The Neural Encoding of Reward in the Striatal-Pallidal Circuitry

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STRIATAL-PALLIDAL CIRCUITRY

by

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ABSTRACT
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Humans and animals are constantly exposed to external stimuli. The ability to process reward value of a stimulus is critical to guiding appropriate behavior and essential for survival. These processes are regulated by neuronal activity and neurochemical signaling in the reward circuitry, particularly in the nucleus accumbens (NAc). The NAc receives dopaminergic inputs from the midbrain ventral tegmental area (VTA) and sends GABAergic projections to the ventral pallidum (VP). Electrophysiological studies have characterized phasic neuronal responses in the NAc that differential encode appetitive and aversive taste stimuli. Exposure to an appetitive taste stimulus evoked predominantly phasic inhibitory responses in the NAc whereas a majority of responses to an aversive taste was excitation. The work presented here focused on investigating how activity in the NAc modulate reward encoding in downstream VP, and the role of dopamine signaling in regulating neuronal responses to reward in the NAc.

Using electrophysiological recording techniques, we present evidence of neural encoding of reward information in the VP. VP neurons responded to appetitive and aversive taste stimuli with primarily inhibitory and excitatory responses, respectively. Furthermore, devaluation of the appetitive stimulus resulted from cocaine-induced taste aversion conditioning revealed that the encoding of sucrose shifted from inhibition to excitation, resembling that of an aversion response. These data suggest that the VP, similar to the NAc, also encode reward information neuronally.

In a subsequent study, the influence of NAc on VP reward encoding was tested by pharmacologically manipulating activity in the NAc while monitoring the neuronal activity in the VP. We demonstrate that by inhibiting activity in the NAc with a GABAergic agonist, the neural encoding to sucrose in the VP was augmented, followed by increased sucrose consumption. These findings support the notion that at least some aspect of reward information processed in VP is modulated by NAc activity.

In the final study, we show that chemogenetically suppressing activity of VTA dopamine neurons inverted the response profile to sucrose from inhibition to excitation in the NAc. This elimination of inhibitory reward encoding in the NAc was accompanied by a dampened motivational state, demonstrated by subjects terminating leverpress behavior for sucrose reward quicker in a progressive ratio test on day that dopamine signaling was chemogenetically suppressed but not in control condition.

Taken together, results from these studies provide insights into how reward information is represented by physiological events in the reward circuitry. We demonstrate that neuronal responses in both the NAc and the VP encode reward and correlate strongly to reward-driven motivated behavior. Furthermore, we used a chemogenetic approach to show that suppressed NAc dopamine signaling models a low motivational state that is represented by altered neuronal responses in the NAc. This endeavor to better understand the neural representation of reward may help us better understand the physiology of both normal and diseased motivational and affective states.

ACKNOWLEDGEMENTS

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Chapter I

INTRODUCTION

Part 1: General Introduction

Reward and affective neuroscience

The experiences of pleasure and displeasure and the emotional states they engender (e.g. joy and sadness) are key components of the human experience. Experience-driven changes in emotional state not only allow us to relish joyful moments in life, but also promote many basic functions that are critical to survival. Positive affective states are triggered by rewarding stimuli, which can be as simple as the smell of coffee in the morning or seeing a loved one. The ability to accurately process reward information is critical to guiding appropriate behavioral response (e.g. consumption of food) promoting experience-based learning (e.g. learning where to find food). These states can be powerful determinants of human behavior in adaptive ways (e.g. bonding with family) and maladaptive ways (e.g. drug seeking). For this to occur there must be a mental representation of environmental stimuli, and the emotions they engender. The focus of this dissertation is on characterizing the nature of reward and aversion encoding in specific brain areas, and the connectivity between these areas, in order to better understand how experiences translate to motivated behavior.

Dysregulation of the reward system contributes to the pathology observed in multiple psychiatric disorders characterized by emotional and motivational deficits, including depression, eating disorders, post-traumatic stress disorder, and addiction (Kent

Berridge, Ho, Richard, & DiFeliceantonio, 2010; Carelli & Wightman, 2004; Peter Kalivas & Volkow, 2005; Russo & Nestler, 2013). In particular, both human and animal studies have demonstrated that depression is directly linked to reduced activity in the limbic reward circuitry (Baumann et al., 1999; Francis et al., 2015; Heller et al., 2009). These disorders pose significant social and economic burdens in addition to straining the lives of people that are affected. A great deal of effort has been expended researching the neurological basis of these disorders, yet therapeutic development has been elusive. Moreover, the comorbidity of these disorders is high, with 30-40% of individuals suffering from addiction also exhibiting symptoms of mood disorders (Conway, Compton, Stinson, & Grant, 2006), suggesting dysregulation of common neural circuits. Developing a better understanding of how affect and motivation are being generated in the reward circuitry can provide valuable insights into the neurobiology of normal and dysregulated affective and motivational states.

Hedonics and motivation

Pleasurable stimuli can induce positive affective states, through a process that is mediated by the reward circuitry. Not only are rewarding stimuli capable of affecting mood and promoting appetitive behaviors, they also promote associative learning that can help guide behavior in the future when encountering the same stimuli. These processes therefore can help guide adaptive behavior that allows animals to interact with the external environment efficiently. Reward as a psychological construct can be viewed as having two dissociable components, namely "hedonic liking" and "motivational wanting" (Berridge & Kringelbach, 2015). Understanding the neural underpinnings of these

elements is crucial to understanding the neurobiology of affect, necessitating the development of experimental approaches that can separate them.

Dissociating "liking" and "wanting", or "hedonic" and "motivational" (terminology that will be used more frequently in this manuscript), in animals requires careful experimental planning since these are inherently intertwined behavioral processes. For example, methods evaluating how much an animal enjoys a certain rewarding stimulus frequently require voluntary approach directed toward a food receptacle or a liquid bottle, confounding hedonic measures with motivated approach behavior. To circumvent this, a taste reactivity test provides a plausible way to experimentally separate "hedonic liking" from "motivational wanting". By intraorally delivering taste stimuli, hedonic behavioral responses can be assessed without engaging other behaviors. While these processes may not be completely separable, this approach likely provides the best way to measure "hedonic liking", without engaging motivational mechanisms.

Measuring hedonics with taste reactivity

Despite Darwin's proposal over a century ago, that behavioral expression can reliably reflect an animal's emotional state (Darwin & Ekman, 1872), this idea remains somewhat controversial. The taste reactivity test has been proposed as a way to access otherwise hidden brain processes, like hedonic processing (Berridge, 2000). Appetitive and aversive taste reactivity were thoroughly characterized by Grill and Norgren (1978). They noted that animals exhibit different stereotypical orofacial and behavioral responses to appetitive and aversive taste stimuli. These responses are observed across various mammalian species and are thought to reflect the palatability of the stimulus (Berridge,

2000; Jankunis & Whishaw, 2013; Kiefer, Hill, & Kaczmarek, 1998; Steiner, Glaser, Hawilo, & Berridge, 2001). In this design, intraoral catheters are surgically implanted to allow passive infusions of solutions, thereby bypassing the need for animals to engage in goal directed action to obtain a reward. The subjective feeling towards a given taste stimulus is evaluated by measuring the frequency of appetitive and aversive taste responses expressed by the animals. Typical appetitive taste response includes lateral tongue protrusions, whereas aversive responses include gapes, wet dog shake, and paw flailing. Rats that receive intraoral catheter surgery and intact rats both exhibit similar taste reactivity responses (Gray & Cooper, 1995), showing that this surgical approach does not impede or change the normal perception of tastants. In addition, behavioral paradigms like conditioned taste aversion (CTA) have been used in combination with taste reactivity to examine how taste reactivity reflects the hedonic valence of a tastant, rather than innate taste properties. Thus this technique is useful for determining how hedonic information is encoded in the brain (Berridge, 2000; O'Doherty, Deichmann, Critchley, & Dolan, 2002). Many studies have demonstrated that the reward system, specifically the ventral striatum and its output targets, are heavily implicated in the processing of hedonic information (Calipari et al., 2016; Ho & Berridge, 2013; Kelley et al., 2002; Pecina, & Berridge, 2000; Smith & Berridge, 2007; Smith, Berridge, & Aldridge, 2011; Xiu et al., 2014), making these structures interesting targets for investigating the potential encoding of hedonic information.

Gauging motivation

Hedonic perception and motivation are related elements of reward. For example, a highly palatable food is capable of creating a stronger motivational drive than a less palatable food, and more likely to modulate animal behavior. The ability of a stimulus to motivate behavior has been characterized as "incentive salience" and this phenomenon is thought to reflect "wanting" (Berridge & Robinson, 1998). Popular methods used to assess motivation include measuring voluntary intake of food or appetitive solutions (e.g. sucrose) from a food bowl or bottle (Bakshi & Kelley, 1993; Galistu & D'Aquila, 2012; Tindell, Smith, Peciña, Berridge, & Aldridge, 2006). These tasks directly measure reward-driven motivation. A derivative of these tasks is to measure motivation by evaluating the level of effort subjects are willing to employ to obtain a reward under different conditions, which has also provided important insights into the functions of dopamine in reward (El-Ghundi, O'Dowd, Erclik, & George, 2003; Randall et al., 2012). Many of these motivational tasks require the animals to be able to form an association between the conditioned stimulus (e.g. sucrose reward, drugs of abuse) and the conditioned response (e.g. lever press, nosepoke), a learning process that requires dopamine (Flagel et al., 2011).

Motivated behavior driven by affect

Animals are naturally driven or motivated to behave in ways that help them maintain a positive affective state (LeDoux, 2012; Thayer, Newman, & McClain, 1994). The relationship between affect and motivation is particularly noticeable in psychiatric disorders such as depression and addiction, with depressed patients possessing both

lowered mood and motivation, and addicted patients reporting the desire to use drugs to self-medicate a low mood state (Fox, Hong, Siedlarz, & Sinha, 2008; Sinha, Fuse, Aubin, & O'Malley, 2000). At the same time, individuals with substance abuse disorder lose interest in activities they had previously enjoyed and have difficulties abstaining from drug use despite the conscious awareness of the negative consequences (American Psychiatric Association, 2013). As demonstrated by self-report data, the strongly aversive affective and physiological states experienced during drug craving are proportional to the euphoric effects produced by drug use (Newton, Kalechstein, Tervo, & Ling, 2003). More importantly, presentation of drug-associated cues alone can induce strong craving that can promote drug use and relapse (Fox et al., 2008; Paliwal, Hyman, & Sinha, 2008; Sinha et al., 2000). Both findings suggest a strong correlation between negative mood state and motivation for drugs of abuse. This idea corresponds to an emerging understanding of the relationship between stress and addiction, in which stress has been implicated as one of the main factors that can facilitate relapse of substance abuse (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004; Fox et al., 2008; Paliwal et al., 2008; R. Sinha et al., 2000; Rajita Sinha, Catapano, & O'Malley, 1999). Clues to whether and how negative affect can modulate motivated behavior potentially lie within the activity of the ventral striatum, where both affect and motivation are being encoded and regulated.

Neural correlates of affective state

The processing of reward information involves midbrain dopaminergic signaling and the activity of various limbic and cortical structures. An essential locus of this system is the ventral striatum, also known as the nucleus accumbens (NAc). Activity in the NAc

is heavily regulated by midbrain dopamine input from the ventral tegmental area (VTA). Dopamine concentration in the NAc increases in response to rewarding stimuli, and decreases in response to aversive stimuli (Figures 1.1A-B) (Roitman, Wheeler, Wightman, & Carelli, 2008). In addition, NAc dopamine is elicited not only by rewarding stimuli, but also by their predictors (Carelli, 2004; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Wheeler et al., 2015). Reward information is also encoded by neuronal activity in the NAc. Electrophysiological recording techniques have been used to capture changes of neuronal activity in response to hedonic experiences or motivational tasks in awake, behaving animals. Appetitive and aversive gustatory stimuli elicit different neuronal response profiles in the NAc, with palatable stimuli eliciting predominantly reduced activity while aversive stimuli predominantly evoke increased activity (Figures 1.1C-D) (Roitman et al., 2008; Roitman, Wheeler, & Carelli, 2005; Twining et al., 2015). Such findings provide a strong foundation for expanding the systems-level characterization of reward encoding presented in subsequent chapters. The following sections are intended to better describe the reward circuit.
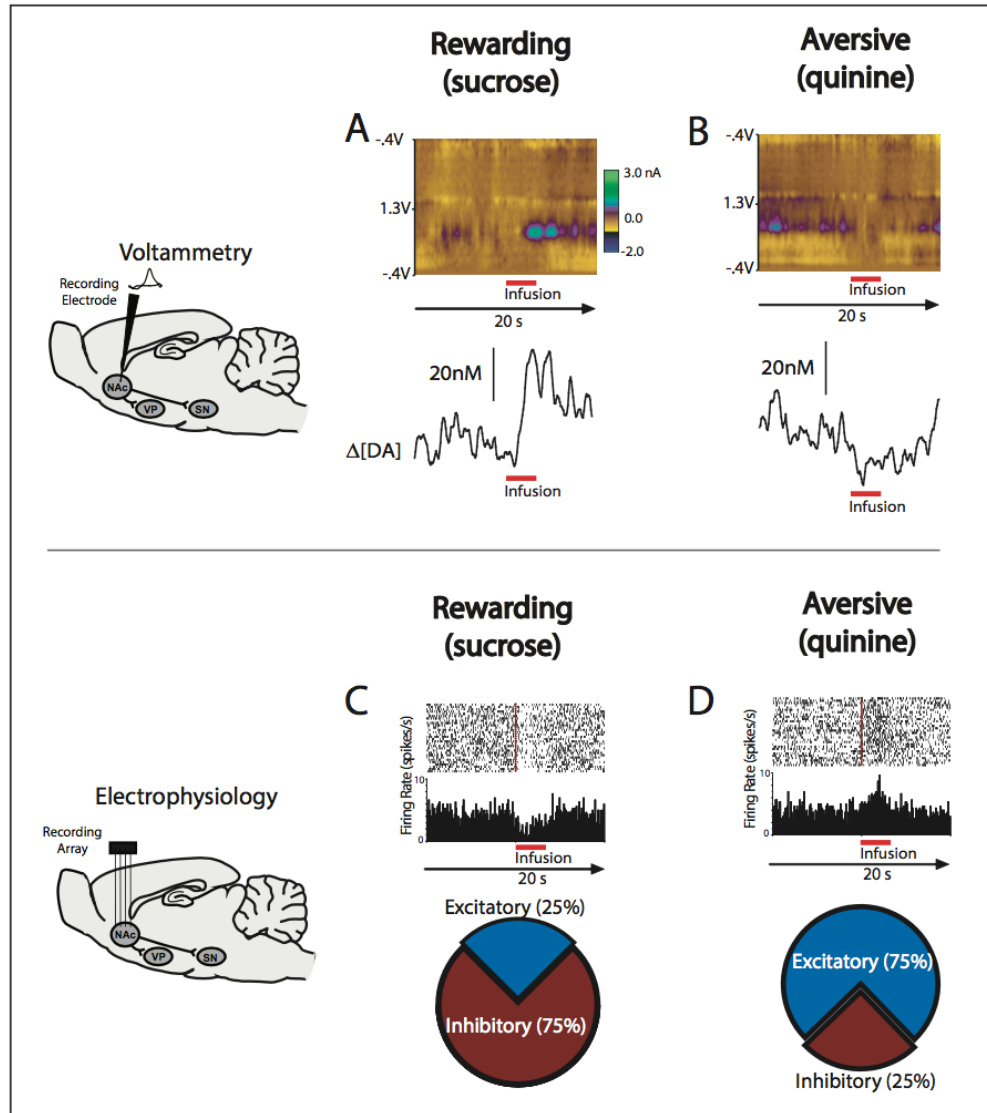
Figure 1.1 Voltammetric and electrophysiological recordings characterized responses to appetitive and aversive taste stimuli in the NAc.
Intraoral infusions of sucrose (A) caused a phasic increase in dopamine level while quinine infusions (B) resulted in a phasic reduction of dopamine. Average dopamine concentration (solid lines) during infusions (indicated by red horizontal bar) are presented. Colorplot above depicts current change in false color (z-axis) plotted against time (x-axis) and voltage ramp (y-axis). Current change reflecting the oxidation of dopamine is observed at approximately 0.64 V. Electrophysiological recordings revealed that sucrose infusions (C) primarily evoked phasic inhibitory responses in the NAc while quinine (D) evoked primarily excitatory responses. Perievent-histograms above represent neuronal firing rate (spikes/sec) across trials (y-axis) that are aligned to tastant infusions (indicated by red horizontal bar, x-axis). Pie charts below represent the proportion of phasic inhibitory and excitatory responses observed in response to solution deliveries.
Figure adapted from (Wheeler et al., 2008, 2011)

