9 mM Mg^{2+}. The food bowl was weighed at the time of injection and at set points later, to determine the amount of food eaten.

Experiment 1: Does activation of $GABA_AR$ with muscimol elicit feeding?

In order to determine if activation of $GABA_A$ receptors in the PVT mediates feeding, a group of 16 rats was given intra-PVT injection of 300 ng muscimol or its aCSF vehicle. Since this dose was effective, a separate group of 15 rats was subsequently given PVT injections of muscimol at 0, 100, 200 and 300 ng. Food intake was measured 60 min after injection.

Experiment 2: Does activation of $GABA_BR$ with baclofen elicit feeding?

In order to determine if activation of $GABA_B$ receptors in the PVT elicits feeding, a group of 10 rats was given intra-PVT injection of baclofen, a $GABA_B$ receptor agonist, at 0, 10, 33 and 100 ng. Food intake was measured 60 min post-injection.

Experiment 3: Does prior blockade of $GABA_A$ receptors prevent muscimol-induced feeding within the PVT?

To test receptor selectivity and confirm that the stimulatory feeding effect was mediated through local GABA receptors, a group of 8 rats was pretreated with the specific $GABA_A$ receptor antagonist, bicuculline methbromide, or the specific $GABA_B$ receptor antagonist, saclofen, 10 min prior to an injection of 300 ng muscimol. Food intake was measured 60 min post-injection.

Statistical Analysis

GraphPad Prism software was used for statistical analysis to determine differences between groups. Data are expressed as mean ± SEM of food intake. Data were analyzed using paired t-test or one- or two-way ANOVA as appropriate. Dunnett's post-hoc test was used to reveal significant differences between groups. A $p<0.05$ was considered significant.

Histology

At the end of behavioral experiments, rats were deeply anesthetized with Fatal-Plus Solution and transcardially perfused with 10% formalin. Their brains were extracted and post-fixed in 10% formalin overnight before being sectioned using a microtome. 100 µm-thick coronal sections around the cannulation site were collected and mounted onto glass microscope slides. After drying, the slides were stained with cresyl violet. Accuracy

of cannula placement was verified by visualizing under a light microscope and compared to size-matched figures from the atlas of Paxinos and Watson (2007).

Results

Experiment 1: Activation of $GABA_A$ receptors in the PVT elicits feeding

Consistent with previous work, injection of the $GABA_A$ receptor agonist muscimol into the PVT elicited a consistent feeding response. Fig 2.1 depicts food intake 1 hr after PVT injection of muscimol (300 ng) or its vehicle. A paired-samples t-test showed a significant increase in food intake 1 hr following injection of muscimol (Mean=10.1, SD=10.5) as opposed to vehicle alone (Mean=0.4, SD=0.6); t(15) = 3.666, p = 0.0023.

As shown in Figure 2.2, the feeding response to muscimol was dose-dependent ($F_{(3,11)}$ = 4.398; p< .05). Post-hoc analysis showed that doses of 200 ng and under were ineffective, but the highest dose of 300 ng significantly increased food intake as compared to the aCSF treatment.

Experiment 2: Activation of PVT $GABA_B$ receptors elicits feeding

Activation of $GABA_B$ receptors in the PVT with the $GABA_B$ receptor-specific agonist baclofen dose-dependently elicited feeding responses in satiated rats ($F_{(3,6)}$ =

3.725; $p < .05$). Post-hoc analysis showed that with lower doses of 33 ng and under baclofen had no effect, but the highest dose of 100 ng significantly increased food intake as compared with aCSF alone (Fig. 2.3). Mean intake 1 hr post-injection was 0.8 g after vehicle injection and 4.0 g following the highest dose of baclofen.

Experiment 3: Blockade of $GABA_A$ receptors fails to suppress muscimol-induced feeding within the PVT

As shown in Figure 2.4, PVT injection of bicuculline or saclofen failed to suppress muscimol-elicited eating. Pretreatment with bicuculline (8.5 g) or saclofen (7.2 g) produced mean intakes comparable to vehicle (8.7 g) (not significant).

Discussion

The main finding of the present study was that injection of GABA receptor agonists directly into the PVT significantly increased food intake in satiated rats. This is in agreement with prior evidence showing that the $GABA_A$ receptor agonist muscimol stimulated feeding with injection into this brain region (Stratford & Wirtshafter, 2013). We also provide new data suggesting that activation of PVT $GABA_B$ receptors, via direct injection of the $GABA_B$ receptor agonist baclofen, is also sufficient to induce feeding. Although the magnitude of eating following activation of $GABA_A$ receptors was larger than that produced by activation of $GABA_B$ receptors, both agonists produced dose-dependent increases in feeding within the 1-hr postinjection period. These results

provide evidence that the PVT, and particularly GABAergic signaling within this nucleus, is part of the circuitry mediating feeding behavior in rats.

In support of this interpretation, a variety of previous studies have linked the PVT to the control of food intake. For example, lesioning the PVT increased cumulative food intake and body weight gain (Bhatnagar & Dallman, 1999). Consistent with that study, pharmacological activation of PVT $GABA_A$ receptors with muscimol, or optogenetic stimulation of zona incerta (ZI) GABAergic neurons projecting to the PVT, potently stimulated feeding (Stratford & Wirtshafter, 2013; X. Zhang & van den Pol, 2017). Common to these studies is the involvement of inhibitory GABAergic input to the PVT, suggesting that inhibition of the PVT stimulates food intake. The PVT is devoid of local GABA interneurons, instead receiving GABAergic input from other nuclei (Arcelli et al., 1997). Some of the most likely sources of feeding-related GABAergic input to the PVT include the reticular thalamic nucleus, ZI and hypothalamic regions such as the LH, arcuate nucleus and suprachiasmatic nucleus (Betley et al., 2013; Li & Kirouac, 2012; L. Zhang et al., 2006; X. Zhang & van den Pol, 2017). Photostimulation of LH GABAergic neurons projecting to the PVT increased food intake, but to a lesser extent compared to stimulation of ZI to PVT neurons (X. Zhang & van den Pol, 2017). Betley (2013) found that photostimulation of discrete subsets of AgRP neurons, some of which have been shown to release GABA (Atasoy et al., 2012), were sufficient to increase feeding, and the PVT was one of these downstream targets. The PVT also receives glutamatergic input from the parasubthalamic nucleus. Photostimulation of this excitatory input to the PVT

decreased food intake, further supporting the idea that decreased activity of the PVT promotes feeding (X. Zhang & van den Pol, 2017).

Previous studies have not investigated whether $GABA_A$ and $GABA_B$ receptors, which are both widely distributed in the PVT, differentially regulate feeding behavior in the PVT. Here, we showed that intra-PVT injections of the $GABA_A$ receptor agonist muscimol or the $GABA_B$ receptor agonist baclofen both elicited feeding in satiated rats, providing the first evidence that $GABA_B$ receptors in the PVT may play a stimulatory role in feeding behavior. $GABA_A$ receptors are fast-acting, ionotropic receptors assembled from subunits to form pentamers, the combination of which determines their distribution and pharmacology. While subunit combination in PVT neurons remains to be identified, they are typically permeable to Cl^- and mediate phasic inhibition on postsynaptic membranes (Farrant & Nusser, 2005). This type of signaling has been observed for SCN innervation to the anterior PVT (L. Zhang et al., 2006). $GABA_B$ receptors are $G_{\alpha i/o}$ protein-coupled receptors that mediate synaptic transmission over a slower timescale. These receptors may be located presynaptically, where they function as autoreceptors to inhibit glutamate input, or postsynaptically where they inhibit neuronal activity by opening G-protein activated K+ channels (Chalifoux & Carter, 2011). Thus, these two GABA receptor subtypes can produce differential actions on PVT neurons that may explain differences in the magnitude of elicited effects. Nevertheless, activation of either receptor subtypes leads to a decrease in excitatory drive to the PVT, and similar behavioral outcomes. It is important to recognize that endogenous GABA

can act on both receptor subtypes, potentially producing more substantial inhibition. Although the neuropharmacology and distribution of GABA receptors in the PVT remain to be fully explored, there is evidence implicating them in the regulation of motivated behaviors, as activation of GABA receptors in the PVT has been shown to induce feeding (Stratford & Wirtshafter, 2013), reduce the expression of cocaine-induce conditioned place preference (Browning et al., 2014) and block cue-induced reinstatement to cocaine (Matzeu et al., 2015).