Part II: The Reward Circuitry

Structures that regulate reward

The subjective experience of pleasure elicited by a diverse array of stimuli involves the recruitment of a distributed neural network. However, several studies have found common brain structures that are associated with pleasure, regardless of the type of experience (Cacioppo, Bianchi-Demicheli, Frum, Pfaus, & Lewis, 2012; Georgiadis & Kringelbach, 2012; Parsons, Young, Murray, Stein, & Kringelbach, 2010; Salimpoor, Benovoy, Larcher, Dagher, & Zatorre, 2011; Xu et al., 2011; Zeki & Romaya, 2010). Pharmacological and behavioral studies over the past decades have revealed a significant amount of information about the neural circuitry of reward. Structures in the reward circuitry such as the NAc have been shown to affect the expression of taste reactivity, demonstrating the importance of specific areas of the reward circuit in regulating hedonic perception (Peciña et al., 2000). The nuclei most frequently implicated in the processing of reward include the NAc, the VTA, the prefrontal cortex (PFC), the ventral pallidum (VP), the hippocampus, the thalamus, and the amygdala (Athos, Impey, Pineda, Chen, & Storm, 2002; Daumas, Halley, Francés, & Lassalle, 2005; Jones, Day, Wheeler, & Carelli, 2010; Papp et al., 2012; Rolls, 2015; Russo & Nestler, 2013; Sesack & Grace, 2010; Tachibana & Hikosaka, 2012; Van Zessen, Phillips, Budygin, & Stuber, 2012). These structures in the reward circuitry regulate various aspects of reward to guide approach behavior and reward learning. It is important to note that the reward circuitry is a complicated and heavily interconnected system in which reciprocal projections are present between most structures. Figure 1.2 demonstrates some of the key nuclei mentioned above and illustrates the critical neurotransmitter systems involved. The

midbrain VTA sends dopaminergic projections to the NAc, the PFC, the amygdala, the VP, and the hippocampus (Gasbarri, Packard, Campana, & Pacitti, 1994; Hnasko, Hjelmstad, Fields, & Edwards, 2012; Kabanova et al., 2015; Oades & Halliday, 1987). However, the VTA projection to the NAc has been a particular focus of reward and motivation research. Indeed, this dopaminergic pathway appears to be critical for initiating motivated behavior for natural rewards and drugs of abuse (Bradberry & Roth, 1989; Pascoli, Terrier, Hiver, & Lüscher, 2015; Roberts & Koob, 1982). The NAc sends strong projections to the VP, making it a main output target. Functionally speaking, the NAc and the VP are both important for hedonic information processing (Berridge & Kringelbach, 2013). Many of the structures that receive dopaminergic inputs from the VTA also appear to receive serotoninergic and noradrenergic projections from other midbrain structures, including the raphe nuclei and pontine locus coeruleus, which can also regulate affect (Venkatraman, Edlow, & Immordino-Yang, 2017).

Overall, a significant amount of information is known about the brain structures, the anatomical projections, and the neurochemical systems that are involved in reward processing, yet the specific neuronal activity required to create mental representations of reward and reward-directed behavior remain mostly undetermined.
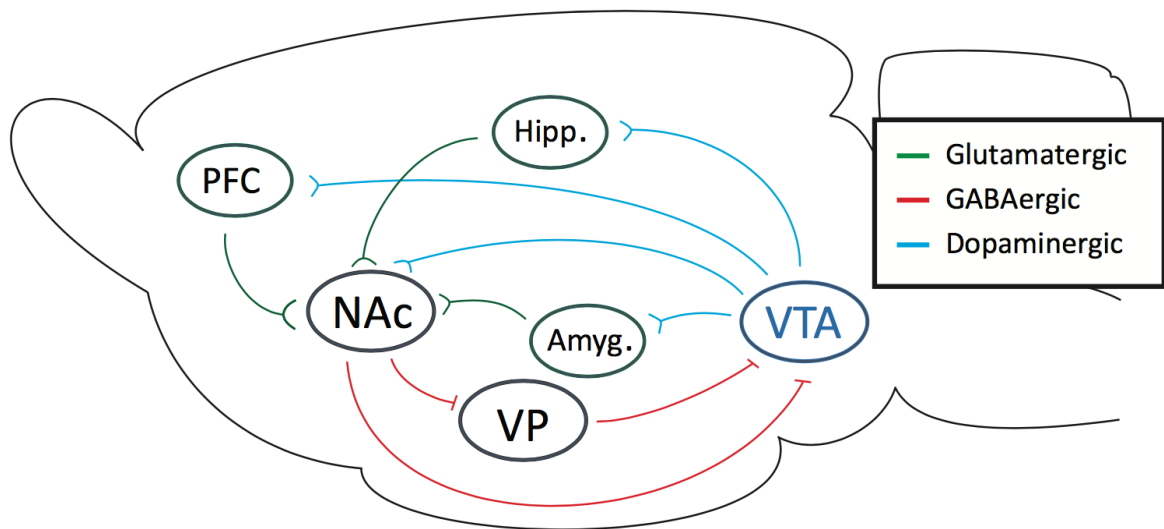
Figure 1.2. Simplified schematic of the reward circuitry implicated in aspects of reward processing and learning.
The generally accepted roles for these nuclei in reward processing are as follows. The NAc and the VP are heavily involved in motivated behavior and hedonic processing, the PFC mediates decision making processes, the amygdala processes emotion-related memories and salient stimuli, while the hippocampus processes associative and salient stimuli. PFC-prefrontal cortex, NAc-nucleus accumbens, VP-ventral pallidum, Hipp.- hippocampus, Amyg.-amygdala, VTA- ventral tegmental area.

Part III: The Nucleus Accumbens

Neuroanatomy

The NAc is a key structure in the basal ganglia that is critically involved in regulating motivated behavior. The NAc is located medial to the septum and forms the ventral striatum together with the olfactory tubercle. As illustrated in Figure 1.2, the VTA, the amygdala, and the PFC all project to the NAc, putting it in an ideal location for integrating reward information to guide motivated behavior. Several decades of research have defined the NAc as being a center of limbic motor integration. This limbic reward center is critical for reward seeking as demonstrated with a wide range of rewarding

stimuli (Aragona et al., 2006; Kelley, 2004; Mogenson, Jones, & Yim, 1980; Stratford & Kelley, 1997). Glutamatergic projections from the PFC, basolateral amygdala (BLA), and the hippocampus converge on the medium spiny neurons (MSNs) in the NAc carrying information about memory, context and emotion (Brog, Salyapongse, Deutch, & Zahm, 1993; Groenewegen, Wright, Beijer, & Voorn, 1999; McGeorge & Faull, 1989; Zahm & Brog, 1992). The principle neurons in the NAc are gama-aminobutyric acid (GABA)-containing MSNs that represent over 90% of the neuronal population in the NAc, with the remaining being GABAergic or cholinergic interneurons. The NAc MSNs can be classified into two distinct populations based on whether they express D1-like receptors (D1R) or D2-like receptors (D2R). Most MSNs express either D1Rs or D2s, with an approximation of 5-17% that express both (Bertran-Gonzalez et al., 2008). It has been estimated that a majority of NAc D2R-MSNs are in a high-affinity state whereas the D1Rs are in a low-affinity state, meaning that most D2Rs are occupied under tonic dopamine neuron firing conditions (Rice, Patel, & Cragg, 2011; Richfield, Penney, & Young, 1989). Histological features and characteristics in afferent and efferent connections have defined two major subregions of the NAc, the core and the shell (Brog et al., 1993; Jongen-Relo, Groenewegen, & Voorn, 1993; Záborszky et al., 1985; Zahm, 1999).

Connectivity

The NAc sends its projections to cortical structures, other basal ganglia structures, thalamic regions, and the VTA (Sesack & Grace, 2010; Watabe-Uchida, Zhu, Ogawa, Vamanrao, & Uchida, 2012). A critical component of the reward pathway is the direct

projection from the NAc to the VTA, often referred to as the "direct pathway". The direct-pathway projection is comprised of D1R-expressing projecting MSNs (Wall, De La Parra, Callaway, & Kreitzer, 2013). Approximately half of the efferent projections from the NAc go through the VP, composing the "indirect-pathway". Unlike the direct-pathway, the indirect pathway consists of both D1R- and D2R- expressing neurons (Kupchik et al., 2015; Wall et al., 2013). Since the VP is a major output target of the NAc, hedonic and incentive signals from other regions that are processed in the NAc are likely to be conveyed in some form to the VP, modulating the activity patterns of VP neurons. This strong connectivity is likely reflective of the critical role that both structures play in regulating hedonic processing and motivated behavior.

Glutamatergic inputs from the PFC, BLA, and hippocampus synapse on both projecting MSNs as well as interneurons in the NAc. These inputs also form an organized but complex network with the NAc, with glutamatergic inputs synapsing onto both the direct pathway neurons and the indirect pathway (Surmeier, Ding, Day, Wang, & Shen, 2007). NAc MSN activity is heavily regulated by dopaminergic input from the VTA. These projections form synapses on the necks of dendritic spines receiving glutamatergic input from the cortex, BLA and hippocampus. This positioning provides dopamine signaling the ability to regulate sensitivity to excitatory drive (Rice et al., 2011; Sulzer, Cragg, & Rice, 2016). Further, recent evidence indicates that some projections from the VTA can co-release glutamate and GABA (Hnasko et al., 2012; Root et al., 2014; Tritsch, Ding, & Sabatini, 2012), adding another layer of complexity to the regulation of reward encoding.

Role in regulating reward

Much of the research aimed at determining the locus of motivational and hedonic

processing has targeted inputs to, and the activity of, the NAc. In animals, microinfusions

of opioid-receptor agonists and GABAergic agonists into the NAc shell drastically

increase feeding and sucrose drinking, with mu-opiate receptor agonists having the

greatest effect (Bakshi & Kelley, 1993; Basso & Kelley, 1999; Kelley et al., 2002; Zhang

& Kelley, 2000; Zhang, Gosnell, & Kelley, 1998; Zhang & Kelley, 1997). The NAc is

divided into core and shell subregions and primary reward information appears to be

preferentially processed in the shell subregion. Pharmacological manipulations that alter

appetitive and consummatory behavior have specific and pronounced effects in the NAc

shell (Kelley & Swanson, 1997; Kelley & Berridge, 2002; Peciña & Berridge, 2005).

Further, the shell is preferentially involved in processing the primary rewarding effects of

drugs of abuse (Carlezon & Wise, 1996). Based on these studies, the NAc shell is thought

to be more involved in mediating the primary reinforcing properties of rewards while the

core is thought to mediate reward-related learning and conditioned responses (Di Chiara

& Bassareo, 2007; Ito, Robbins, & Everitt, 2004).

The "Hedonic hotspot" in the NAc

Immediate early gene expression studies have identified a hedonic hotspot in the

anterior portion of the NAc shell that takes up approximately 10% of the overall NAc

volume (Peciña & Berridge, 2005). Stimulation of opioid signaling through

microinfusions of the mu-opioid receptor selective agonist (DAMGO) in this part of the

NAc strongly augments the expression of appetitive taste reactivity to a sweet solution

(Peciña & Berridge, 2005; Smith et al., 2011). Interestingly, agonists for kappa and delta-opioid receptors administered in this restricted area also resulted in the same appetitive effect (Castro & Berridge, 2014). These studies also discovered that microinfusion of opioid agonists anywhere else in the NAc resulted in either no effect or the opposite effect on taste reactivity, indicating that the appetitive effect is specific to this hedonic hotspot. The unique role of the NAc hedonic hotspot in reward processing could be the result of a combination of the features of neurochemical interaction and connectivity patterns. For instance, mu-opioid signaling has a stronger effect in modulating dopamine signaling in the NAc hedonic hotspot (Britt & McGehee, 2008). It has also been demonstrated that the hedonic hotspot expresses distinct connectivity features when compared to the rest of the NAc (Thompson & Swanson, 2010; Zahm, Parsley, Schwartz, & Cheng, 2013).

Aversion in the NAc

Imaging studies provided evidence that the NAc is involved in aversion (Levita et al., 2009), with changes in striatal activity observed in response to both punishment and reward (Delgado, Nystrom, Fissell, Noll, & Fiez, 2000; Delgado, 2007). Studies using invasive techniques in rats also support this idea. Recent studies using a reversible pharmacological neurotransmission blocking technique (RNB) demonstrated evidence that the striato-pallidal indirect pathway is important for aversion learning (Hikida et al., 2013; Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010). Furthermore, activity in the NAc has been implicated in mediating negative affect, with microinfusions of GABA agonists in the caudal NAc causing fear behaviors (Reynolds & Berridge, 2001, 2002,

2003; Salamone, 1994). The caudal part of the NAc where aversion can be evoked

pharmacologically was therefore termed the hedonic coldspot. In the same studies, when

microinfusions took place between the anterior hedonic hotspot and the caudal hedonic

coldspot, a mixed of appetitive and fearful behaviors were observed. Intriguingly, while

both a GABAA agonist and a glutamatergic antagonist caused defensive behaviors when

microinfused into the caudal part of the NAc, only GABAergic manipulations affected

hedonic processing (taste reactivity). This observation suggests that GABAergic and

glutamatergic signaling, at least in this specific part of the reward circuitry, are not

opposingly regulating all behaviors. Taken together, these observations suggest that the

NAc is a functionally heterogeneous structure, with the anterior region processing reward

and the posterior region processing aversion. In accordance with this idea, human studies

have also found evidence that the ventral striatum responds to reward and aversion with a

rostral-caudal topographical representation (Seymour, Daw, Dayan, Singer, & Dolan,

2007).