Unexpectedly, pretreatment with the $GABA_A$ receptor antagonist bicuculline was ineffective in suppressing the feeding produced by muscimol. What might explain bicuculline's ineffectiveness? Given that a high dose of muscimol was used to elicit a maximal eating response, one possibility is that the dose of bicuculline was insufficient to overcome the binding of the agonist to its receptor. This possibility cannot be dismissed since bicuculline is a competitive $GABA_A$ receptor antagonist. Another possibility is that muscimol-elicited feeding is an artifact, and that $GABA_A$ receptors in the PVT are not involved in physiological mechanisms controlling feeding behavior. While this possibility should not be dismissed, it seems unlikely in view of the wealth of previously-discussed evidence supporting a role for PVT $GABA_A$ receptors in these mechanisms. Nevertheless, the data in the literature support a role for GABA, and $GABA_A$ receptors in the PVT, in the control of feeding. As previously mentioned, GABAergic pathways arising from multiple sources make synaptic connections to neurons in the PVT. $GABA_A$ receptors are also widely expressed in the PVT, allowing

GABA and its agonists to influence neuronal activity within this region (Gao et al., 1995). We have shown here that microinjection of muscimol into the PVT elicits feeding in satiated rats. This replicates prior studies showing a similar effect with the $GABA_A$ agonist and supports a role of PVT GABA specifically in feeding (Stratford & Wirtshafter, 2013). As to be discussed in Chapter 4, we have also shown that bicuculline suppressed feeding in food-deprived rats, confirming the effectiveness of the $GABA_A$ antagonist and suggesting the involvement of endogenous GABA. Together, these findings suggest that GABA released from other brain regions can act on GABA receptors in the PVT, and that inhibition of neuronal activity in this region can have a distinct influence on feeding behavior.

Given that GABA is the primary inhibitory neurotransmitter in the brain, it is not surprising that GABA receptors in other brain areas have also been implicated in the control of food intake. For example, blockade of $GABA_A$ receptors in the LH increased food intake, while activation of either $GABA_A$ or $GABA_B$ receptors in the NAc shell stimulated feeding, in satiated rats (Stratford & Kelley, 1997; Turenius et al., 2009). A functional connection between the LH and the NAc has previously been established. Specifically, feeding elicited by blockade of AMPA/kainate receptors in the NAc shell was abolished by inactivation of the LH by muscimol or D-AP5 (Maldonado-Irizarry et al., 1995; Urstadt et al., 2013). The LH projects to the PVT, and the PVT in turn sends a glutamatergic projection to the NAc (Christie et al., 1987). The direction of these feeding effects induced by GABA is in line with a proposed LH to PVT to NAc circuit. Accordingly,

inhibitory inputs from the LH or other sources to the PVT would lead to decreased glutamate release in the NAc, ultimately leading to the onset of feeding (Maldonado-Irizarry et al., 1995). Consistent with this, optogenetic stimulation of PVT glutamatergic neurons reduced food intake, while blockade of glutamate receptors in the NAc shell with the AMPA/kainate receptor antagonists DNQX, NBQX or CNQX elicited feeding (Maldonado-Irizarry et al., 1995; X. Zhang & van den Pol, 2017).Thus, the PVT may represent an indirect pathway by which the LH and NAc mediate feeding.

In summary, we find that inhibition of the PVT, via $GABA_A$ or $GABA_B$ receptor activation, is sufficient to evoke feeding behavior. However, it is important to be aware of the complexity with which the PVT can potentially regulate these behaviors. For example, orexin input from the LH, which has been shown to excite PVT neurons, also appears to stimulate food intake (Ishibashi et al., 2005). Genetic knockdown of the OX1R receptor specifically in the PVT reduced consumption of a high-fat diet, while posterior PVT microinjection of an OX-A agonist increased sucrose drinking (Barson et al., 2015; Choi et al., 2012). Intra-PVT OX-A also increased dopamine (DA) levels in the NAc, indicating that DA signaling may be involved in PVT regulation of motivated behaviors. In addition, hypothalamic CART fibers preferentially innervate the PVT compared to adjacent thalamic nuclei, and these PVT neurons project to the NAcS shell (Parsons et al., 2006). CART, which frequently colocalizes with GABA, has been shown to inhibit feeding (Vrang et al., 1999). Thus, the PVT can integrate various signals from other

neuropeptide and neurotransmitter systems, allowing it to play a complex role in the control of food intake and related behaviors.

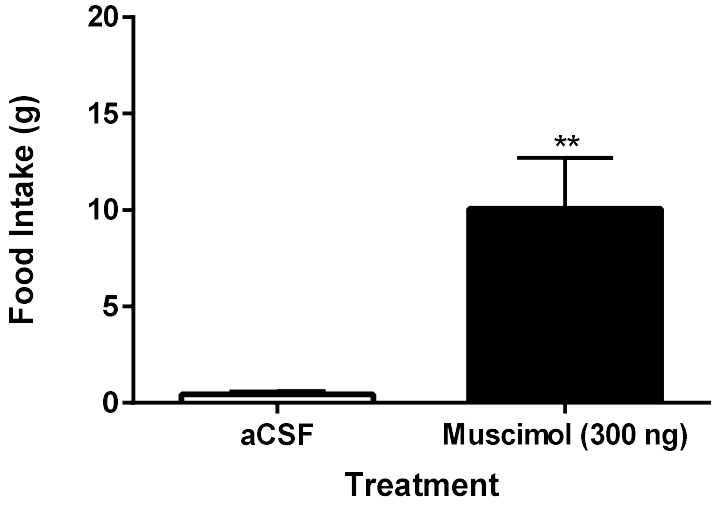

Figure 2.1 Mean food intake 1 hr after PVT injection of muscimol (300 ng) or its vehicle. Data are expressed as mean (g) (SD). n = 16 rats. **p<0.01 compared to aCSF-treated group by paired t-test.

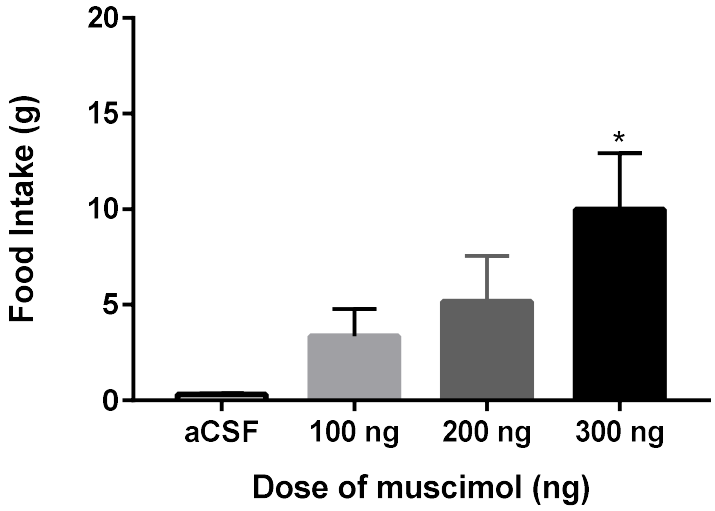

Figure 2.2 Mean food intake 1 hr after PVT injection of muscimol as a function of dose. Data represent mean food intake in g ± SEM. n = 15 rats. *p<0.05 compared to aCSF-treated controls, by one-way ANOVA followed by Dunnett's post hoc test.

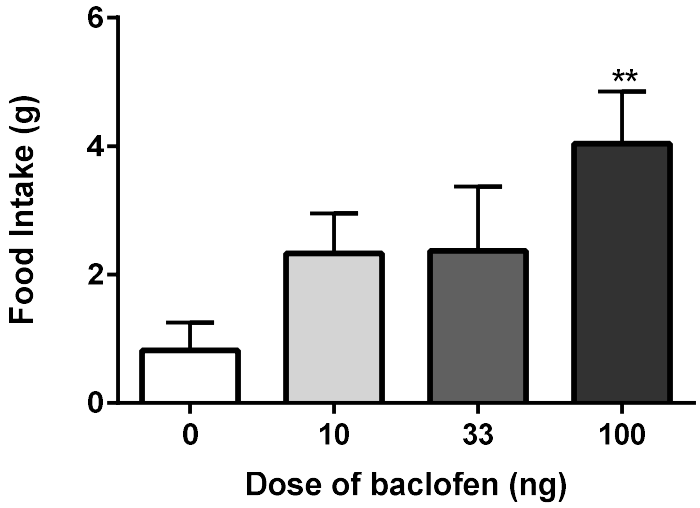

Figure 2.3 Mean food intake 1 hr after PVT injection of baclofen as a function of dose. Baclofen dose-dependently elicited food intake (mean g ± SEM) in satiated rats within 1 hr of injection into the PVT. n = 10 rats. The food intake was significant at the highest 100 ng dose. **p<0.01 compared to aCSF-treated controls, by one-way ANOVA followed by Dunnett's post hoc test.

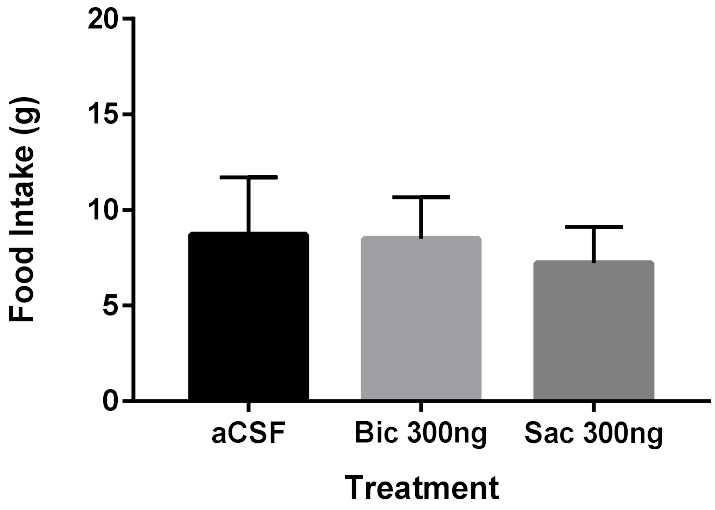

Figure 2.4 Food intake (mean g + SEM) 1 hr after PVT injection of muscimol (300 ng) following a prior injection of bicuculline, saclofen or vehicle. n = 8 rats.

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Chapter 3: Regional specificity of GABA's feeding stimulatory effects in the PVT

Abstract

The paraventricular nucleus of the thalamus (PVT) receives hypothalamic inputs and projects to accumbal neurons known to be involved with reward and motivation, including the control of feeding behavior. The PVT contains distinct anterior (aPVT) and posterior (pPVT) subregions, as derived from recent evidence highlighting differences in projections as well as responses to pharmacological manipulations. This distinction, until recently, has remained relatively understudied. The purpose of this study was to determine whether the aPVT and pPVT differentially mediate the feeding response induced by GABA agonist injection into the PVT. To examine this, adult male Sprague-Dawley rats were stereotaxically implanted with cannulae directed at anterior and posterior portions of the PVT. We injected the $GABA_A$ receptor agonist muscimol or the $GABA_B$ receptor agonist baclofen and measured food intake in satiated rats. Muscimol elicited feeding in both the aPVT and pPVT 60 min post-injection, but produced a smaller response in the aPVT. Baclofen also induced feeding in the pPVT, but failed to have any effect when injected into the aPVT. These results indicate that the pPVT is the most sensitive site of GABA's actions and suggest that there are functional differences between the anterior and posterior divisions of this nucleus in the control of feeding.

Introduction

The paraventricular nucleus of the thalamus (PVT) is increasingly being recognized as part of a neural system regulating feeding behavior. The previous experiment showed that injections of GABA receptor agonists into the PVT elicited eating in satiated rats. Increases were produced by either $GABA_A$ or $GABA_B$ receptor agonist injections. These results suggest that inhibition of neurons within this brain region ultimately leads to the initiation of feeding. However, the anatomical specificity of this effect has not been fully explored. This is particularly important given that, while the PVT has traditionally been viewed as a single structure, recent studies have begun to highlight both functional and anatomical differences between the anterior (aPVT) and posterior (pPVT) subregions.

The PVT varies along the rostral-caudal axis, being bordered by the mediodorsal thalamic nucleus more posteriorly, while the paratenial thalamic nucleus is immediately adjacent at the anterior aspect. Although the overall outputs of the aPVT and pPVT are similar, with major targets to limbic subcortical structures such as the nucleus accumbens, bed nucleus of the stria terminalis and amygdala, there are some differences in the degree of distribution that may have functional implications. For example, both retrograde and anterograde tracer studies have identified a dense projection from the pPVT to the central extended amygdala, while connections from the aPVT appear relatively weak. The aPVT, on the other hand, projects more heavily to

various hypothalamic nuclei including the suprachiasmatic nucleus, ventromedial hypothalamus and lateral hypothalamic area (Li & Kirouac, 2008; Moga et al., 1995; Vertes & Hoover, 2008).

Furthermore, previous studies have found differences in response to behavioral manipulations across the anteroposterior axis. In chronically-stressed rats exposed to a novel restraint stressor, an increase in Fos reactivity, a marker for neuronal activity, was observed in the pPVT but not aPVT. A functional role for this difference was suggested by data showing that lesions to the pPVT facilitated ACTH responses in chronically stressed but not control rats (Bhatnagar & Dallman, 1998). Similarly, food deprivation increased Fos expression in the pPVT earlier than in the aPVT (Timofeeva & Richard, 2001). Lesions of the aPVT affected the entrainment of circadian rhythms to light, whereas lesions of the pPVT did not (Salazar-Juarez et al., 2002). In relation to feeding, differential c-Fos expression along the rostral-caudal axis was observed in rats in response to presentation of a food cue that had previously acquired incentive value (Flagel et al., 2011). Orexin receptor 2 signaling in the aPVT, but not pPVT, was correlated with voluntary ethanol drinking. Conversely, injections of orexin-A into the pPVT specifically stimulated sucrose intake, but injections into the aPVT had no effect (Barson et al., 2015). In another study, inactivation of the aPVT with muscimol increased bar pressing for a cued sucrose reward when the reward was omitted, but had no effect in the pPVT (Do-Monte et al., 2017).

These studies suggest that neurons in the aPVT and the pPVT may have different functions. Given that many experiments to this point, including those focused on feeding behavior, have targeted a single site within the PVT, the question of whether the aPVT or pPVT differentially influence food intake remains. Therefore, the effect of microinjection of GABA receptor agonists targeting more rostral and caudal ends of the PVT, representing the anterior and posterior regions of the nucleus, was compared.

Materials and Methods

Subjects

Adult male Sprague-Dawley rats, weighing 350-500 g at the time of surgery, were used in this experiment. They were individually housed in wire mesh cages and kept in a temperature- and humidity-controlled room with standard 12 hr light/dark cycle, with lights on at 9 AM. Food and water were available *ad libitum,* unless otherwise stated. All procedures were in accordance with the Institutional Animal Care and Use Committee (IACUC) of The University of California, Riverside (Riverside, CA).

Stereotaxic surgery

Rats under pentobarbital anesthesia (50 mg/kg body weight, i.p.) were stereotaxically implanted with an 18 mm, 26-gauge stainless steel guide cannula aimed to terminate 1 mm above the anterior PVT. A separate group of rats received a single

cannula directed at the posterior PVT. The coordinates were as follows: aPVT - 0.0 mm lateral, 1.5 mm posterior to bregma and 4.4 mm ventral to the surface of the skull; pPVT - 0.0 mm lateral, 3.0 mm posterior to bregma and 4.4 mm ventral to the surface of the skull, with the incisor bar 3.3 mm below the ear bars. Cannulas were held in place using cold-cure dental acrylic secured to the skull with stainless steel screws, and kept clear with a 33-gauge stainless steel obturator. Each rat was allowed to recover for a minimum of 7 days before the start of behavioral testing.

Experimental procedure and measurement of food intake

At least 3 days before testing, standard food pellets were replaced with a fresh mash diet consisting of 500 g Purina rat chow powder, 400 g sugar and 354 mL evaporated milk. On test day, this fresh mash diet was given at least 1 hr prior to injection in order to ensure satiation. A single injection of drug or vehicle into the PVT, at a volume of 0.3 µL, was given via a 33-gauge stainless steel injector that protruded 1 mm beyond the base of the cannula. The food bowl was weighed at the time of injection and 60 minutes later, to determine the amount of food eaten. Rats received either drug or vehicle in counterbalanced order, with 48 hr allowed between injections, during which rats had *ad libitum* access to food and water.