In the NAc, dopamine has been implicated in both reward and aversion (Kravitz,

Tye, & Kreitzer, 2012; Lobo et al., 2010). Phasic reductions in dopamine concentration in

the NAc are associated with the perception of an aversive taste stimulus (Roitman et al.,

2008). While terminal measures of dopamine using microdialysis have produced mixed

results, electrophysiological recordings of putative dopamine neuron activity indicate that

most dopamine neurons increase firing rate for rewarding stimuli and reduce firing rate

for punishing stimuli (Bassareo, De Luca, & Di Chiara, 2002; Matsumoto & Hikosaka,

2009; Tobler, Dickinson, & Schultz, 2003; Ungless, Magill, & Bolam, 2004).

Electrophysiological studies also demonstrate that the NAc encodes aversion, as

unpalatable and devalued taste stimuli both evoke characteristic excitatory responses that differ significantly from the inhibitory response profile observed in response to rewarding stimuli, see figure 1.1 (Roitman, Wheeler, Tiesinga, Roitman, & Carelli, 2010; Wheeler et al., 2008).

It is not surprising that both reward and aversion are being represented in NAc activity, as they both guide motivated behavior. Aversive stimuli or associated cues possess the power to modulate mood and drive behavior under certain situations, and both of these processes are regulated in the reward circuitry (Carlezon & Thomas, 2009; McCutcheon, Ebner, Loriaux, & Roitman, 2012). The impact of exposing animals to aversive stimuli can be studied using a simple Pavlovian paradigm. For example, an aversive stimulus such as an electric shock can cause fear and subsequent avoidance of the location that the shock took place. In more sophisticated experimental designs, aversive stimuli (e.g. footshock) can be used as stressors to examine their effects on drug-seeking behavior. This behavioral design is thought to model the predictive relationship between stress, drug-associated cues, and negative affective states that predict relapse (Fox et al., 2008). In both of these designs, exposure to aversive stimuli results in an active behavioral response. However, the relationship between negative affect and behavioral redirection, as well as the underlying neuronal mechanism, remains elusive.

Part IV: The Ventral Pallidum as a NAc Output Target

Discovery as a hedonic generator

The role of the VP as a regulator of hedonic and motivated behavior was discovered when the lateral hypothalamus (LH) was lesioned to study its effect on

feeding behavior. The LH is a hypothalamic region adjacent to the VP and plays a key

role in the regulation of food intake and metabolism (Elmquist, Elias, & Saper, 1999;

Sohn, Elmquist, & Williams, 2013). Lesions of the LH result in severe hypophagia, to the

extent that animals would die of starvation (Elmquist et al., 1999). Electrical stimulation

of the LH drastically increases food consumption, and surprisingly, also can also increase

aversive taste responses (Berridge & Valenstein, 1991). It was later discovered that the

VP but not the LH was responsible for the effects on inducing aversive taste reactivity.

Careful mapping studies have shown that the magnitude of the aversion corresponds

better to the selective lesion of the VP (Cromwell & Berridge, 1993; Morgane, 1961;

Schallert & Whishaw, 1978; Teitelbaum & Epstein, 1962). The LH and its connectivity

to the VP are important components of the feeding circuitry (Castro, Cole, & Berridge,

2015), with orexin signaling between the two structures playing an especially important

role (Baldo, Daniel, Berridge, & Kelley, 2003; Marcus et al., 2001; Peyron et al., 1998).

Nevertheless, it is now well established that the VP is involved in regulating hedonics in

addition to consummatory behavior (Ho & Berridge, 2013; Shimura, Imaoka, &

Yamamoto, 2006; Smith & Berridge, 2005).

Neuroanatomy

The VP receives strong projections from the NAc and plays an important role in

hedonic processing. The pallidum is a morphologically diverse structure, containing

predominantly GABAergic neurons (Zahm, Zaborszky, Alones, & Heimer, 1985) and

some glutamatergic neurons (Hur & Zaborszky, 2005). Neurons in the VP receive direct

GABAergic inputs from the NAc as well as local GABAergic connections (Zahm et al.,

1985). Within the VP, there are cholinergic and GABAergic interneurons (Duque, Tepper, Detari, Ascoli, & Zaborszky, 2007). The entire VP expresses strong immunoreactivity for substance P as well as enkephalin (Groenewegen & Russchen, 1984; Heimer, Harlan, Alheid, Garcia, & de Olmos, 1997; Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991). Broadly speaking, the VP can be divided into the ventromedial VP (vmVP) and the much smaller dorsolateral VP (dlVP), which receive projections from the NAc shell (NAcS) and core (NAcC), respectively. The vmVP comprises the majority of the VP and expresses strong neurotensin immunoreactivity (Geisler & Zahm, 2006; Zahm, Williams, & Wohltmann, 1996). On the other hand, the dlVP exhibits strong calbindin-d28k immunoreactivity (Tripathi, Prensa, Cebrián, & Mengual, 2010; Tripathi, Prensa, & Mengual, 2013; Zahm et al., 1996). It has been proposed that the vmVP subregion which receives afferents from the NAc shell is involved in consumption, reward stimulus processing, and working memory (Root, Melendez, Zaborszky, & Napier, 2015). In contrast, based on the motor-related output targets of the dlVP, such as the subthalamic nucleus (STN) and the substantia nigra pars reticulata, it is thought to be involved in mediating reward motivated behavior (Root, Melendez, Zaborszky, & Napier, 2015). There is evidence demonstrating that VP neuronal activity is modulated by dopamine in slice preparation, which is likely released by midbrain projections (Napier & Potter, 1989). However, the manner by which rewarding and aversive stimuli modulate VP dopamine is, as yet, uncharacterized.

Roles in hedonic processing and motivation

Since the initial studies implicating the VP in hedonic processing, several additional studies have further characterized the role of the VP more broadly in reward processing. Neuroimaging studies in humans demonstrate a positive correlation between brain activity in the VP and self-reports of pleasure, including responses to images of appetizing food and the idea of winning money (Pessiglione et al., 2007; Simmons et al., 2014). Lesion and pharmacological inactivation studies using rodents indicate that both the NAc and VP are critical to generating motivation and hedonics (Kalivas & Volkow, 2005). Lesions of the VP eliminated appetitive responses and elicited aversive behavioral responses to a palatable sucrose solution administered intraorally (Cromwell & Berridge, 1993). This observation indicates a strong reversal of hedonic processing to a taste stimulus, an effect that was unique to the VP.

The VP and the NAc are similar in the reward functions they regulate. Similar to the NAc, the VP regulates motivation, feeding, and motor function, suggesting that the VP serves as a functional interface between the limbic and motor systems (Mogenson et al., 1980). Disruption of the activity in the VP leads to altered hedonic responses and reduced goal-directed behavior (Berridge & Cromwell, 1990). Notably, the role of the VP in hedonic processing is not limited to natural rewards. Opioid neurotransmission in the VP is also required for cocaine-induced reinstatement of cocaine seeking behavior (Tang, McFarland, Cagle, & Kalivas, 2005). Furthermore, lesion or pharmacological inactivation of the VP disrupts motivated behavior in several addiction paradigms, including cocaine-induced conditioned place preference (CPP) (Gong, Neill, & Justice, 1997), cocaine self-administration (Robledo & Koob, 1993), and cocaine-induced reinstatement (McFarland

& Kalivas, 2001; Tang et al., 2005). Berridge and Kringelbach (2013) interpret these findings as an indication that these subcortical structures (i.e. NAc and VP) are responsible for the generation of affect and motivation, while cortical structures involved in this circuit (e.g. OFC) may regulate other related higher cognitive functions such as appraisal, memory, and decision making. This suggestion is consistent with some clinical literature. For example, a case study of a patient with severe damage to the limbic neocortex (OFC, and ventral anterior cingulate), hippocampus, and amygdala exhibited no deficit in the ability to experience the normal range of human emotions, suggesting that these functions are regulated elsewhere in the reward system (Damasio, Damasio, & Tranel, 2013).

The VP "hedonic hotspot"

The VP has been characterized as a "hedonic generator" based on the observation that it possesses the unique ability to augment aversive taste reactivity when inactivated or lesioned (Ho & Berridge, 2014; Shimura et al., 2006; Smith & Berridge, 2005). The VP also possesses a "hedonic hotspot" located in the posterior end of the structure, and manipulations in this hotspot can modulate taste reactivity in both directions. Similar to the NAc hedonic hotspot, opioid stimulation in the VP hedonic hotspot enhances hedonic responses to sucrose (Castro & Berridge, 2014; Peciña & Berridge, 2005; Smith & Berridge, 2007). In other words, stimulating mu-opioid neurotransmission at either NAc or VP hotspot results in enhancement of "hedonic liking" responses. Therefore, the VP hedonic hotspot modulates appetitive taste reactivity, and opioid stimulation can enhance appetitive responses. These findings demonstrate that, like the NAc, the VP processes the

palatability of a pleasurable taste.

Connectivity between the NAc and VP hedonic hotspots

Microcircuits that process different aspects of reward are likely to exist between the NAc and the VP. For example, the GABAergic projection from the NAc to the VP exerts profound effects on regulating motivated food-seeking. The best evidence of this is the common observation that both GABAergic stimulation in the NAc and GABAergic inhibition in the VP induce feeding, supporting the idea that the NAc projection inhibits VP activity and regulates consummatory behavior (Stratford & Kelley, 1997, 1999; Stratford & Wirtshafter, 2012). On the other hand, opioid signaling between the NAc and VP appears to modulate the expression of hedonic responses, an effect that is not observed with GABAergic manipulations (Smith & Berridge, 2007). Experimental results from a cFos study showed that the effects of VP hedonic hotspot stimulation were attenuated when pharmacologically inhibiting the activity of the NAc hedonic hotspot (Smith & Berridge, 2007). Figure 1.3 depicts a summary diagram of hedonic circuits between the NAc and VP, demonstrating that the NAc and the VP are anatomically and functionally heterogeneous structures. Several studies discussed above suggest a topographical distribution of the encoding of rewarding and aversive stimuli in the NAc and the VP, with anterior NAc and posterior VP being the hedonic hotspots (for review, see Berridge & Kringelbach, 2013). However, it should be noted that this functional map in the NAc is sensitive to environmental stimuli and does not contain definite anatomical boundaries (Reynolds & Berridge, 2008).
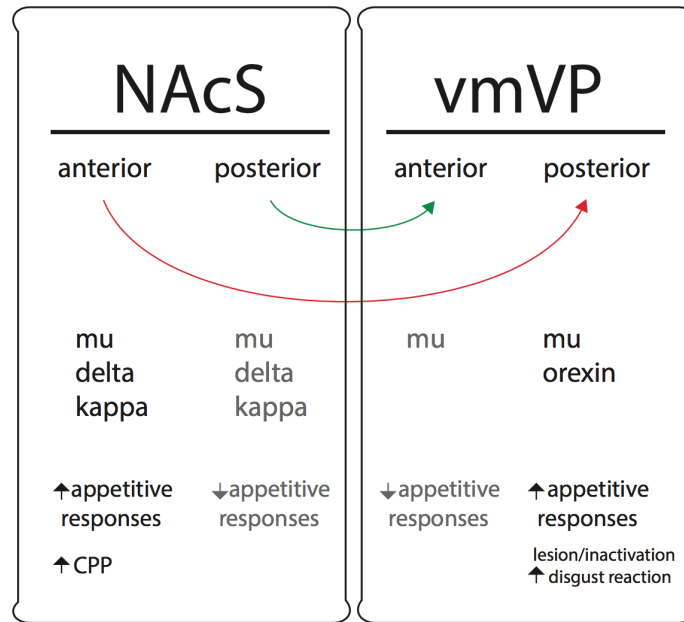
Figure 1.3. Hedonic circuits between the NAc and the VP.
Hedonic hotspots are located in the anterior NAc shell and the posterior VP. Opioid stimulation in either hedonic hotspot increases the expression of appetitive taste reactivity. The hypothesized hedonic circuit is indicated in red. Regions outside the hedonic hotspot have been identified as the hedonic coldspots, since opioid stimulation in these sites reduces appetitive taste reactivity. The VP hedonic hotspot is particularly important for appetitive taste reactivity as lesions of this area dramatically increase aversive responses.

Part V: Reward Encoding by Dopamine and Neuronal Activity

Striatal dopamine signaling promotes incentive

VTA dopamine terminals in the NAc release dopamine and regulate incentive and motivation. Early microdialysis studies demonstrated that rewarding stimuli increase dopaminergic signaling in the NAc (Hernandez & Hoebel, 1988). Studies using pharmacological manipulations showed that dopamine antagonists significantly reduce the reinforcing properties of food, cocaine and amphetamine (De Wit & Wise, 1977; Ettenberg & Camp, 1986; Gerber, Sing, & Wise, 1981; Yokel & Wise, 1976). Administration of mu opioid receptor agonists into the VTA increase striatal dopamine

level (Devine, Leone, Pocock, & Wise, 1993) and enhance the rewarding properties of hypothalamic brain stimulation (Jenck, Gratton, & Wise, 1987). These reward-related increases in terminal dopamine release in the NAc are the result of increased dopaminergic neuronal firing rates in the VTA, in response to the perception of rewarding stimuli or reward-predictive cues (Mirenowicz & Schultz, 1996). More recent studies have demonstrated that optogenetic stimulation of dopaminergic neurons is sufficient to induce place preference conditioning (Tsai et al., 2009) while suppression of their activity through increasing the activity of VTA GABAergic interneurons results in place aversion (Tan et al., 2012; Van Zessen et al., 2012). Furthermore, striatal dopamine responses are dependent on physiological state, suggesting a role of dopamine on sensing physiological needs in order to guide appropriate behavioral response (Cone et al., 2016; Cone, McCutcheon, & Roitman, 2014).