Experiment 1: Do the aPVT and pPVT differentially mediate feeding elicited by muscimol?

In order to examine whether the anterior and posterior regions of the PVT differentially mediate feeding elicited by injection of the $GABA_A$ agonist muscimol, rats

with a cannula directed at the aPVT (-1.5 mm posterior to bregma) or pPVT (-3.0 mm) were given either aCSF or 300 ng muscimol, a dose which was previously found to elicit maximal a feeding response. Satiated rats were tested as described above.

Experiment 2: Do the aPVT and pPVT differentially mediate feeding elicited by baclofen?

To test whether the anterior and posterior regions of the PVT differentially mediate feeding elicited by injection of the $GABA_B$ agonist baclofen, a separate group of rats with cannula directed at either the aPVT or pPVT were tested. They were injected with 100 ng baclofen, the dose that was previously shown to produce a statistically significant increase in feeding. Satiated rats were tested as described above.

Statistical Analysis

Data are expressed as mean ± SEM of food intake. Data were analyzed using independent samples t-test. A $p<0.05$ was considered significant.

Histology

Following behavioral testing, accuracy of injection sites was verified with Nissl staining. These sections were compared to matching templates from the Paxinos and Watson rat brain atlas (2006). Representative photomicrographs can be seen in Fig. 3.1.

Results

Experiment 1: Muscimol was more effective in eliciting eating in the pPVT compared to aPVT

Figure 3.2 depicts food intake 1 hr following injection of 300 ng of muscimol or its vehicle into the aPVT or pPVT. There was a significant increase in food intake produced by an aPVT injection of muscimol (Mean=2.3, SD=1.6) compared to vehicle alone (Mean=0.5, SD=0.5); $t(7) = 2.906$, $p=0.0228$. Muscimol also elicited feeding in the pPVT (Mean=10.1, SD=10.5) compared to aCSF (Mean=0.4, SD=0.6);$t(15) = 3.666$, $p=0.0023$. An independent t-test was conducted to compare the magnitude of the feeding response in the two subregions. Importantly, muscimol elicited greater cumulative eating when injected into the pPVT compared to the aPVT, $t(22)=2.074$, $p=0.05$ suggesting that, although this dose of muscimol was able to stimulate food intake in both subregions, it was more effective in the pPVT.

Experiment 2: Baclofen specifically elicited feeding in the pPVT but not aPVT

As shown in Figure 3.3, baclofen at 100 ng elicited feeding only when injected into the pPVT. There was no significant difference in cumulative intake for rats injected in the aPVT with baclofen compared (Mean=0.4, SD=0.7) to vehicle (Mean=0.2, SD=0.06). $t(6)=0.927$, $p=0.390$. On the other hand, rats implanted with cannula in the pPVT ate significantly more food following injection with baclofen (Mean=4.0, SD=2.5)

compared to aCSF (Mean=0.8, SD=1.4), t(9)=5.152, p=0.0006. These data suggest that the feeding-stimulatory effects of baclofen are regionally specific to the pPVT.

Discussion

The main findings of the present study are that GABA receptor agonists elicited feeding in the pPVT but were either ineffective or less effective when injected into the aPVT. These results are in agreement with previous findings that GABA receptors within the PVT are involved in mediating ingestive behavior, but also provide new evidence that these effects may be regionally specific. Specifically, the $GABA_A$ receptor agonist muscimol elicited a strong feeding response when injected into the pPVT, but the same dose produced a reduced effect in the aPVT. In contrast, while the $GABA_B$ receptor agonist baclofen was effective in dose-dependently stimulating eating in the pPVT, it had no effect when injected into the aPVT. Together, these results suggest that the posterior subregion is the most sensitive site of GABA's feeding stimulatory effect in the PVT.

Although muscimol elicited feeding when injected into either the aPVT or pPVT, the magnitude of the response was much greater in the pPVT. Injections into the pPVT elicited 10.1 g food intake in 1 hr, compared to 2.3 g in the aPVT. Since these injections were 1.5 mm apart along the rostrocaudal axis of the PVT, it is likely that this GABA agonist was acting on local receptors within each subregion to produce eating, with the

pPVT being the more sensitive site. However, the extent of drug diffusion was not measured in this experiment. Although it is possible that a drug may exert its effect by diffusing to nearby tissue, it seems unlikely that the small feeding effect observed following muscimol injection into the aPVT is a result of diffusion into the pPVT. Previous studies injecting various dyes into the thalamus found that an injection of 0.5 µL, a volume greater that the 0.3 µl we used, spread 1 mm on average and concluded that injections of this volume can be attributed to the specific nucleus within the brain where it was injected (Myers, 1966). A separate experiment injected 0.3 µL methylene blue dye into the aPVT and pPVT and found that the radial spread of diffusion was ~0.5 mm, never overlapping between one subregion and the other (Barson et al., 2015). Nevertheless, different chemical substances can diffuse to different degrees, based on their physical properties, as well as the site of injection, and therefore it would be useful to trace the drug to more accurately determine its spread.

The ability of muscimol to elicit feeding when injected into the posterior region of the PVT is in agreement with previous findings (Stratford & Wirtshafter, 2013), who in addition found that feeding was not observed following injections aimed dorsally into the third ventricle, or laterally into the mediodorsal nucleus. On the other hand, we found that baclofen failed to elicit feeding when injected into the aPVT, indicating that $GABA_B$ receptors here are not relevant to the feeding stimulation we measured. Together, these findings suggest that the pPVT is the specific site of GABA's actions.

Further studies are needed to determine if feeding can be elicited with injections into adjacent structures more caudal or ventral to the PVT.

These results that the pPVT is most sensitive to the effects of GABA suggest that GABA may be acting in anatomically or functionally distinct ways between the subregions of the PVT. Although the distribution of GABA receptors within the two subregions has not been extensively studied, it is unlikely that the aPVT is devoid of functional GABA receptors given how broadly distributed they are within the brain (Margeta-Mitrovic et al., 1999). While it is possible that the anatomical specificity is due to differential GABA receptor distribution between the subregions, a more likely scenario is that activation of GABA receptors in the aPVT has a different functional role that failed to manifest as increased feeding in our study.

The aPVT and pPVT may mediate different aspects of food-seeking behavior. For example, activity in the aPVT has been associated with cues signaling the availability or timing of food. c-Fos expression is increased prior to delivery of food in food-restricted rats, or by cues predicting delivery of a reward (Igelstrom et al., 2010; Poulin & Timofeeva, 2008). On the other hand, the pPVT has been associated with intake of a high-fat diet or standard chow (Choi et al., 2012; Stratford & Wirtshafter, 2013).

A difference in response to GABA receptor activation may be accounted for by differences in the subcortical targets innervated by the two subregions of the PVT. Anterograde and retrograde tracing studies have identified a topographical organization

of PVT projections to the nucleus accumbens (NAc), with the aPVT preferentially innervating the dorsomedial part of the NAc shell, and the pPVT projecting more to the ventromedial NAc shell (Dong et al., 2017; Li & Kirouac, 2008). This may be important given that these distinct parts of the NAc have been shown to mediate opposing behaviors. For example, optogenetic stimulation of dynorphin-expressing cells in the dorsal NAc induced a conditioned place preference whereas excitation of these cells in the ventral NAc induced an aversive state (Al-Hasani et al., 2015). In general, the dorsomedial shell has been linked to reward-related behaviors (Reed et al., 2015). Some studies have indicated that the aPVT and pPVT may differentially mediate distinct aspects of consummatory behavior. For example, activation of orexin receptors in the aPVT, but not the pPVT, increased ethanol consumption, but not food or water, while orexin receptor activation in the pPVT specifically increased sucrose intake (Barson et al., 2015). Interestingly, orexin receptor 1 knockdown targeting a more posterior portion of the PVT decreased consumption of a high-fat diet (Choi et al., 2012).

In addition, many of the PVT neurons projecting to the NAc also collateralize to innervate other targets such as the bed nucleus of the stria terminalis, central nucleus of the amygdala and prefrontal cortex (Dong et al., 2017). Although the function of these collateralizations is virtually unknown, these neurons that project to multiple targets may be able to coordinate the activity of different brain regions involved in mediating diverse aspects of behavior. Some differences regarding the extent of collateralization exist along the anteroposterior axis of the PVT. For example, one study using dual

retrograde tracers found that tracer deposits into the PFC and septal pole of the NAc only double-labeled cells in the aPVT (Bubser & Deutch, 1998). Thus, aPVT and pVT cells, whose primary output is the NAc, may still be able to differentially signal between different populations of cells or brain regions.

Our findings indicate that activation of GABA receptors in the posterior subregion of the PVT is sufficient to elicit feeding, while the aPVT is less sensitive to $GABA_A$ receptor activation. These results point to the functional complexity of the PVT as a whole, and suggest that inhibition of neurons within the aPVT versus pPVT may lead to different downstream substrates mediating different behavioral responses. The pPVT appears to be part of a neural circuit regulating eating mechanisms where inhibition of neurons ultimately leads to feeding stimulation.

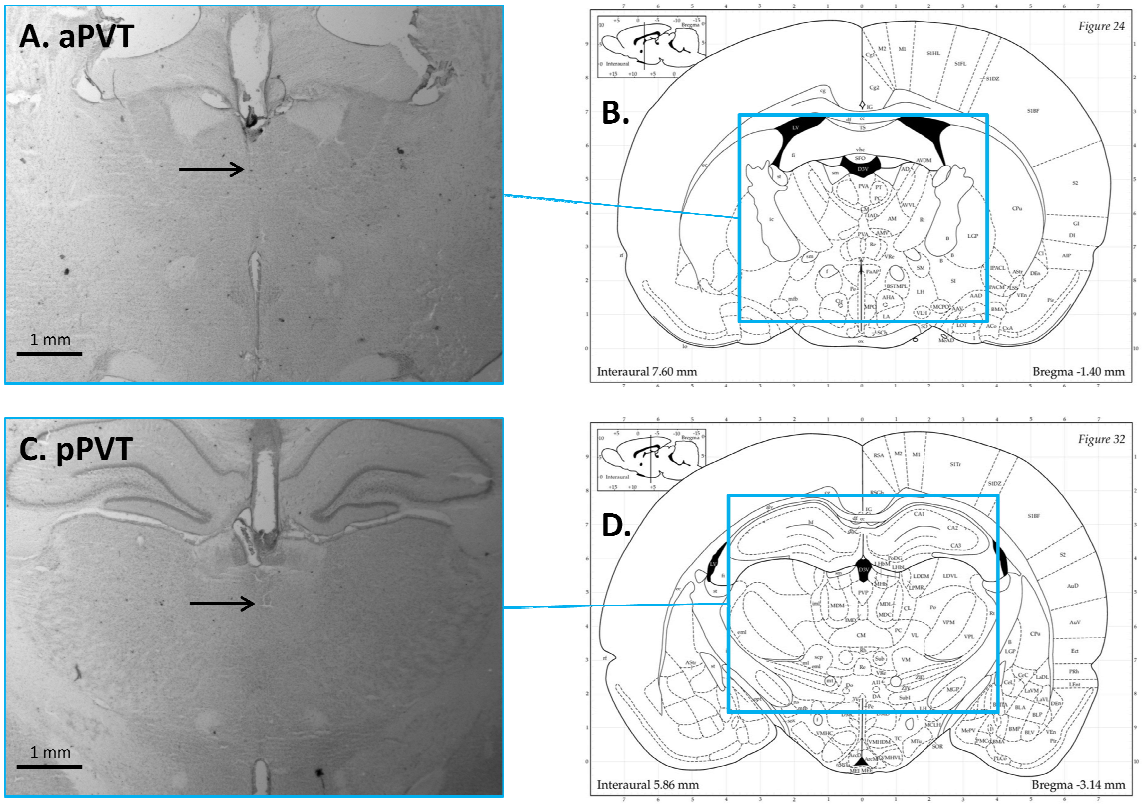

Figure 3.1 Representative images showing cannula placement. A) Anterior PVT, C) Posterior PVT. B, D) Example coronal section diagrams approximating the target of interest (1.5 mm and 3.0 mm posterior to bregma for the aPVT and pPVT, respectively). Atlas sections adapted from Paxinos and Watson (2006).

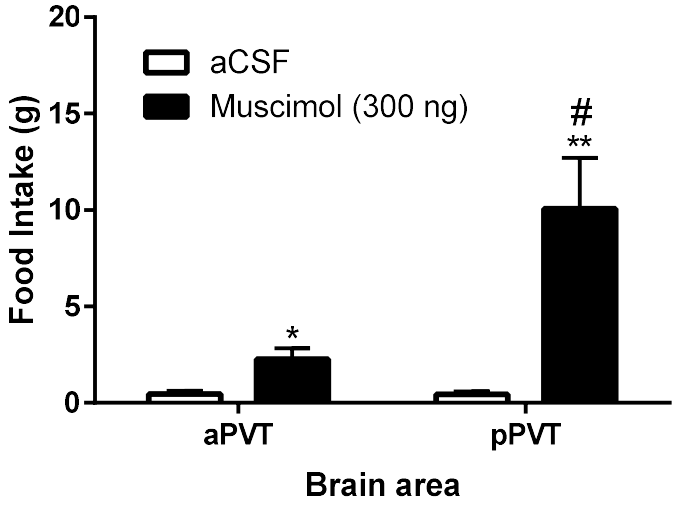

Figure 3.2 Mean food intake as a function of injection site. 300 ng muscimol increased mean 1 hr food intake (g ± SEM) in the pPVT (n = 16) and aPVT (n = 8), but the response was larger with pPVT injections. *p<0.05; ** p<0.005 compared to aCSF-treated group by paired t-test. #p<0.05 compared to aPVT with independent t-test.

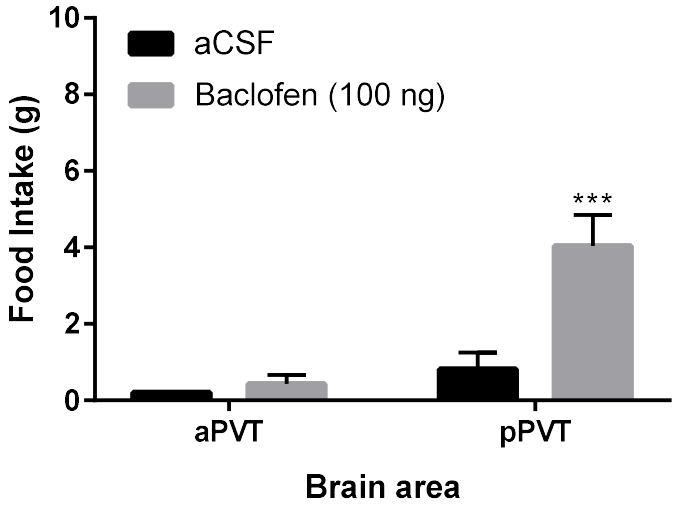

Figure 3.3 Mean food intake (g + SEM) 1 hr after injection of 100 ng baclofen or its vehicle into the aPVT (n = 7) or pPVT (n = 10) of satiated rats. Baclofen increased food intake within 1 hr of injection specifically in the pPVT. ***p<0.001 by paired t-test compared to aCSF injection.

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Chapter 4: The role of GABA receptors in the PVT in natural feeding

Abstract

Several studies provide evidence that manipulations of the PVT, in particular inhibition of neurons via the GABAergic system, can influence feeding behavior. However, the question remains whether GABA and GABA receptors within the PVT play a role in natural feeding. To this end, we sought to characterize the effect of pharmacological blockade of PVT $GABA_A$ receptors on deprivation-induced feeding. Adult male Sprague-Dawley rats with indwelling guide cannula directed at the PVT were fasted for 24 hr and then received injection of the $GABA_A$ receptor antagonist bicuculline (BIC) immediately before refeeding. Deprivation produced a consistent feeding response, with rats consuming approximately 12.5 g 1 hr after refeeding. This response was dose-dependently suppressed by BIC at 1 hr postinjection, with the 200 ng dose producing a statistically significant 41.6% reduction in food intake. This finding suggests that endogenous PVT GABA is involved with the physiological control of eating and points towards the involvement of $GABA_A$ receptors in the PVT in this effect.