Dopamine signaling in the NAc mediates motivated behavior

Reward information is encoded by neuronal activity in various signaling pathways in the reward system, although the specific physiological mechanisms that result in the generation of an affective state require further experimentation. Earlier studies have noted a correlation between dopamine inhibition by neuroleptics and self-reported emotional indifference, which contributed to the idea that dopamine could be important for the subjective experience of pleasure (Belmaker & Wald, 1977; Healy, 1989). However, further research suggested that while mesolimbic dopamine signaling plays a critical role in motivation, its involvement in the actual subjective hedonic feeling appears to be more tenuous (Wise, 2008). Pharmacological blockade of dopamine fails to affect appetitive

taste reactivity in rats (Peciña, Berridge, & Parker, 1997; Peciña & Berridge, 2013; Peciña, Cagniard, Berridge, Aldridge, & Zhuang, 2003; Treit & Berridge, 1990; Wyvell & Berridge, 2000). Further, experimentally lesioning the mesostriatal dopamine projection in rats results in reduced motivation to eat but no change in taste reactivity (K C Berridge, Venier, & Robinson, 1989; Kent C. Berridge & Robinson, 1998). More recently, an elegant study using optogenetic techniques demonstrated clear evidence that midbrain dopamine neurons are involved in regulating behavioral responses following a stressful experience that produces depressive-like symptoms (Tye et al., 2013). This study showed that optogenetic inhibition and excitation of VTA dopamine neurons can bidirectionally increase or decrease depression-related behaviors, and modulate the neural activity of NAc neurons.

Accumulating evidence also demonstrates the importance of dopamine in the motivation for food and drugs of abuse (McCutcheon, 2015; Volkow & Morales, 2015). Despite having a very limited role in modulating hedonic liking, dopamine does appear to regulate motivation. Human imaging studies suggest that dopamine is released in the ventral striatum when subjects expect monetary or taste rewards (Knutson, Adams, Fong, & Hommer, 2001; O'Doherty et al., 2002). Similarly, many animal studies have demonstrated dopamine release in response to natural rewards, drugs of abuse, and reward-predicting cues (Aragona et al., 2006; Cameron, Wightman, & Carelli, 2014; Hernandez & Hoebel, 1988; Martel & Fantino, 1996; Roitman et al., 2008; Roitman et al., 2004; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005). Moreover, suppression of dopamine dramatically reduces the motivation for food or sucrose reward (Galistu & D'Aquila, 2012; Muscat & Willner, 1989; Towell, Muscat, & Willner, 1987; Yokel &

Wise, 1976; Zhou & Palmiter, 1995) and dopamine supplementation restores normal

feeding behavior (Szczypka et al., 2001). Rewarding stimuli cause a phasic increase in

dopamine concentration in the NAc while aversive stimuli cause a phasic reduction

(Figure 1.1). Dopamine signaling appears to be more strongly associated with the

incentive that drives subsequent motivated behavior. A recent study comparing dopamine

signaling measured with both microdialysis and voltammetry recording techniques

showed that tonic level of striatal dopamine is closely correlated to motivational state,

whereas phasic dopamine responses serve as learning signal when a change of reward

value is detected (Hamid et al., 2016). Taken together, the idea that dopamine is a

pleasure chemical gradually dissipated over the decades as we gained an understanding of

the more complex roles of dopamine in the modulation of motivation.

*The patterned activity of NAc neurons encodes reward information and motivated
behavior*

The encoding of hedonic and reward information is reflected in NAc neuronal

activity, in addition to dopamine signaling. The activity of NAc neurons responds to

appetitive and aversive tastes with differential neural encoding profiles (Roitman et al.,

2005). To appetitive solutions such as sucrose or saccharin, most responsive NAc

neurons respond with a phasic reduction of firing rate. In contrast, a bitter quinine

solution elicits an excitatory response, in which the majority of the responsive neurons

increase firing rate, corresponding with the expression of aversive taste reactivity.

Consistent with the findings of this study, NAc neurons exhibit primarily inhibitory

responses to appetitive stimuli in other experimental designs (Taha & Fields, 2005;

Wheeler et al., 2008). Since the behavioral and neuronal responses to sucrose and quinine

are well correlated, this result has been interpreted as mental representations of hedonic information in the brain (Roitman et al., 2005). Similar inhibitory response profiles were observed in the NAc in response to other rewarding stimuli, including ethanol (Janak, Chang, & Woodward, 1999), cocaine (Peoples & West, 1996), heroin (Chang, Janak, & Woodward, 1998), and food (Carelli, 2002). These neural data suggest a potential causal relationship between decreased activity in the NAc and increases in motivated behavior for a given stimulus (Carlezon & Wise, 1996).

An important question is whether differential neural encoding of taste stimuli represents hedonic value or other intrinsic properties of the stimuli (e.g. sensory sweetness or bitterness). Aspects of this question can be tested by using a CTA paradigm to devalue an otherwise appetitive taste stimulus by associating it with an aversive stimulus (often visceral illness). CTA is a well-documented phenomenon of induced-aversion resulting from a taste stimulus becoming associated with aversive outcome. Originally, CTA studies were conducted by pairing a palatable stimulus such as saccharin solution (the conditioned stimulus, CS) with experimentally-induced visceral discomfort (the unconditioned stimulus, UCS) to cause robust taste devaluation. More recently, it has been found that drugs of abuse such as cocaine can also be used as the UCS to cause conditioned aversion (Davis & Riley, 2010). Overall, CTA paradigm provides a means to alter the hedonic value of a stimulus, making studies of hedonic neural encoding in the brain possible.

Importantly, the neuronal response profile in the NAc to a saccharin taste track the change in hedonic value in a CTA paradigm, demonstrating that the response does not reflect basic taste properties. In this experiment, saccharin tastes were paired to either

cocaine or saline. As depicted in Figures 1.4A-C, a cocaine-predictive saccharin taste

(CS+) elicited significantly more aversive taste reactivity compared to saline-paired

saccharin taste (CS-). It was found that saccharin elicited the same inhibitory response in

the NAc when paired with saline (Figure 1.4D-E) but a significant profile shift toward

excitatory activity was observed when it came to predict cocaine availability (Figure 1.4F)

(R. A. Wheeler et al., 2008). This response pattern for cocaine-predictive saccharin

resembles the unlearned response to aversive quinine (Figure 1.4G) and is associated

with the same aversive behavioral response. Other electrophysiology studies also

supported the hypothesis that NAc neuronal activity encodes motivational information

and tracks changes in hedonic value (Krause, German, Taha, & Fields, 2010; Loriaux,

Roitman, & Roitman, 2011). Interestingly, while cocaine-induced conditioned taste

aversion is a robust and well-documented phenomenon, it is difficult to explain. It is not

intuitive that cocaine (which typically reinforces behaviors), would devalue and cause

avoidance of a predictive natural reward. One hypothesis proposed that while drugs of

abuse such as cocaine create highly euphoric experience, drug-predictive cues cause

physiological shifts in the opposite direction in an attempt to accommodate the

anticipated drug effects. With this view, drug craving can be viewed as a conditioned

response that reflects an anticipatory need state that results from learning processes. This

model has been used to study drug-craving for a variety of abused substances, including

alcohol, opiates and cocaine (Eckardt, 1976; McDonald, Parker, & Siegel, 1997; Nyland

& Grigson, 2013; Siegel, 2005; Tandon, Keefe, & Taha, 2016; Turenne, Miles, Parker, &

Siegel, 1996; Wheeler et al., 2008). This theory explains the intense negative affective

state caused by drug cues that are associated with craving, and predicts that there are

identifiable physiological shifts that correspond with the cue-induced increase in drug

seeking behavior. It has been hypothesized that these compensatory responses are

reflected in altered dopamine signaling and the firing rates of NAc neurons (Wheeler et

al., 2011; Wheeler et al., 2008).
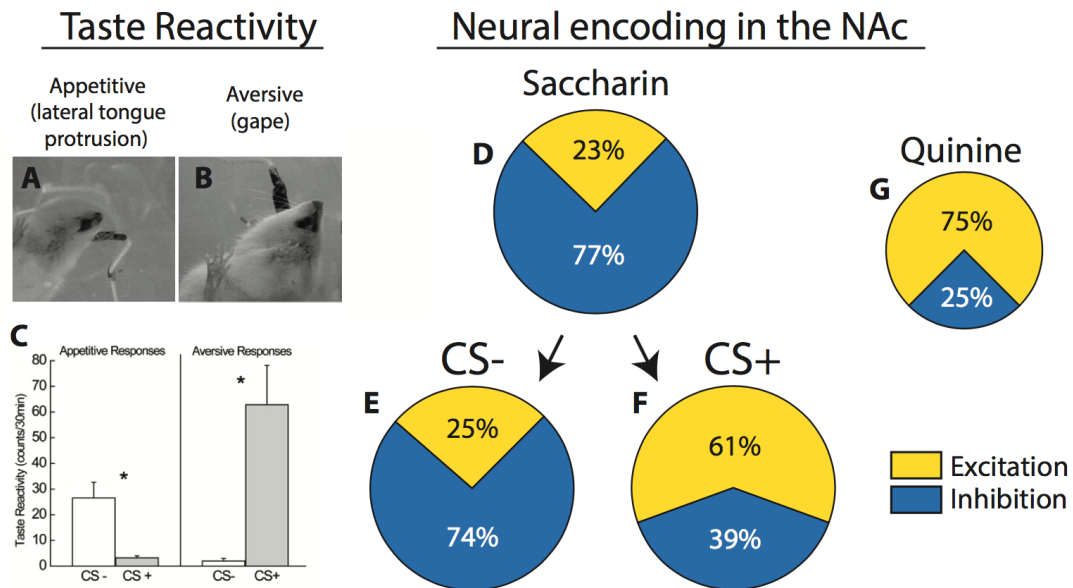
Figure 1.4. Neuronal encoding of hedonic information in the NAc.
Two saccharin tastes were paired with either saline (CS-) or cocaine (CS+) availability in
a modified conditioned taste aversion (CTA) paradigm. (A-C) Taste reactivity was
measured before and after conditioning and it was found that cocaine-predictive
saccharin was devalued and became aversive. (D-G) Electrophysiological recordings in
the NAc found that pairing with saline caused no change in neural encoding profile but
association with cocaine shifted the encoding profile from primarily inhibitory to
excitatory, resembling the innate aversive response. Pie charts illustrate the proportion of
phasic excitatory and inhibitory neuronal responses in the NAc observed during
recording sessions.
Figure adapted from (Wheeler et al., 2008).

The discovery that the NAc encodes hedonic information raises questions

regarding how this neuronal information is being relayed within the limbic reward

circuitry. It is possible that NAc activity directly modulates the activity of neurons in a

downstream structure such as the VP. Relating to this, a NAc-VP functional circuit has

been hypothesized, predicting that exposure to rewarding stimuli reduces activity in the NAc and disinhibits the VP due to reduced GABAergic input (Berridge, 2012; Taha & Fields, 2005). This perspective is based on the anatomical and connectivity positions of the VP as a locus of integration of hedonic and motivational information downstream from the NAc (Groenewegen et al., 1999; Inui, Inui-Yamamoto, Yoshioka, Ohzawa, & Shimura, 2011; Inui, Yamamoto, & Shimura, 2009; Kalivas, Churchill, & Romanides, 1999; Lim, Murphy, & Young, 2004).

Neural encoding of reward information by VP neurons

VP neurons are responsive to rewards and their predictors. VP neurons have been shown to increase their firing rates in response to palatable sucrose and associated stimuli. The pairing of a tone with a sucrose reward leads to an excitatory response to both the tone as well as the sucrose (Tindell et al., 2006). This supports the idea that VP neurons track the incentive salience of stimuli. A recent study showed that the activity of VP neurons not only encodes incentive salience, but also the instrumental response for reward (Richard, Ambroggi, Janak, & Fields, 2016). Further, phasic firing patterns have been observed in both the ventromedial and dorsolateral VP during approach and lever press responses for cocaine (Root et al., 2012, 2013; Root, Fabbricatore, Ma, Barker, & West, 2011). Together, these data indicate that activity in the VP encodes appetitive stimuli involved in goal-directed behaviors, consistent with the concept that the VP acts as a limbic-motor interface. However, it remains unknown whether a change in the value of a stimulus is encoded in the VP.

Gustatory processing and reward

Consumption of nutritious food is an essential behavior required to support biological functions in animals. The ability to accurately evaluate taste qualities and learn from the experience plays a role that is critical for survival. Knowledge of the position of the gustatory neural circuitry with respect to the reward circuitry is therefore crucial to understanding how reward information can exert top-down modulatory effects on taste perception and motivated behavior.

Gustatory information is transmitted from the taste buds to the rostral part of the nucleus of solitary tract (rNST) and medullary reticular formation (RF) through cranial nerves. While the RF pathway is important for generating reflex and oromotor functions, the rNST projection is involved in higher taste discrimination (Carleton, Accolla, & Simon, 2010; Zaidi, Todd, Enquist, & Whitehead, 2008). The rNST projects to the pontine parabrachial nucleus (PBN) which then project reciprocally to the lateral hypothalamus, basolateral amygdala (BLA), bed nucleus of stria terminalis (BNST), and the thalamus. The gustatory fibers in the thalamus (specifically the ventral posteromedial nucleus of the thalamus) continue to several cortical regions, including the primary gustatory cortex, the somatosensory cortex, and the orbitofrontal cortex (OFC). These cortical structures project back to the PBN and thus form a loop that allows integration of other sensory (e.g. OFC input) and limbic (e.g. amygdala input) information to modulate taste processing (Ongür & Price, 2000; Tokita, Inoue, & Boughter, 2009). In addition to amygdala and cortical inputs being integrated into the gustatory circuit, the NAc projects to the thalamus and brainstem, and indirectly (through the VP) to the thalamus, providing

additional points of entry to the gustatory circuit where limbic reward information could

regulate gustatory responses as demonstrated in Figure 1.5.
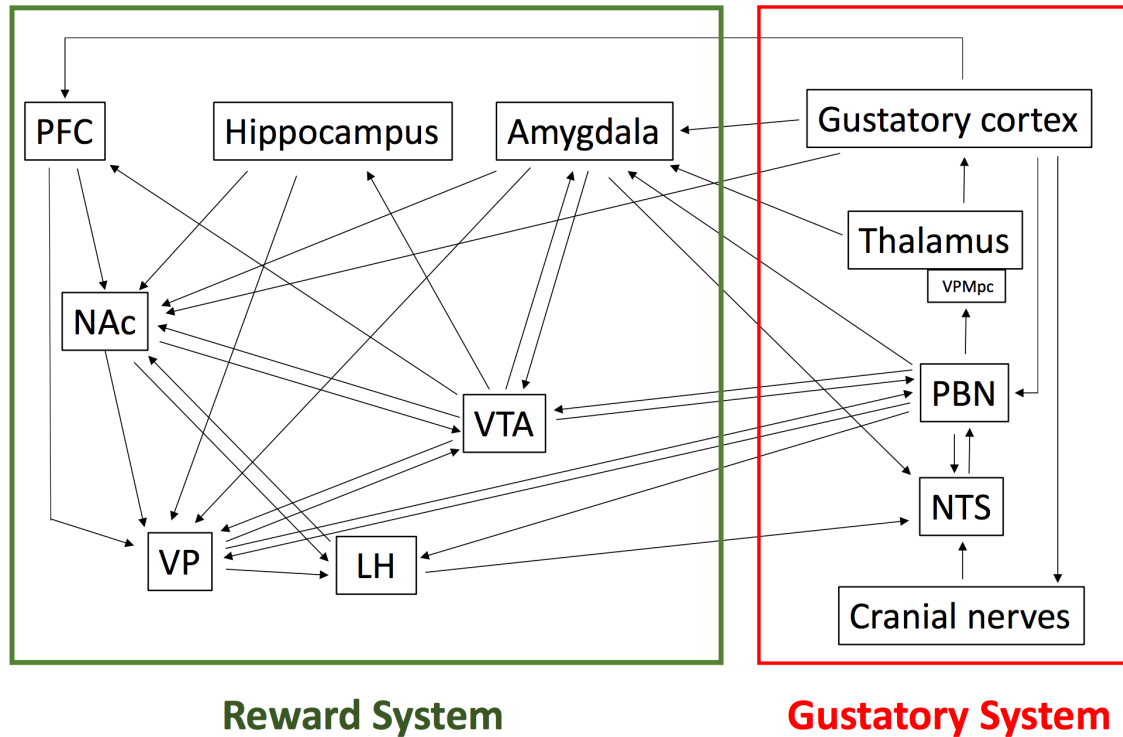
Figure 1.5. Top-down modulation of taste processing by the reward circuitry.
Brain structures broadly grouped based on functions. Structures inside the green box (left)
represent key structures of the reward circuitry and structures inside the red box (right)
represent the gustatory system. Information from reward and gustatory systems are likely
to interact at the NAc, the VP, the amygdala, the LH, the VTA, and various cortical
regions (e.g. PFC, GC). While the anatomical connections of the interface structures with
the reward and gustatory systems are mostly bidirectional, it is hypothesized that higher
cognitive reward information exerts top-down modulation of the gustatory system to
produce appropriate behavioral responses.
*PFC = prefrontal cortex, NAc = nucleus accumbens, VP = ventral pallidum, LH =
lateral hypothalamus, VTA = ventral tegmental area, PBN = parabrachial nucleus, NTS =
nucleus of the solitary tract, GC = gustatory cortex

Hedonic responses to gustatory stimuli are regulated by structures in this circuit.