Introduction

Early evidence that the PVT was a brain region that supported intracranial self-stimulation suggested that this nucleus was involved in circuits controlling motivated behaviors such as feeding and reward (Clavier & Gerfen, 1982). In Chapter 2, we demonstrated a role for GABA in the PVT in the control of food intake, as injection of agonists for the $GABA_A$ and $GABA_B$ receptors directly into the PVT elicited feeding in satiated rats. This is consistent with literature showing that lesions of the PVT, or inhibition of PVT neurons through pharmacological or chemogenetic means, is capable of stimulating eating behavior (Bhatnagar & Dallman, 1999; Stratford & Wirtshafter, 2013; Zhang & van den Pol, 2017).

The role of GABA and GABA receptors in the PVT in natural feeding, however, remains unanswered. Therefore, the ability of pharmacological blockade of these receptors to suppress natural feeding was examined. Rats spontaneously increase their food intake following a period of deprivation, to compensate for a general metabolic deficit, or at the onset of the dark phase, in response to circadian factors. Thus, rats were given injections of the $GABA_A$ receptor antagonist bicuculline following food deprivation, a condition that has previously been shown by our lab to elicit robust feeding (Turenius et. al. 2009, Stanley et. al. 2009). If blockade of these receptors suppresses food intake in fasted rats, it would provide evidence that *endogenous* GABA, acting on PVT $GABA_A$ receptors, plays a physiological role in mediating natural feeding.

Materials and Methods

Subjects

Adult male Sprague-Dawley rats, weighing 350-500 g at the time of surgery, were used. Rats were housed individually in wire mesh cages and given *ad libitum* access to food and water, unless otherwise noted. They were kept in a temperature- and humidity-controlled room with standard 12 hr light/dark cycle, with lights on at 9 AM. All procedures were in accordance with the Institutional Animal Care and Use Committee (IACUC) of The University of California, Riverside (Riverside, CA).

Stereotaxic surgery

Stereotaxic implantation of an 18 mm, 26-gauge stainless steel guide cannula targeting the posterior PVT was performed under pentobarbital anesthesia (50 mg/kg, i.p.). The coordinates were 0.0 mm lateral, 3.0 mm posterior to bregma and 4.4 mm ventral to the surface of the skull, with the incisor bar 3.3 mm below the ear bars. Coordinates were based on the rat brain atlas of Paxinos and Watson (2007). Cannulas were held in place using dental acrylic secured to the skull with stainless steel screws, and kept clear with a 33-gauge stainless steel obturator. Rats were allowed to recover for 7 days and were handled and mock injected during this time in order to habituate to behavioral testing.

Experimental procedure and measurement of food intake

Rats were given mash diet in place of the standard food pellets 3 days prior to testing. Injections were given in a volume of 0.3 µL per injection through 33-gauge stainless steel injectors protruding 1 mm beyond the base of the cannula to reach the PVT. Injectors were left in place for an additional 10 seconds in order to minimize leakage up the cannula tract. When possible, a within subjects design was used, with all subjects tested in all conditions in counterbalanced order, with 48 hr between injections. The $GABA_A$ antagonist bicuculline methbromide, purchased from Sigma Aldrich, was dissolved in aCSF before use. The food bowl was weighed at the time of injection and at set time points later, to determine the amount of food eaten.

Experiment 1: Does antagonism of $GABA_A$ receptors in the PVT suppress spontaneous food intake?

In order to examine whether selective blockade of PVT $GABA_A$ receptors can suppress food intake in fasted rats, rats were injected with the $GABA_A$ receptor antagonist, bicuculline, or vehicle, following a period of food deprivation. Specifically, rats were tested in the light phase after being fasted for 24 hr. On test day, they were given PVT injections of BIC (100 ng or 200 ng) or aCSF. Immediately after, fresh mash diet was provided. Food intake was measured at 1, 2, 4 and 24 hr post-injection.

Statistical Analysis

GraphPad Prism software was used for statistical analysis to determine differences between groups. Data are expressed as mean ± SEM of food intake. Data were analyzed using two-way ANOVA. Dunnett's post-hoc test was used to reveal significant differences between groups. A $p<0.05$ was considered significant.

Histology

At the end of behavioral testing, rats were deeply anesthetized with Fatal-Plus Solution and transcardially perfused with 10% formalin. Their brains were extracted and post-fixed in 10% formalin overnight. 100 μm coronal sections around the cannulation site were collected, stained with cresyl violet in order to verify the accuracy of cannula placement.

RESULTS

Experiment 1: Blockade of $GABA_A$ receptors in the PVT suppressed feeding in food-deprived rats

As shown in Figure 4.1, animals which were food-deprived for 24 hr demonstrated a strong feeding response following the reintroduction of food, eating 11.6 g during the first hour. BIC injected into the PVT produced a dose-dependent suppression of food intake. This effect was immediate, with a 41.6% reduction in food

intake following the 200 ng dose at the 1-hr time point. By 2 hr post-injection the suppression declined to 18.2%. Repeated measures ANOVA revealed no significant effect of treatment ($F(2, 42) = 1.063$, $p > .05$), but there was a significant effect of time ($F(2, 84) = 92.38$, $p < .0001$) and a significant interaction between the two factors ($F(4, 84) = 5.701$, $p < .0004$). Post hoc analysis showed that 200 ng BIC produced a significant overall suppression of food intake during the first hour of the experiment. Cumulative intake from 2 hr to 24 hr (not shown) did not differ as they ate 40.5 g after BIC and 40.3 g after vehicle injection, 24-hr postinjection (Fig. 4.2).

Discussion

The role of GABA in the PVT in the physiological control of food intake has previously been unexplored. To examine how PVT GABA and its receptors might be involved in natural feeding, PVT injections of the $GABA_A$ receptor antagonist bicuculline were administered just before eating induced by 24 hr of food deprivation. It was found that BIC suppressed deprivation-induced eating. The previous finding that a GABA receptor agonist stimulates feeding in the PVT, combined with the present finding that an antagonist suppresses deprivation-induced feeding, provides evidence that endogenous GABA acts on GABA receptors in the PVT to regulate natural feeding.

There is evidence that PVT neurons can integrate information related to energy balance and circadian rhythms. For example, PVT neurons are activated after refeeding

following a period of deprivation, as measured by c-Fos expression (Timofeeva & Richard, 2001). They also display diurnal changes in firing activity, displaying increased spontaneous burst firing during the night, when rats are most active and consume most of their daily food intake (Kersten et al., 1980; Kolaj et al., 2014). Furthermore, the PVT contains a population of NAc-projecting neurons expressing the glucose transporter Glut2 that are sensitive to hypoglycemia; optogenetic activation of these neurons lead to increased sucrose-seeking (Labouebe et al., 2016). PVT neurons also express glucagon-like-peptide-1 receptors, and GLP-1R signaling is important for satiety. These GLP-1 inputs arise from preproglucagon neurons of the NTS, which are activated by food intake (Ong et al., 2017). Together, these studies suggest that the PVT can integrate various signals that can ultimately govern feeding behavior.

In the present study, pharmacological blockade of PVT $GABA_A$ receptors with bicuculline decreased the cumulative food intake in rats that were re-fed following 24 hr of food deprivation, suggesting the involvement of endogenous GABA and $GABA_A$ receptors in the PVT in mediating this form of natural feeding. Microdialysis studies have shown that initiation of a meal is accompanied by a decrease in glutamate levels in the nucleus accumbens (Rada et al., 1997). The PVT is a possible source of this glutamatergic input, as the PVT projects strongly to the NAc (Labouebe et al., 2016; Parsons et al., 2006). Thus, increased excitability of a PVT to NAc glutamatergic pathway may account for the reduced feeding following $GABA_A$ receptor blockade in this

experiment. This is in line with evidence showing that blockade of AMPA/kainate receptors in the NAc shell elicits feeding (Maldonado-Irizarry et al., 1995).

The source of GABAergic input to the PVT that is mediating deprivation-induced feeding remains to be determined. One possible source is the arcuate nucleus of the hypothalamus, which contains AgRP neurons that are sensitive to metabolic signals such as ghrelin (Cowley et al., 2003). Some AgRP neurons co-express GABA, and optogenetic stimulation of AgRP neuron projections to the PVT elicits feeding (Betley et al., 2013; Horvath et al., 1997). GABAergic signaling also arises from the zona incerta, and photostimulation of PVT-projecting GABAergic neurons from the ZI also increased food intake (Zhang & van den Pol, 2017).

That the feeding was not completely abolished and was only reduced 1 hr postinjection suggests that 1) the $GABA_A$ receptor blockade may not have been complete or long-lasting or 2) other factors are also involved in the regulation of deprivation-induced feeding. For example, GABA may still act on $GABA_B$ receptors which are present in the PVT (Bischoff et al., 1999); thus, it is possible that inactivation of the PVT with a combination of $GABA_A$+$GABA_B$ receptor antagonists may be more effective in suppressing deprivation-induced feeding. Alternatively, feeding may be influenced by GABA-independent mechanisms. For example, orexin fibers originating from the lateral perifornical regions of the hypothalamus innervate the PVT, and orexin receptors are highly expressed in the PVT (Kirouac et al., 2005; Marcus et al., 2001). Orexin levels

increase during the dark when rats are most active and eating (Yoshida et al., 2001). Orexin has been shown to depolarize PVT neurons, and OX1 receptor knockout attenuated consumption of a high fat diet (Choi et al., 2012; Ishibashi et al., 2005). That orexin produces an antagonistic effect suggests it may act on a distinct population of neurons in the PVT. Nevertheless, the findings of this study provide further support for the involvement of GABA in feeding behavior. Specifically, the observation that blockade of $GABA_A$ receptors in the PVT significantly reduced eating in fasted rats suggests that GABA and GABA receptors in the PVT play a role in the physiological control of feeding.

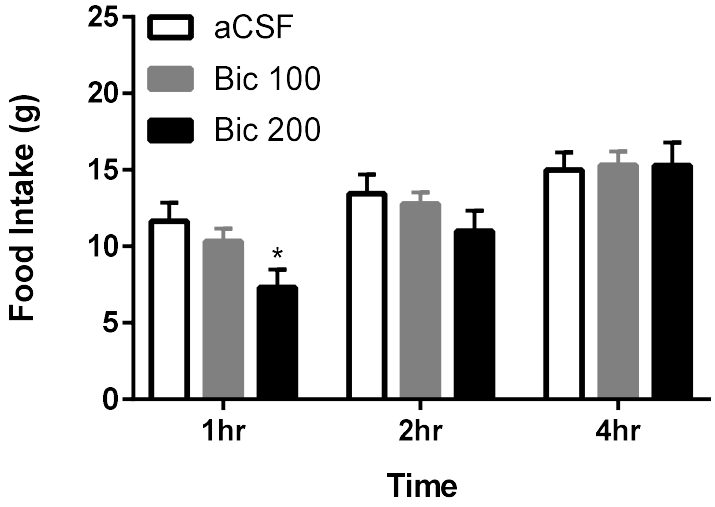

Figure 4.1 Mean cumulative food intake of 24-hr fasted rats following PVT injection of BIC or vehicle given just before refeeding. BIC dose-Dependently suppressed food intake (g ± SEM) 1 h after injection into the PVT. n = 15 rats. * $p<0.05$ compared to vehicle at matched postinjection time.

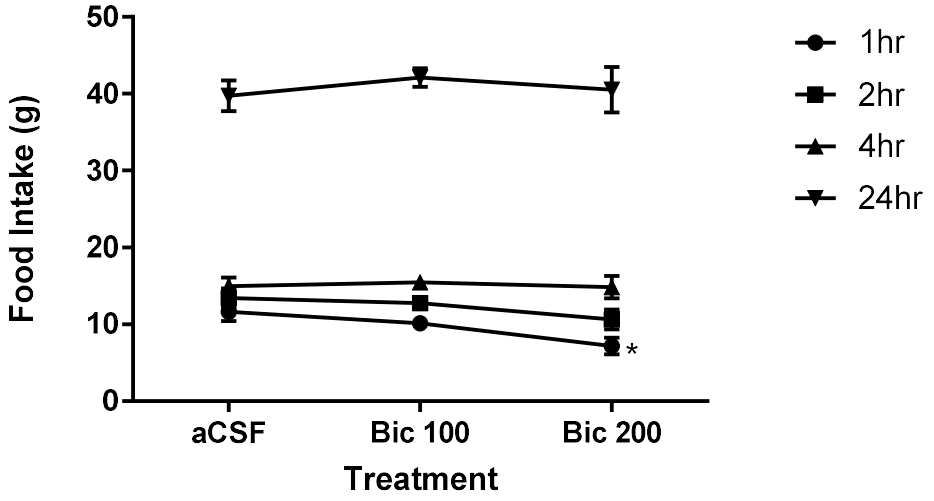

Figure 4.2 Mean cumulative food intake (g ± SEM) as a function of hours postinjection in 24-hr food-deprived rats given PVT injection of BIC (0, 100 or 200 ng). n = 15 rats. *p<0.05 compared to vehicle at matched postinjection time.

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Summary and Conclusion

Obesity, in its simplest terms, is excess body weight for a given height, brought about by an imbalance between energy intake and energy expenditure. Given this broad view, public health strategies have promoted changes in lifestyle, from improving eating habits to increasing physical activity, in an effort to combat the rise in this epidemic. However, it is becoming increasingly clear that the propensity for obesity to develop in some people but not others is not simply due to a lack of individual effort or choice. Various avenues of scientific research have demonstrated that obesity is a complex disease, and its underlying cause is due to interplay between genetic and environmental factors acting on brain systems that regulate feeding and energy homeostasis. In recognizing that obesity is a disease and that dysregulation of these systems is in part responsible, continued research and a better understanding of these central mechanisms is essential in the effort to discover more effective therapies towards fighting obesity.

The goal of the present studies was to elucidate the role of GABA in the PVT in the control of feeding. Despite being the predominant inhibitory amino acid neurotransmitter in the mammalian brain, previous studies have found that GABA plays a fundamental and specific role in the regulation of food intake. For example, muscimol stimulated food intake when injected into the ventromedial hypothalamus; conversely, blockade of $GABA_A$ receptors in the LH also elicited feeding (Grandison & Guidotti, 1977;

Turenius et al., 2009). Importantly, the opposite effects of GABA seen in these studies demonstrate that the role of GABA in feeding is highly dependent upon the brain region upon which it is acting. We investigated GABA in the paraventricular nucleus of the thalamus (PVT), a region of interest given that it receives multiple feeding-related peptidergic inputs from the hypothalamus and in turn projects to ventral striatal regions such as the amygdala and nucleus accumbens. The PVT is therefore situated in an anatomically unique location to integrate information related to energy balance control and to influence motivated behaviors including feeding and reward.

In our initial experiments, we confirmed previous findings that the $GABA_A$ receptor agonist muscimol elicited feeding in the PVT (Stratford & Wirtshafter, 2013). This was accomplished by using the central injection technique in order to administer the agonist directly into the PVT of satiated rats and observe its effect on feeding behavior. We then proceeded to ask the following questions: 1) Are different GABA receptor subtypes differentially involved in mediating feeding in the PVT? In other words, are $GABA_B$ receptors, also widely expressed within this brain area, also involved with food intake control? 2) Was the observed effect anatomically specific? That is, do the anterior and posterior subregions of the PVT both mediate feeding in similar ways? 3) Are $GABA_A$ receptors in the PVT involved in natural feeding? We thus determined if pharmacological blockade of $GABA_A$ receptors in the PVT would block deprivation-induced feeding.

Our results showed that the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen both produced a dose-dependent increase in feeding in satiated rats, suggesting that inhibition of the PVT by GABA results in feeding behavior and that it does so via actions on both $GABA_A$ and $GABA_B$ receptor subtypes present within the PVT. One issue of concern is that these agonists are producing nonspecific behavioral effects which then manifest as an increase in feeding behavior. This is especially notable since GABA receptors are ubiquitously expressed. In addition, the PVT has been linked to multiple other behaviors and inhibition of a brain area with GABA agonists could lead to changes in any of these behaviors. While feeding was dose-dependently increased, and the animals appeared to be engaged in feeding behavior with a short latency, it would still be appropriate to conduct a behavioral profile on the rats following injection with the GABA receptor agonists to rule out any significant changes in, for example, water intake or locomotor activity, that may confound a specific role in stimulating feeding.