Interestingly, early studies of decerebrate rats found no impairment on the expression of

orofacial responses to tastes (Grill & Norgren, 1978), however, taste reactivity is by no means merely reflexive (H J Grill & Kaplan, 2001). Unlike normal rats, decerebrate animals failed to adjust their expression of taste reactivity to changes in sweetness and physiological state accordingly, suggesting the inability to update reward information (Kaplan, Roitman, & Grill, 2000). In addition, it has been shown that the activity of limbic structures such as the NAc and VP modulate both appetitive and aversive taste reactivity (Ho & Berridge, 2013; Peciña et al., 2000). These observations suggest that the brainstem (left intact in the decerebration procedure) is sufficient to produce taste responses, while other structures such as the PFC, NAc, and VP exert a top-down modulation of the expression of taste reactivity.

Part VI: Techniques Used to Characterize Reward Encoding in the Brain

Manipulating brain activity and dissecting reward circuitry

An array of techniques has been used to investigate brain circuitries, ranging from lesions, to pharmacological manipulations, to the more recently established chemogenetic manipulations. Chemogenetic approaches have gained significant attention in recent years due to their capability to target a specific cell type or circuit.

Although being gradually replaced by chemogenetics and other techniques, administration of pharmacological reagents directly into a targeted brain area is one of the most commonly used methods to investigate brain functions. Much of our understanding of the brain's regulation of behavior is built on studies using such pharmacological manipulations. Microinfusions of agonists and antagonists of various

receptors can induce robust behavioral effects, including changes in hedonic response and food consumption (Baldo, Sadeghian, Basso, & Kelley, 2002; Basso & Kelley, 1999; Castro & Berridge, 2014; Maldonado-Irizarry, Swanson, & Kelley, 1995; Reynolds & Berridge, 2002; Richard & Berridge, 2011). Moreover, mapping studies have demonstrated that subregion level-specificity can be achieved when a manipulation is calibrated and verified carefully (Ho & Berridge, 2013; Peciña & Berridge, 2005; Peciña et al., 2000).

On the other hand, chemogenetic techniques allow pharmacological activation or inhibition of targeted neurons that express DREADD (Designer Receptor Exclusively Activated by Designer Drugs) to control neural activity in vivo. DREADD receptors are G protein-coupled designer receptors that are activated specifically by a designer pharmacological reagent that is otherwise inert, such as clozapine N-oxide (CNO). Cells are first transfected virally to express the DREADD receptor of choice. Depending on the experiment, stimulatory Gs/Gq or inhibitory Gi DREADDs can be used to alter the excitability of neurons (Roth, 2016). With chemogenetic techniques, cell-type specific transfection can be achieved. For example, transgenic animals expressing Cre recombinase in tyrosine hydroxylase- (TH) expressing neurons can be selectively targeted using a Cre-dependent DREADD. In this way, chemogenetic manipulations can be used to specifically increase or reduce the activity of midbrain dopamine neurons to modulate dopamine signaling in the reward circuit.

Characterizing the Neural Encoding of Reward in the NAc-VP Circuitry

Current knowledge of the NAc and the VP strongly suggests that these structures are key regions that encode the neural representations of reward. While electrophysiology studies have shown evidence that patterned neuronal responses in the NAc encode reward information, little is known about how these neural responses are being regulated and how structures communicate with one another. The dissertation projects described here use in vivo electrophysiological recording techniques to characterize and explore how reward is being encoded in the limbic reward circuitry. In the first study, neuronal responses in the VP to appetitive and aversive stimuli were characterized. The second experiment examined the effects of altered NAc activity on neuronal responses in the VP during hedonic and motivation behavioral tasks. Finally, based on recently published data from our laboratory suggesting a potential correlation between dopamine signaling and neuronal activity in the NAc (Figure 1.6), the last project tests for a causal role of dopamine signaling in modulating the encoding of reward in the NAc using a chemogenetic approach. The body of work presented here provides data that further our understanding of how reward information is represented by neuronal activity in the limbic circuitry and, importantly, how structures in the reward circuit communicate.
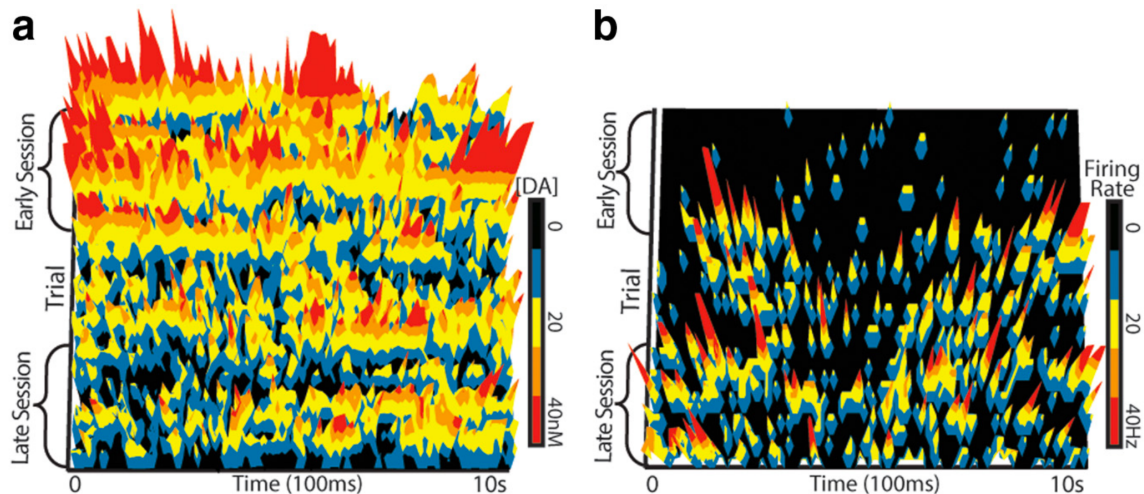
Figure 1.6. An aversive stimulus suppresses dopamine signaling and enhances neuronal excitability in the NAc.

(A) An example voltammetry recording illustrating dampened dopamine signaling in the NAc in response to a devalued and aversive taste stimulus. Dopamine concentration is presented in false color (z-axis). Changes in dopamine concentration are presented across trials (y-axis) and within a trial (x-axis). (B) A representative NAc neuron increased firing rate response to an aversive taste stimulus on the same timescale. Neuronal activity is presented in false color (z-axis). Changes in firing rate are presented across trials (y-axis) and within a trial (x-axis).

Figure adapted from (Wheeler et al., 2015).

Chapter II

Neural Encoding of Reward Information in the Ventral Pallidum

Abstract

Cocaine experience affects motivation structures such as the nucleus accumbens (NAc) and its major output target, the ventral pallidum (VP). Previous studies demonstrated that both NAc activity and hedonic responses change reliably as a taste cue comes to predict cocaine availability. Here we extended this investigation to examine drug-experience induced changes in hedonic encoding in the VP. VP activity was first characterized in adult male Sprague-Dawley rats in response to intraoral infusions of palatable saccharin and unpalatable quinine solutions. Next, rats received 7 daily pairings of saccharin that predicted either a cocaine (20 mg/kg, ip) or saline injection. Finally, the responses to saccharin and quinine were again assessed. Of 109 units recorded in 11 rats that received saccharin-cocaine pairings, 71% of responsive units significantly reduced firing rate during saccharin infusions and 64% increased firing rate during quinine exposure. However, as saccharin came to predict cocaine, and elicited aversive taste reactivity, VP responses changed to resemble quinine. After conditioning, 70% of saccharin-responsive units increased firing rate. Most units that encoded the palatable taste (predominantly reduced firing rate) were located in the anterior VP, while most units that were responsive to aversive tastes were located in the posterior VP. This study reveals an anatomical complexity to the nature of hedonic encoding in the VP.

Introduction

Drug addiction is a neurological disorder that burdens societies with a significant economic cost and individuals with profound suffering. One of the hallmarks of addiction is the high rate of chronic relapse observed in individuals attempting to remain abstinent. For addicts, stressors and dug-associated cues can spark episodes of drug craving and aversive physiological responses, fueling cycles of relapse following periods of abstinence (Fox et al., 2008; Newton et al., 2003; Paliwal et al., 2008). Therefore, understanding the neural systems that mediate both drug associations and affective responses are likely to be necessary steps in understanding how to effectively disrupt this cycle.

The measurement of a conditioned change in the affective state of a nonhuman is extremely difficult, but some animal models allow for the study of neural systems that regulate affective state. One such design pairs an otherwise palatable taste with investigator-delivered cocaine administration or availability (self-administration) to generate taste aversion (Colechio, Imperio, & Grigson, 2014; Grigson, 1997). Using this design, the patterned neuronal activity in the nucleus accumbens (NAc) has been shown to encode not only the observed change in hedonic state but also the motivation to seek cocaine (Wheeler et al., 2015; Wheeler et al., 2008). Specifically, while the predominant neuronal response to palatable taste stimuli is generally a decrease in firing rate, aversive stimuli evoked largely increased firing rates, regardless of whether the aversive stimulus is innately aversive (Roitman et al., 2005) or devalued by a drug of abuse (Wheeler et al., 2015; Wheeler et al., 2008).

The NAc participates in a network of nuclei that modulate both hedonic perception and motivated behavior. The recipient of the majority of GABA-ergic output projections from the NAc is the ventral pallidum (VP), which also has functional microcircuits that regulate hedonic processing and motivated behavior (Smith & Berridge, 2007). In humans, functional imaging studies have shown that the activity of the VP is strongly associated with positive affective responses (Childress et al., 2008; Pessiglione et al., 2007; Simmons et al., 2014). In rodents, several studies have demonstrated that the VP not only plays a critical role in the normal expression of hedonic responses, but also in ingestive behavior (Gong et al., 1997; Stratford & Kelley, 1997), and drug seeking (Kemppainen, Raivio, & Kiianmaa, 2012; Mahler et al., 2014; Stefanik, Kupchik, Brown, & Kalivas, 2013). Furthermore, in vivo electrophysiological experiments have aligned specific patterns of VP neuronal activity with hedonic processing and drug-seeking behavior (Root et al., 2012, 2013, 2011; Smith et al., 2011). Based on this intimate relationship, and coincident function, we hypothesized that VP neurons encode hedonic information and are sensitive to changes of hedonic values as a result of cocaine-induced conditioned taste aversion.

An objective of this study was to characterize the neural encoding of appetitive and aversive tastants throughout the VP. Pharmacological manipulations at various sites in the VP indicate that it is a functionally heterogeneous structure, similar to the NAc (Ho & Berridge, 2013; Ho & Berridge, 2014), which receives relevant NAc input throughout its rostral-caudal extent (for review see Root, Melendez, Zaborszky, & Napier, 2015). Therefore, recordings were conducted with an attempt to cover as much of the VP as possible along this axis. Initial recordings were conducted to characterize the neuronal

responses to palatable saccharin and aversive quinine intraoral infusions. Then, we examined a potential change in the encoding of the saccharin solution, reflective of a change in the perceived palatability, as saccharin was devalued through association with cocaine. Our results indicate that the VP encodes the drug-induced devaluation of natural rewards, and illustrate a regional organization of hedonic encoding.

Material and Methods

Animals

Male, adult Sprague-Dawley rats (Harlan Laboratories, IN) weighing between 300-350 g were used in this study. Animals were individually housed in AAALAC-accredited vivarium on a 12 h reversed light/dark cycle. All experimental procedures and testing took place during the dark phase, which is the naturally active phase of rats. Every animal had ad libitum access to food and water throughout the entirety of the study. A total of 16 animals were used in this study (11 from the Cocaine group and 5 from the Saline group) for both electrophysiological recordings and taste reactivity behavior. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Marquette University in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

Surgical Procedures

Animals were deeply anesthetized with ketamine hydrochloride (100mg/kg) and xylazine hydrochloride (20 mg/kg) and intraoral catheters were implanted as previously described (R. A. Wheeler et al., 2008). The catheters were implanted bilaterally, lateral to

the first maxillary molar and exteriorized on the top of the animal's head. Plastic washers were used to secure catheter placement. Microelectrode arrays (NB Labs, TX) were implanted bilaterally at AP +0, ML ±0.8 relative to bregma, and DV -7.8 relative to brain surface. Each array contained 2 rows of 4 microwires (diameter 50 μm, 0.25 mm between wires, rows separated by 0.5 mm). During surgeries, the ground wire for each microelectrode was wrapped around a skull screw and inserted approximately 1 mm into the brain. The stereotaxic coordinates were chosen to ensure array placement across the VP. The implanted arrays were held in place using acrylic dental cement. For all surgical procedures, rats were treated with the anti-inflammatory meloxicam (1% oral suspension) the day of, and for three days following surgery to reduce inflammation and postoperative pain. Animals were allowed to recover for a minimum of 7 days prior to the initiation of experimental procedures.