Another question with regards to the role of GABA in the PVT in the control of feeding relates to the anatomic specificity of the effect. Specifically, we wanted to determine whether the aPVT and pPVT would differentially mediate feeding induced by GABA. Recent studies have identified differences in connectivity between the two subdivisions of the PVT; however, the functional significance of this in relation to feeding has not been consistently addressed. Injection of the $GABA_A$ receptor agonist muscimol into the aPVT and pPVT increased food intake, but the effect was more

pronounced in the pPVT. On the other hand, the $GABA_B$ receptor agonist baclofen produced a consistent eating response in the pPVT but failed to have any effect in the aPVT. The results of this study suggest that the pPVT is the primary site mediating GABA-induced feeding in the PVT and that there are differences in activity across the aPVT and pPVT. The possibility that these two subregions play distinct roles related to feeding remains to be determined. One concern not presently addressed is the extent to which diffusion of the injections to neighboring sites might account for the observed feeding stimulatory effects. Although our results are in agreement with others' that the PVT, at least the posterior portion, is involved with feeding, a cannula mapping study would be required to rule out the possibility that adjacent areas are responsible.

Although we demonstrated that pharmacological inhibition of the PVT with a $GABA_A$ receptor agonist stimulated food intake, a third question was whether the PVT and GABA receptors within the PVT play a role in natural eating behavior. We found that blockade of $GABA_A$ receptors with PVT injection of bicuculline suppressed feeding induced by 24 hr period of food deprivation. These results suggest that endogenous GABA plays a physiological role in regulating eating behavior. One hypothesis is that food deprivation causes the release of endogenous GABA and this GABA is normally acting on PVT $GABA_A$ receptors to induce feeding. The involvement of PVT $GABA_B$ receptors in deprivation-induced eating, or of either receptor subtype in the spontaneous feeding at the onset of the dark cycle, was not addressed in these experiments. Given that $GABA_A$ blockade produced only a short-term reduction in the

feeding effect, it is possible that GABA was still acting on $GABA_B$ receptors to increase feeding; alternatively, deprivation may produce other unaccounted changes, through other neurotransmitters or brain areas, to prevent a complete suppression of the effect.

Taken together, the results of the current experiments suggest that the PVT is involved in the regulation of eating behavior and that, in particular, inhibition of a population of neurons within the PVT, through $GABA_A$ and/or $GABA_B$ receptors, is sufficient to induce feeding. In addition, there are regional differences across the anteroposterior axis of the PVT, at least with regards to food intake, where pPVT neurons are more sensitive to GABA. That a $GABA_A$ receptor antagonist suppressed deprivation-induced eating also indicates that the PVT is involved in natural eating. Although the afferents and efferents of the PVT are diverse, it appears to be precisely situated to communicate between key brain structures that can modulate motivated behaviors, including feeding. A summary of relevant connections is shown below in Fig. 5.1. The PVT receives input from hypothalamic nuclei that convey signals related to energy balance and circadian rhythms, and in turn sends excitatory, glutamatergic projections to limbic areas including the shell of the NAc and CeA, known to be involved in reward, motivation and mood. Inhibition of the PVT, particularly its posterior subregion, through multiple GABAergic sources, including the LH, ArcN and zona incerta, stimulates feeding. This inhibition would halt glutamatergic input to the NAcSh, consistent with previous findings that glutamate levels are decreased in the NAc during feeding (Saulskaya & Mikhailova, 2002). The PVT also receives neurochemically diverse

monoaminergic and neuropeptide inputs related to feeding. PVT excitation through LH orexinergic innervation also stimulates feeding, presumably through a different population of neurons. In summary, the PVT is situated to integrate signals from brain areas related to energy and internal states and output to structures related to the emotional saliency of stimuli, thereby guiding behavioral responses such as towards feeding. Many of the studies relevant to this proposed circuit are summarized in Table 1.

Obesity is a complex disorder in part because the neural circuits controlling ingestive behavior and energy homeostasis are very complex. This control arises from the coordinated activity of multiple brain regions, forming distinct networks that govern different aspects related to eating, from hunger and satiety to the palatability of food to learning and memory. In focusing even on an individual nucleus such as the PVT, there is still a great deal of research that needs to be done. For example, there are other neurotransmitter and neuropeptide inputs to the PVT that have received less attention, while connections to or from the PVT are still being investigated. Future studies are needed to incorporate these and other systems into our understanding of the circuits regulating appetite and how dysregulation of these circuits ultimately lead to obesity.

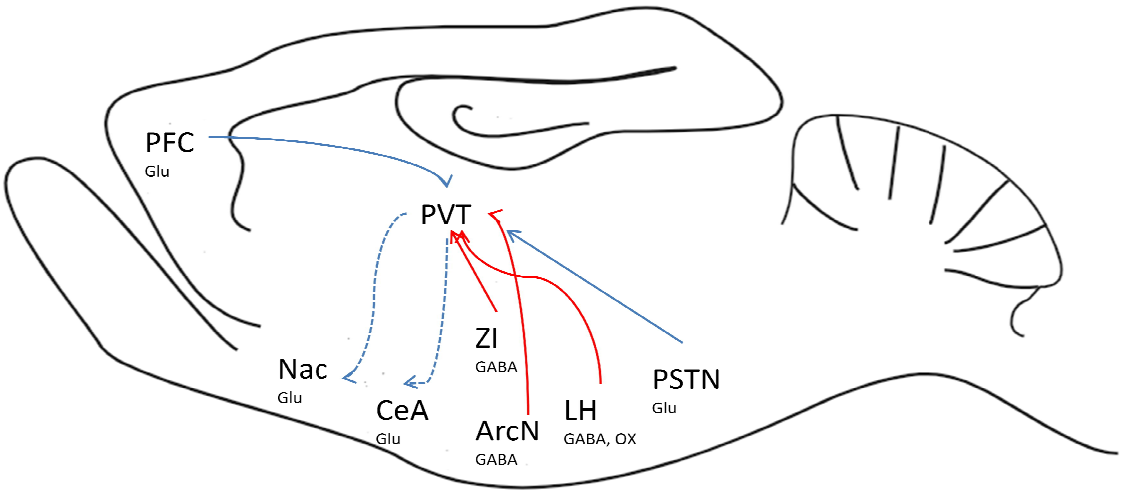

Figure 5.1 Schematic diagram of paraventricular thalamic nucleus (PVT) circuitry mediating food intake. The PVT receives GABAergic input from multiple sources including the lateral hypothalamus (LH), arcuate nucleus of the hypothalamus (ArcN) and zona incerta (ZI). The LH also sends excitatory projections containing orexin to the PVT. In turn, the PVT sends glutamatergic (Glu) projections to the nucleus accumbens (NAc) and central nucleus of the amygdala (CeA) to differentially modulate intake. The PVT is also innervated by glutamatergic signals arising from the parasubthalamic nucleus (PSTN) and prefrontal cortex (PFC). Red lines: GABAergic, blue lines: glutamatergic, Dashed lines: outputs.

Table 1 PVT Manipulations and their Effect on Feeding Behavior

Manipulation	Effect	Study
Ibotenic acid lesion	↑food intake	(Bhatnagar & Dallman, 1999)
Muscimol	↑food intake	(Stratford & Wirtshafter, 2013)
OX-A	↑sucrose intake	(Barson et al., 2015)
Chemogenetic inhibition	↑food intake	(Zhang & van den Pol, 2017)
Optogenetic stimulation of PVT VGlut2	↓food intake	
Optogenetic stimulation of ZI_{GABA}→PVT LH_{GABA}→PVT	↑food intake	
Optogenetic stimulation of Arc AgRP→PVT	↑food intake	(Betley et al., 2013)
OX1R knockdown	↓High-fat diet intake	(Choi et al., 2012)
Optogenetic stimulation of PVT Glut2→Acb	↑sucrose seeking	(Labouebe et al., 2016)
Muscimol (when reward omitted)	↑sucrose seeking	(Do-Monte et al., 2017)
Photoinhibition aPVT→NAc	↑sucrose seeking	
Photoinhibition aPVT→amygdala	↓sucrose seeking	
Optogenetic inhibition PFC→PVT	↑anticipatory licking	(Otis et al., 2017)

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