Apparatus

Both electrophysiological recording and taste-drug pairings took place in a 43 x 43 x 53 cm Plexiglass chamber (Med. Associates, VT) housed within a sound-attenuating box. An infusion line connecting to a 20 ml syringe in a syringe pump was installed and passed through a swivel and a commutator (Crist Instruments, MD) located above the Plexiglass chamber. The infusion pump was controlled by MedPC computer program to allow for the automated delivery of tastants, and the generation of event-related timestamps that were coordinated with electrophysiological recording equipment. Under each chamber, a camera was positioned to allow for recording behavioral responses (taste reactivity).

Taste-Drug Pairings

Following recovery from surgery, electrophysiological responses to saccharin (0.15%), and quinine (0.001 M), were initially recorded to establish a baseline population response. In each recording session, 45 intraoral infusions of each tastant (0.2 ml/6 s) were delivered with randomized intertrial intervals (30-90 s) within a maximum of 52 min for a given session. After baseline responding was established, animals received 7 days of daily saccharin-cocaine (Cocaine group), or saccharin-saline (Saline group) pairings. During each training session, rats in the Cocaine-paired condition received 45 infusions of saccharin followed by a cocaine injection (20 mg/kg, ip). Following daily saccharin exposure, animals in the Saline-paired condition received an injection of saline of equivalent volume. Following conditioning, a test session occurred in which electrophysiological and behavioral responses to both saccharin and quinine were again assessed using the same parameters. The design of the test session was identical to baseline testing.

Taste reactivity scoring/analysis

Taste reactivity was analyzed in a frame-by-frame analysis using digital video recorded on the test sessions before and after conditioning. The appetitive and aversive responses were counted using the technique described in Grill and Norgren (1978). Instances of rhythmic tongue protrusions and paw-licking behavior were counted as appetitive responses. Aversive behavioral responses include gaping (mouth movements that matched a triangle shape for a duration exceeding 90 ms), paw flailing, wet dog shakes, and passive drips. The rates of aversive and appetitive events (per trial) were

computed for each animal. Taste reactivity data were analyzed with mixed ANOVA and subsequent planned comparisons of appetitive and aversive taste reactivity (events/trial) in the Cocaine and Saline groups.

Electrophysiological recording procedures and neuronal response analysis

To familiarize the rats with the recording situation, all animals were connected to a flexible recording cable (Plexon Inc., TX) attached to a commutator (Crist Instruments) for 2 hours on the day before the experiment was initiated. On the following day, animals were again connected to the flexible recording cable attached to a commutator and their intraoral catheters were connected to the infusion pump. This allowed unrestrained movement in the chamber while neuronal activity in the VP was recorded. Unit activity was recorded differentially between each active wire (recording channels) and an inactive wire chosen for the absence of unit activity (reference channel).

Online isolation and discrimination were accomplished using a commercially available neurophysiological system (OmniPlex system; Plexon Inc., TX). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal events to a computer. Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each of these events to the OmniPlex to be time-stamped along with the neural data. Criteria for identifying different neurons on a single wire have been

described in detail elsewhere (Roitman et al., 2005). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template and principle component analysis procedures provided by the PlexControl software system. The template analysis procedure involves taking a sample of the waveform and building a template of that extracellular waveform. Subsequent neurons that match this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment was finished using additional principle components analysis in Offline Sorter V3.3.2 (Plexon Inc., TX).

Phasic encoding of the tastants was characterized by generating perievent response histograms (100 ms bins) 10 sec prior to and following intraoral infusions using NeuroExplorer (Nex Technologies, MA). Each histogram was divided into a baseline epoch and an effect epoch, each 10 sec in duration. A two-tailed, paired t-test was conducted on each unit's firing rate change to determine reliable changes in activity elicited by tastant infusion. With this analysis, unit responses were categorized as either increased cell firing, decreased cell firing, or non-responsive. After neuronal responses were characterized, Fisher's exact tests were used to detect any differences in phasic responses between Cocaine and Saline conditions as well as differences in encoding along the rostral-caudal extent of the VP.

Histology

Following testing, animals were euthanized with CO_2, and the location of each unit was verified. A current (20 μA) was run through each implanted microwire, and brains were incubated in 4% potassium ferrocyanide, 10% formaldehyde solution. All

brains were sliced into 40-μm sections and mounted. The slides were then stained with either 0.25% thionin, neutral red, or left unstained before coverslipping. Electrode placements were determined using Paxinos and Watson (2006). Figure 2.1 shows electrode placements for recorded units.
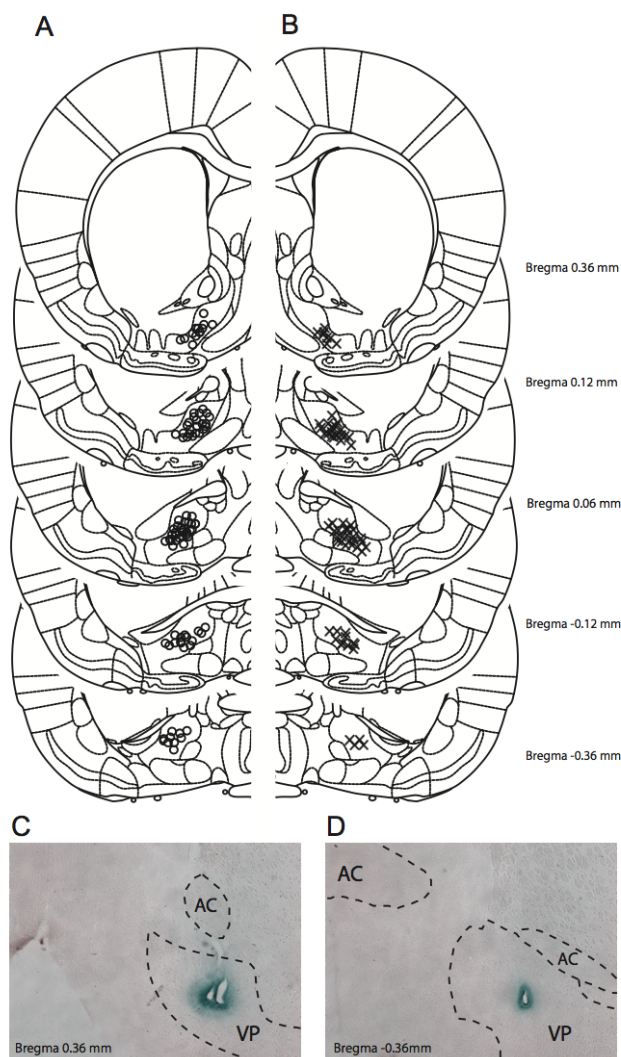
Figure 2.1. Electrophysiological recording sites in the VP.
Histologically identified placements of electrode tips are depicted as Os for animals in the Cocaine group in (A), and Xs for animals in the Saline group in (B). Histological examples of electrode placements in the anterior (C) and posterior (D) VP are presented.

Results

Behavioral responses to appetitive and aversive tastants

Consistent with previous studies (Harvey J. Grill & Norgren, 1978; M. F. M.

Roitman et al., 2008; R. A. Wheeler et al., 2008), saccharin and quinine solutions evoked

differential behavioral responses in rats. Upon initial exposure, saccharin elicited several

appetitive responses (mean = 0.95 counts/trial, SEM = 0.20) and few aversive responses

(mean = 0.08 counts/trial, SEM = 0.02) in Cocaine-paired animals. In contrast, quinine

induced few appetitive responses (mean = 0.02 counts/trial, SEM = 0.01) but many

aversive responses (mean = 1.74, SEM = 0.34). Similar behavioral response patterns

were observed in Saline-paired animals, for both saccharin (appetitive: mean = 1.92

counts/trial, SEM = 0.40; aversive: mean = 0.07 counts/trial, SEM = 0.02) and quinine

(appetitive: mean = 0.07 counts/trial, SEM = 0.02; aversive: mean = 1.08 counts/trial,

SEM = 0.23). All taste reactivity data are summarized in Table 1.

Table 1		Cocaine group				Saline group			
		Pre		Post		Pre		Post	
		Appetitive	Aversive	Appetitive	Aversive	Appetitive	Aversive	Appetitive	Aversive
Saccharin	Average	0.947	0.076	0.291	0.397	1.915	0.074	1.774	0.080
	SEM	0.205	0.019	0.074	0.117	0.396	0.024	0.315	0.029
Quinine	Average	0.017	1.740	0.002	1.309	0.072	1.077	0.031	1.056
	SEM	0.011	0.338	0.002	0.119	0.017	0.233	0.015	0.100

Table 1. Cocaine-induced conditioned taste aversion.

Following initial behavioral assessment, the otherwise palatable saccharin

solution was paired with either cocaine or saline and the resulting change in hedonic

perception was evaluated. Following conditioning, results of a mixed ANOVA showed

main effects of group (Cocaine vs Saline: $F_{(1,14)}$ = 12.92, p < .01) and response type

(appetitive vs aversive: $F_{(1,14)} = 42.71$, p < .001) as well as an interaction (Conditioning x

Response type: $F_{(1,14)} = 9.70$, p < .01), indicating a change in the perceived palatability of

saccharin in the Cocaine group. Planned comparisons of this interaction indicated that

cocaine experience significantly reduced appetitive responses ($F_{(1,14)} = 12.05$, p < .01) and

increased aversive responses ($F_{(1,14)} = 10.47$, p < .01; Figure 2.2).
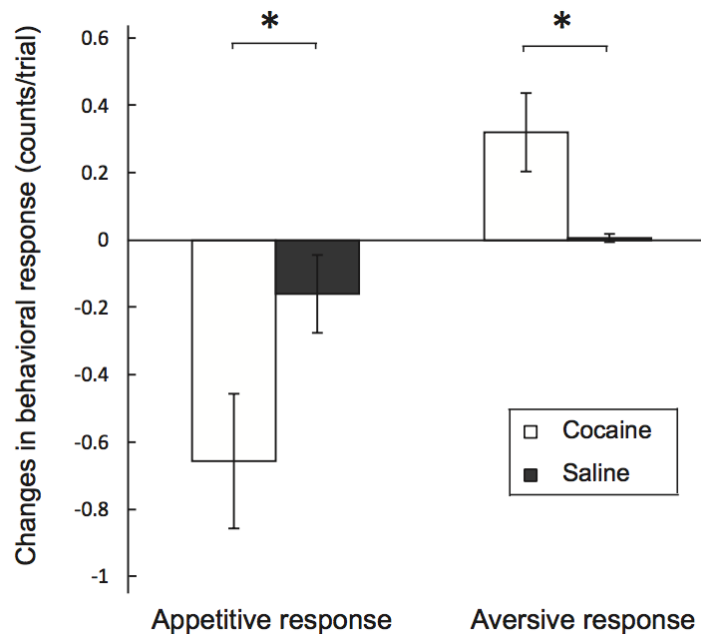
Figure 2.2. Cocaine-predictive saccharin elicits aversive behavioral responses.
The expression of appetitive (left) and aversive (right) responses was measured in
Cocaine- and Saline-paired animals. Following conditioning, Cocaine-paired animals
demonstrated significantly fewer appetitive and more aversive responses at test compared
to Saline animals, indicating that association with cocaine resulted in conditioned taste
aversion. Data are presented as mean ± SEM. Asterisks indicate significant differences
(p < .05).

Behavioral responses to quinine were assessed before and after saccharin conditioning as well. A separate mixed ANOVA comparing responses to quinine revealed only a main effect of response type (appetitive vs aversive: $F(1,14) = 61.92$, $p <$ .001). No other effects or interactions were observed (all p values > .14), indicating that rats responded similarly to quinine at the beginning and the end of the experiment.

Neural encoding of unconditioned appetitive and aversive tastants in the VP

Saccharin and quinine elicited different patterned activity in the VP. In this experiment, 109 (Cocaine) and 48 (Saline) histologically verified VP units were recorded and analyzed. Phasic responses were characterized based on the direction of change in firing rate, and most recorded units exhibited either saccharin- or quinine-specific responses (Figures 2.3A-B).
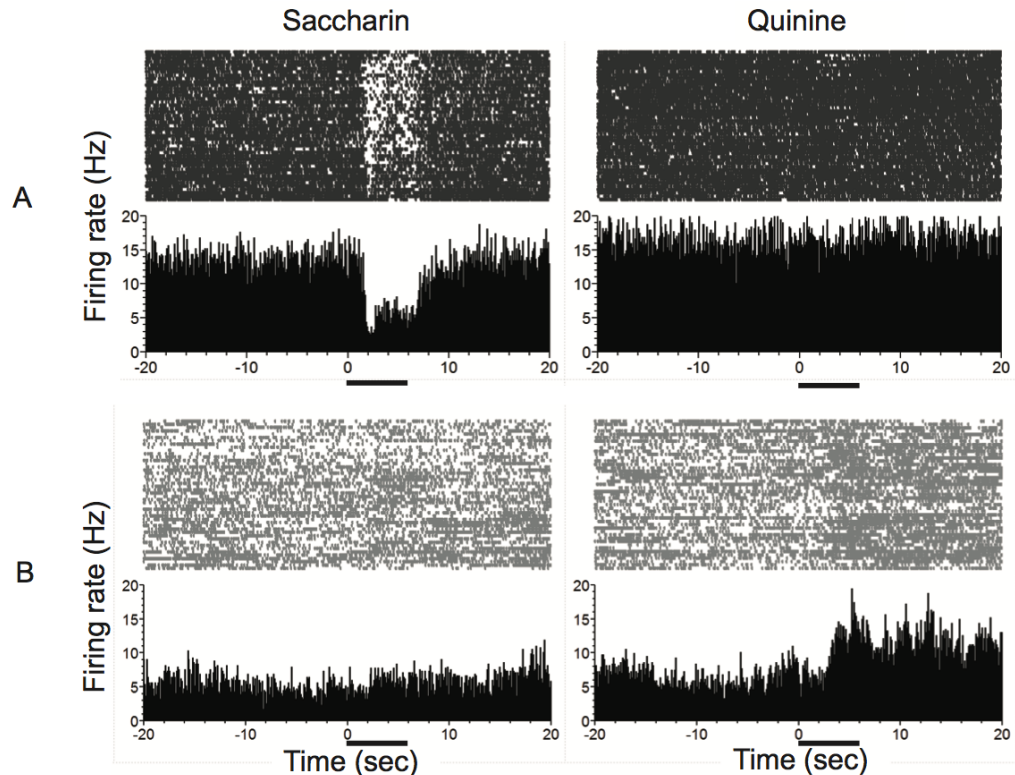
Figure 2.3. Representative taste-selective neuronal responses in the VP.
Two individual VP units are shown here as examples (A and B). Rasters and histograms depict examples of reduced (A) and increased (B) firing rate time-locked to the selective response to a presentation of saccharin (left) or quinine (right). Individual action potentials during each tastant presentation are shown in the raster, and firing rate (Hz) is shown in the histogram below. The black bar under each histogram indicates the duration of the intraoral infusion.

A variety of neuronal responses were observed in this study, with different response onsets and durations that could reflect different aspects of gustatory, hedonic, and motor processes. This is not surprising based on the VP's position in relaying limbic information to behavioral output regions. Because of the limited number of taste-specific responses, these different responses were combined for the following analyses. Use of the 10 second pre/post infusion epochs allowed for the categorization of phasic responses (see Methods for more detail). Most VP units responded to saccharin with decreases in firing rate and to quinine with increases in firing rate. In saccharin-encoding neurons,

71% of the responses (12/17) from the Cocaine animals were decreases in firing rate

(75% from Saline, 9/12). In all of the quinine-encoding neurons, 64% (9/14) of the

responses were excitatory (100% from Saline, 3/3). All observed and categorized

neuronal responses are presented in Table 2. A comparison of saccharin and quinine

encoding across both groups (Cocaine and Saline) before conditioning revealed a

difference in the proportions of cells that significantly increased or decreased firing rate

(Fisher exact, $p < .007$).

Table 2		Selective				Non-selective				
		Saccharin		Quinine						
Response type		Increase	Decrease	Increase	Decrease	Diff.	Same	R	NR	Total
Cocaine-	Pre	5	12	9	5	7	2	40	69	109
Paired	Post	16	7	5	0	4	0	32	60	92
Saline-	Pre	3	9	3	0	1	1	17	31	48
Paired	Post	3	5	3	1	4	2	18	27	45

Table 2. Neural encoding of appetitive and aversive taste stimuli in the VP.
Note* Increase = significantly increased cell firing; Decrease = significantly decreased
cell firing; Diff. = different for saccharin and quinine; R = responsive units; NR = non-
responsive units

Neural encoding of an aversively conditioned tastant in the VP

As a saccharin taste was devalued by its predictive association with cocaine, it

elicited different patterned activity in the VP. When saccharin was paired with saline

vehicle injections, intraoral infusions of saccharin continued to elicit predominantly

reductions in firing rate, (Fisher exact, $p > .64$; Figure 2.4A, D). In contrast, Cocaine

animals exhibited a significant shift in neuronal response pattern toward increased firing

rates in the VP following conditioning, compared to baseline testing (Fisher exact, $p <$

.03). Specifically, devaluation caused the number of units that responded to saccharin

infusions with decreased firing rates to decrease from 71% to 30% (Figures 2.4B, 2.4E),

resembling the population response for quinine, (Figure 2.4C). No shift was observed in the population response profile for quinine before and after the conditioning procedure in either the Cocaine group (Fisher exact, p > .26) or the Saline group (Fisher exact, p > .99). Interestingly, a greater proportion of neurons selectively encoded devalued saccharin relative to quinine before and after conditioning (Fisher exact, ps < .03). This raises the possibility that VP neurons are more engaged by conditioned aversive stimuli than inherently aversive stimuli.
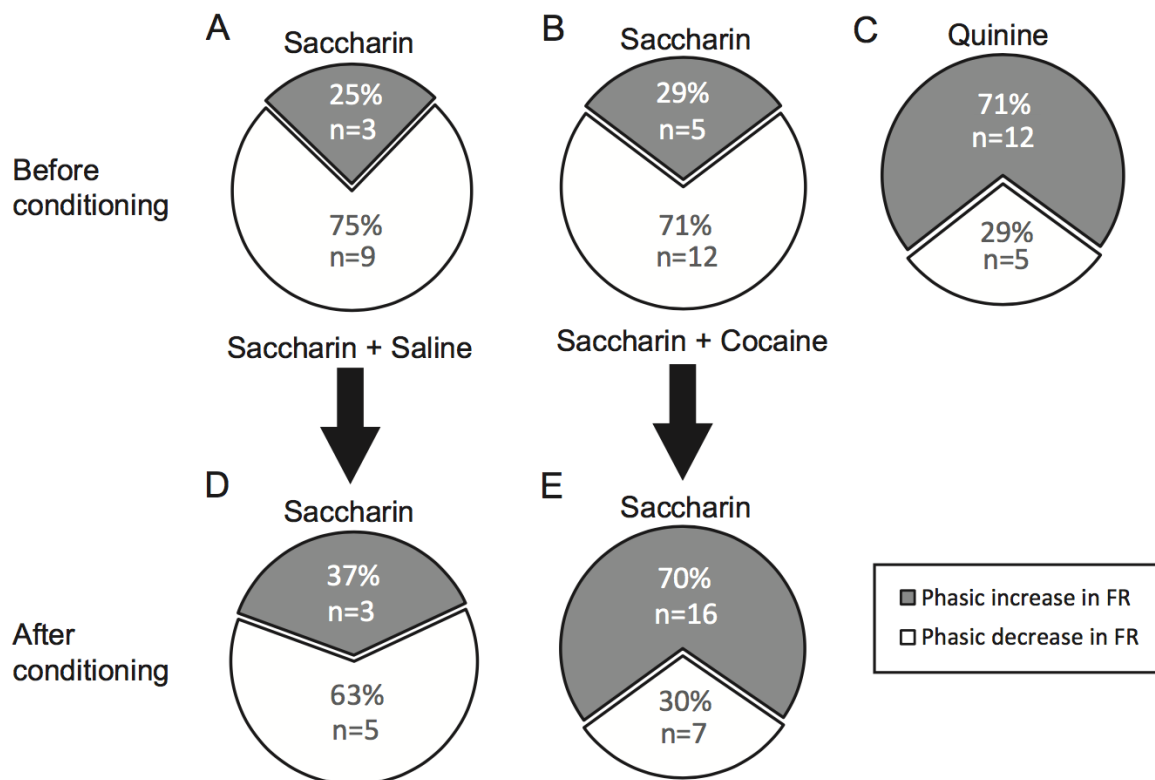
Figure 2.4. Categorization of responses to saccharin and quinine in all phasically active neurons.
(A and B) The predominant phasic response to saccharin exposure was a decrease in firing rate in VP neurons in the Saline (A) and Cocaine (B) conditions prior to conditioning. (C) The predominant response to quinine was an increase in firing rate. (D) In Saline-paired animals, the predominant response to saccharin remained decreased firing rates. (E) In Cocaine-paired animals, the response shifted to become predominantly increased firing rates (p < .03).

Regional differences in neural encoding of rewarding and aversive tastants

One of the goals of this study was to characterize the encoding of taste responses across the rostral-caudal extent of the VP. To compare the distribution of saccharin and quinine neuronal responses, the VP was divided into anterior (rostral to bregma) and posterior VP (caudal to bregma). The VP was divided at $AP = 0$ because it is the approximate middle of the structure (Paxinos & Watson, 2006), and all electrode placements were histologically verified to be within the VP. For analysis purposes, units recorded prior to conditioning from the Cocaine and Saline groups were combined (Figures 2.5A-B). Before conditioning, 86% (25/29) of units that selectively encoded saccharin were located in the anterior region of the VP. In contrast, 59% (10/17) of quinine-selective units were located in the posterior VP. After conditioning, the distribution of saccharin responsive units in the Cocaine condition shifted in a manner consistent with the change in value. Following conditioning, 52% (12/23) were located in the anterior VP (Figure 2.5C). Fisher exact tests revealed that both the differences in the distribution of saccharin and quinine responsive units, as well as the difference in saccharin responsive units following conditioning were significantly different (Fisher exact tests, $ps < .02$). Overall, we found that VP neurons that encode intrinsically appetitive taste reside largely anterior to bregma in the VP while responses for aversive quinine are located posterior to bregma.

A — Pre Sac

86% n = 25

14% n = 4

B — Pre Quin

41% n = 7

59% n = 10

C — Post Sac

52% n = 12

48% n = 11

☐ Anterior
■ Posterior
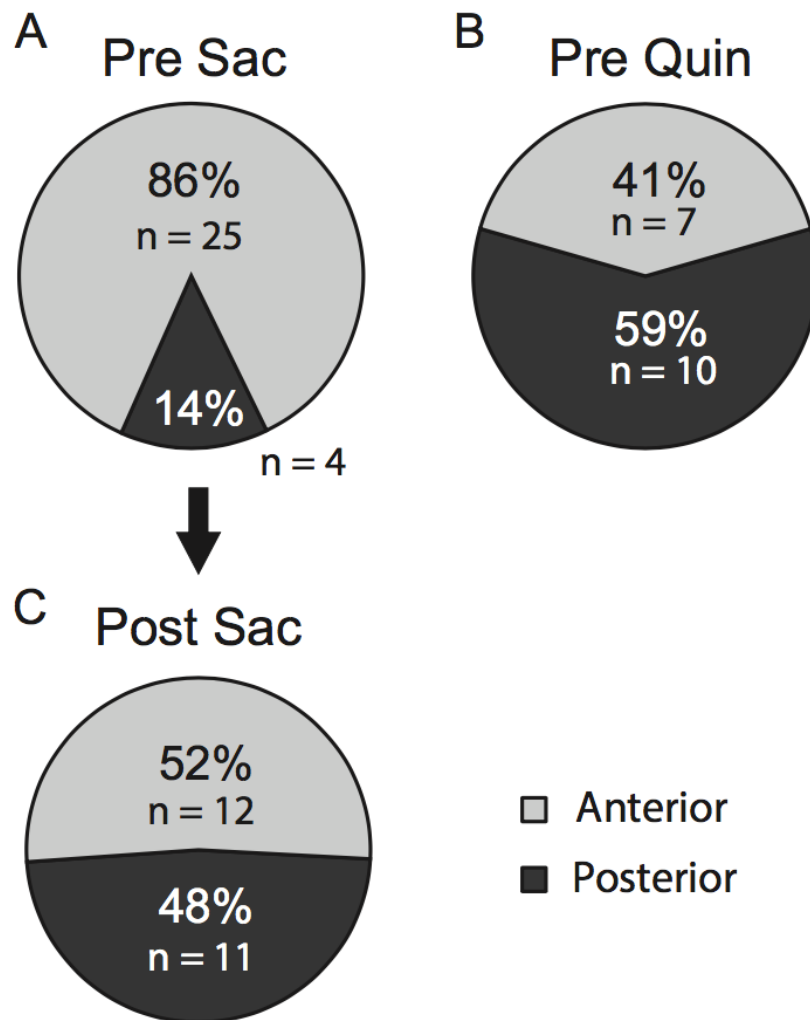
Figure 2.5. Categorization of regionally distributed responses to saccharin and quinine. (A) An examination of all saccharin-responsive units recorded prior to conditioning indicates a regionally selective response in the anterior VP. (B) In contrast, quinine-selective responses were found predominantly in the posterior VP (C) Following saccharin-cocaine pairings, there was a significant increase in the proportion of units recorded in the posterior VP that selectively responded to the aversive saccharin tastant ($p < .02$).

Discussion

These results contribute to a literature characterizing the patterned activity of VP

neurons in response to rewarding and aversive gustatory stimuli. Specifically, the change

in encoding of an otherwise palatable saccharin taste was recorded as it was paired with, and devalued by, its association with cocaine. In general, aversive taste reactivity was accompanied by phasic increases in firing rate both inherently aversive quinine and devalued saccharin. Appetitive saccharin was encoded primarily with phasic decreases in firing rate. The observed activity in the VP is very similar to previous reports of the neural encoding of appetitive and aversive stimuli in the NAc (Roitman et al., 2005; Wheeler et al., 2008). However, selective responses in the VP likely did not merely reflect hedonics or palatability, as devalued saccharin was encoded by a larger proportion of neurons compared to quinine.

The present finding appears to be at odds with previous research examining firing patterns of VP neurons for rewarding and aversive stimuli, as previous reports have not observed a reliable pattern of neuronal responses characterized with decreases in firing rate to rewarding or aversive gustatory stimuli (Tindell et al., 2006). Specifically, it has been reported that the magnitude of the neuronal response in the VP correlates with the perceived palatability of the tastant. The most likely possible explanation for inconsistencies between this report and prior reports is the difference in electrode placement. While all of our electrodes were placed within the VP, they were predominantly represented in the more anterior aspect, not overlapping perfectly with more posterior placements from prior investigations detailing different patterns of encoding rewarding and aversive tastants (Tindell, Smith, Berridge, & Aldridge, 2009; Tindell et al., 2006). It is important to note, however, that a goal of this study was to assay a significant amount of the rostral/caudal aspect of the VP, since we noted that the NAc broadly encodes rewarding and aversive tastants (Roitman et al., 2005; Wheeler et

al., 2008) and projects broadly throughout the VP (David H Root et al., 2015). Our analysis of differences in rostral/caudal encoding of palatability revealed that saccharin-responsive units were predominantly recorded in the anterior VP, while aversive encoding (i.e. responses to quinine) was predominantly found in the posterior VP. Specifically, association with cocaine dramatically altered the VP neuronal response profile to saccharin, resulting in more neurons that showed increased activity in response to saccharin, and abolishing the topological difference along the rostral-caudal axis of the VP. This regional complexity of encoding is provocative and could be the focus of future studies.

The similarity between VP and NAc encoding demonstrates the complexity of the functional connection between the two structures. Because the NAc-VP projections are GABAergic, one might predict that phasic excitations in the NAc would drive a phasic decrease in firing rate in the VP (Wheeler & Carelli, 2006). In contrast to what might be expected based on this simple understanding of NAc-VP connectivity, in vivo studies have demonstrated that stimulation of the NAc can result in various types of responses in the VP. Lavin and Grace (1996) observed three types of neurons in the VP, which all exhibited increases in firing rates in response to accumbens stimulation. Others have characterized NAc stimulation-induced increases or decreases in firing rates, as well as biphasic responses (Chrobak & Napier, 1993; Mogenson, Swanson, & Wu, 1983). Heterogeneous VP responses were also observed during operant responding for cocaine. Interestingly, most phasically active VP neurons were found to reduce firing rate during approach behavior (Root et al., 2012). This response is similar to the predominant response of NAc neurons during appetitive responding (Sharif A. Taha & Fields, 2006),

and is not the predicted result of VP disinhibition resulting from reduced GABA input by the NAc. Although in our studies, we are not examining approach behavior, it is possible that we are observing similar overlapping responses, as the animals are experiencing stimuli that would (in an instrumental design) elicit approach and avoidance, and the VP encoding could reflect the engagement of different aspects of these behaviors (Root et al., 2015). The pattern of responses of decreased cell firing characterized in the NAc and VP could reflect the involvement of other common inputs to both regions (e.g. VTA). Additionally, the neuronal activity of the VP is modulated by different neurotransmitter systems and inputs other than the NAc (Mitrovic & Napier, 1998), which can contribute to the complicated physiological response patterns observed in the VP. In sum, the current report reveals a degree of complexity to the encoding of rewarding and aversive stimuli by VP neurons and additional studies will be necessary to reveal the critical inputs that drive motivated behavior.

Chapter III

Striatal Modulation of Pallidal Reward Encoding

Abstract

Both the nucleus accumbens (NAc) and the ventral pallidum (VP) are essential regulators of appetitive behavior. Patterned neuronal activity that tracks the hedonic value of taste stimuli and motivated behavior has been documented in both the NAc and VP, yet little is known about how accumbens activity can modulate pallidal neural encoding of reward. In this study, 12 male Sprague-Dawley rats were used to directly examine the NAc-VP connectivity by pharmacologically manipulating NAc activity and recording the resulting changes in VP neuronal activity. The GABAA receptor agonist muscimol, the GABAA receptor antagonist bicuculline, and saline were microinfused into the NAc bilaterally before a hedonic test that assessed subjective "liking" and a consumption test that examined motivational "wanting". Results of the hedonic test showed that muscimol increased the inhibitory encoding of sucrose in the VP without affecting the behavioral hedonic response. In the consumption test, muscimol significantly increased sucrose consumption and, correspondingly, the number of neurons encoding sucrose-seeking behavior. Interestingly, none of the pharmacological treatments affected either the baseline firing rate or response magnitude of recorded VP units. The dose of bicuculline used in this study had little effect on reward-related behavior or VP neural encoding. These data support a role for NAc activity preferentially regulating the encoding of motivation by VP neurons.

Introduction

An important yet still elusive aspect of the neuroscience of reward is how hedonic and motivational information are neurophysiologically encoded and relayed through striatal circuitry. The ventral pallidum (VP) receives strong GABAergic projections from the nucleus accumbens (NAc) and both structures are critically involved in hedonic processing and motivated behavior for both natural rewards and drugs of abuse. Pharmacological studies have shown that the NAc and VP play important roles in affective regulation (Castro & Berridge, 2014; Reynolds & Berridge, 2002; K. S. Smith & Berridge, 2007), reward learning (Chang, Wheeler, & Holland, 2012), pair bonding (Aragona et al., 2006; Bales, Mason, Catana, Cherry, & Mendoza, 2007; Resendez et al., 2013), motivated feeding behavior (Stratford & Wirtshafter, 2012; Will, Franzblau, & Kelley, 2003; Zhang, Balmadrid, & Kelley, 2003) and drug-seeking behavior (Cruz et al., 2014; McFarland & Kalivas, 2001; Stefanik et al., 2013). Disconnection studies further provided behavioral evidence that communication between the NAc and VP is critical for motivated behavior and reward learning (Leung & Balleine, 2013; Smith & Berridge, 2007; Torregrossa, Tang, & Kalivas, 2008).

Studies using *in vivo* electrophysiological recordings in behaving animals provide the ability to characterize behaviorally-relevant neuronal responses. Such investigations indicate that the NAc and VP both encode positive and negative valence differentially, and these neural encoding patterns are sensitive to changes in the reward value of the stimulus (Chan, Wheeler, & Wheeler, 2016; Tindell et al., 2006; Wheeler et al., 2008). These two structures also encode behavioral responses evoked by reward (Krause et al., 2010; Richard et al., 2016; Root et al., 2012, 2011; Tindell et al., 2006). Together, these

findings strongly suggest an intimate functional connectivity between the NAc and VP for reward processing.

The activity of striatal neurons may encode hedonic valence, motivational significance, or both. Since these components of reward have been experimentally dissociated (Cannon & Palmiter, 2003; Susana Peciña et al., 2003; Schultz, Dayan, & Montague, 1997; Smith et al., 2011), discrete striatal circuits may differentially regulate them. Pharmacological manipulations indicate that suppressing NAc activity increases reward responding (Baldo, Alsene, Negron, & Kelley, 2005; W A Carlezon & Wise, 1996; Chartoff, Pliakas, & Carlezon, 2006). Correspondingly, electrophysiology studies demonstrate that NAc neurons respond to appetitive gustatory stimuli with primarily phasic reduction of cell firing (Nicola, Yun, Wakabayashi, & Fields, 2004a, 2004b; Roitman et al., 2005; Taha & Fields, 2005; Wheeler et al., 2008). In general, the experience of rewarding stimuli and reward-directed behavior is associated with decreased NAc activity (Carlezon & Thomas, 2009; McCutcheon et al., 2012). However, how suppression of NAc activity may affect the hedonic or motivational representations of reward by VP neuronal activity is less clear.

Considering the clear anatomical link between the NAc and VP, and the possibility of these structures encoding discrete aspects of reward-driven behavior, their functional connectivity deserves a detailed characterization. Based on the inhibitory nature of the GABAergic projection from the NAc to the VP, we hypothesized that pharmacologically inhibiting the activity in the NAc will exert a disinhibiting effect on the neural encoding of reward in the VP. In a counterbalanced experimental design, muscimol, bicuculline, and saline were bilaterally microinfused into the NAc shell of 12

rats before recording single-neuron activity in the VP in 1) a hedonic test in which animals received intraoral sucrose delivery and in 2) a sucrose self-administration consumption test. The results showed that increasing GABAergic activity in the NAc shell did not affect taste reactivity, but increased the inhibitory encoding of sucrose in the VP. Subsequently, pharmacological inhibition of the NAc increased both sucrose consumption as well as the neural encoding of this behavior in the VP. These data demonstrate a functional connectivity between the NAc and VP and characterize the specific influence of the NAc on the encoding of reward processing in the VP.

Materials and Methods

Animals

Fifteen male adult Sprague-Dawley rats (Harlan Laboratories, IN) weighing between 320-380 grams received bilateral guide cannula implantation aimed at the top of the NAc shell, bilateral electrode array implantation targeting the VP, as well as intraoral cannula implantation. Three subjects were removed from the study due to mild but noticeable motoric effects caused by bicuculline microinfusions, an unexpected result considering the use of a low dose (T R Stratford & Kelley, 1997). No seizure or non-specific behavioral effects were observed after bicuculline administration in the remaining 12 animals, and all behavioral and neuronal data collected from these subjects were included in the analyses. Following surgery, animals were individually housed in an AAALAC-accredited vivarium on a 12-hour reversed light/dark cycle. Food was removed 24 hours prior to the initial sucrose self-administration training to encourage learning. Animals were then maintained at 90% of their pre-training body weight during

the first 2 days of training. Upon completion of initial sucrose self-administration training, *ad libitum* access to food and water were provided throughout the remainder of the study. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Marquette University following the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

Surgical procedures

Animals were deeply anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) for surgical implantations of electrode arrays and intraoral (IO) catheters following established procedures (Chapter II). Details of the implantation and use of IO catheters for intraoral solution delivery have been described previously (Chapter II). Microelectrode arrays (NB Labs, TX) for electrophysiological recordings were stereotaxically lowered into the VP bilaterally, targeting at AP 0, ML ±2.0, DV -7.6 in 7 animals, and AP -0.5, ML ±2.8, DV -7.5 in 8 animals, to ensure a characterization of all areas of the VP. The microelectrode arrays contained 8 microwires arranged in 2 columns (columns separated by 0.5 mm and 0.25 mm between rows). Ground wire for each microarray wrapped around a skull screw and was inserted into the brain approximately 1mm. In the same surgery, animals also received bilateral guide cannula implantations aimed at the top of the NAc shell (AP +1.7, ML ±1.9 with a 9° lateral to medial angle, DV -5.9; microinjector tip extends 0.5mm further). The implanted arrays and cannulas were secured with dental cement anchoring to skull screws. The anti-inflammatory meloxicam (1% oral suspension) was given to animals after surgery and for 3 additional days post-surgery to suppress inflammation and reduce pain. Postoperative

health was monitored daily for 14 days, and a minimum of 7 postoperative recovery days elapsed prior to training and testing.

Apparatus

All training and testing procedures took place in a 43 x 43 x 53 cm Plexiglass chamber (Med. Associates, VT) housed in a faraday cage insulated with sound-attenuating foam. A rotary commutator (Crist Instruments, MD) connected to a headstage cable (Plexon Inc., TX) and fluid swivel for IO tastant delivery were positioned directly above the chamber. These moving parts rotate and allow animals to move freely in the chamber during the recording sessions. The IO tubing was connected to an infusion pump, which was programmed for sucrose delivery. Electrophysiological data were recorded on a computer interfacing with a 64 channel OmniPlex system (Plexon Inc., TX). A camera was positioned directly under the clear acrylic chamber floor for recording hedonic responses (taste reactivity) during test.

Sucrose self-administration training

In order to examine motivated behavior, animals were trained in 90 min sessions to nosepoke for intraoral sucrose infusions on a fixed ratio 1 (FR1) schedule of reinforcement prior to testing. Food restriction was applied prior to training and animals were maintained at 90% of pre-experiment body weight during initial training. Animals were returned to *ad libitum* feeding after sucrose self-administration had been acquired, typically at the end of the second training day. Each response yielded a single intraoral sucrose infusion (20%, 0.2 ml over 6 seconds). Testing occurred following the

establishment of stable daily training sessions (<15% variance in sucrose consumption between days).

Pharmacological manipulations of NAc neuronal activity

Every animal received a mock microinfusion, in which no drug was delivered into the NAc before testing to habituate animals to the testing procedure and to verify that guide cannulas were unobstructed. In a counterbalanced experimental design, animals received bilateral microinfusions of the GABAA agonist muscimol (100 ng/side), the GABAA antagonist bicuculline (50 ng/side), or saline infusions on each test day. To verify that the neural and behavioral effects of the pharmacological manipulations were due to the acute effects of the drugs, an additional saline test day (Saline2) was conducted for all subjects after all drug testing. Animals were gently held by the experimenter during the microinfusion (0.3 µl, at rate of 0.3 µl/min). After the microinfusion, animals were connected to the electrophysiology equipment and the IO tubing in the recording chamber 15 min before electrophysiological recordings began. The same procedures were repeated on subsequent test days, and free movement in the chamber was ensured during each test session.

Hedonic test and Consumption test

The goal of this study was to examine the neural encoding of both hedonic information processing and motivated behavior. Therefore, two tasks were given to animals on each test day after NAc microinfusion. In the first test, the processing of hedonic information was assessed. In this test, 20 passive infusions of a 20% sucrose

solution (0.2 ml over 6 seconds, 30 seconds intertrial interval) were delivered to subjects intraorally. Orofacial responses were digitally recorded and analyzed offline. Taste reactivity was analyzed in a frame-by-frame analysis. Appetitive and aversive responses were counted using the technique initially described by Grill and Norgren (1978). Instances of rhythmic tongue protrusions and paw-licking behavior were counted as appetitive responses. Aversive behavioral responses include gaping (mouth movements that matched a triangle shape for a duration exceeding 90 ms), paw flailing, wet dog shakes, and passive drips. The rates of aversive and appetitive events (per trial) were computed for each animal. Motivated behavior for sucrose was tested following the Hedonic test. Animals were allowed to nosepoke at FR1 for the same 20% sucrose infusion (0.2 ml, 6 seconds) for 90 minutes with no cap. In this design, animals exhibited active sucrose-seeking behavior during the first 30 minutes, and then became noticeably sated and inactive. Therefore, analyses were restricted to the first 30 minutes of the session. All behavioral data were analyzed using repeated measures ANOVAs.

In vivo electrophysiological recordings and data analysis

All animals were habituated to the recording chamber, the headstage cable, and IO tubing for 1 hour prior to the first test day. A commercially available neurophysiological system (OmniPlex, Plexon Inc., TX) was used to carry out online unit isolation and recording. The neurophysiological system provides a continuous digital output of neuronal events to a computer. At the same time, behavioral events (i.e. sucrose pump initiation, operant response) were registered by a different computer (Med Associates, VT) and corresponding transistor-transistor logic signals (TTLs) were

communicated with the electrophysiological system. Discrimination of patterned

waveform activity for neuronal units was accomplished using a principle component

analysis (PCA) procedure. In brief, sample of waveforms from a channel were manually

selected and the waveform properties were used to as a template to identify and record

any subsequent waveforms matching the template. This provides the capability to record

neuronal activity from distinct units at each channel. Following recording procedures,

additional PCA processing was conducted using Offline Sorter (Plexon Inc., TX) to

ensure proper unit isolation.

Unit activity was aligned with TTL timestamps of behaviorally relevant events,

specifically, sucrose infusions during the Hedonic test and responses registered during the

Consumption test. Phasic neuronal responses encoding these events were characterized

using the same procedures described in Chapter II. For sucrose encoding in the Hedonic

test, the average firing rate of each unit during the sucrose epoch (6 seconds after pump

initiation) was compared to the baseline epoch (6 seconds before infusions) using a t-test.

All significant increases and decreases in firing rate following stimulus presentation that

had a magnitude change of at least 10% were classified as phasic excitations and

inhibitions, respectively. For the neural data obtained during the Consumption test, based

on the observation that phasic encoding of an operant response often preceded the

response itself (see Figures 3.5A-B), we chose a baseline period of -3 to -1 seconds and a

response period of -1 to +1 seconds, with 0 being the operant response. t-test and 10%

response magnitude criteria were again used to identify phasic responses. As with the

Hedonic test, significant increases and decreases in firing rate (determined by t-test)

during the response period that had a magnitude change of at least 10% were classified as phasic excitations and inhibitions, respectively.

Effects of pharmacological manipulations were analyzed using repeated measures ANOVAs separately on excitatory and inhibitory responses. Planned comparisons were used to identify statistically significant differences after main effects or interactions were detected. Between-group differences in the proportions of cells that exhibited excitatory or inhibitory neuronal responses were compared using Chi-square tests.

Histology

After testing was completed, animals were euthanized with CO_2, and the location of each unit was verified. A current (20 μA) was run through each implanted microwire, and brains were incubated in 4% potassium ferricyanide, 10% formaldehyde solution. All brains were sliced into 40-μm sections and mounted onto glass slides. The slides were then stained with either 0.25% thionin, or neutral red, or left unstained before coverslipping. All placements were determined using Paxinos and Watson (2006) as a reference. Figures 3.1C, 3.2A-B detail all cannula and array placements. For the subsequent analyses, all units recorded anterior to bregma (AP ≥ 0) were classified as anterior VP units, while more caudal units (AP < 0) were considered posterior VP units.

Results

The effect of GABAergic manipulations in the NAc on hedonic responses

To directly examine how natural reward information is processed in the NAc-VP circuitry, muscimol (GABAA agonist), bicuculline (GABAA antagonist), and a saline

vehicle were bilaterally microinfused into the NAc shell before a hedonic test and a consumption test. Subjects received all pharmacological manipulations in a counterbalanced order, with minimum of 48 hours between tests. In the Hedonic test, 20 trials of passive sucrose solution infusions were delivered intraorally and the behavioral taste response (taste reactivity) was examined. Repeated measures ANOVA was used to analyze the expression of appetitive and aversive taste responses between drug conditions. No significant effect was observed on the expression of either appetitive (Figure 3.1A: $F_{(2,22)} = 1.98, p = 0.162$) or aversive taste reactivity (Figure 3.1B: $F_{(2,22)} = 0.16, p = 0.851$), indicating that no pharmacological manipulation produced a change in the hedonic perception of the animals. Histological verification indicated that all microinfusions were in the shell of the NAc (Figure 3.1C).
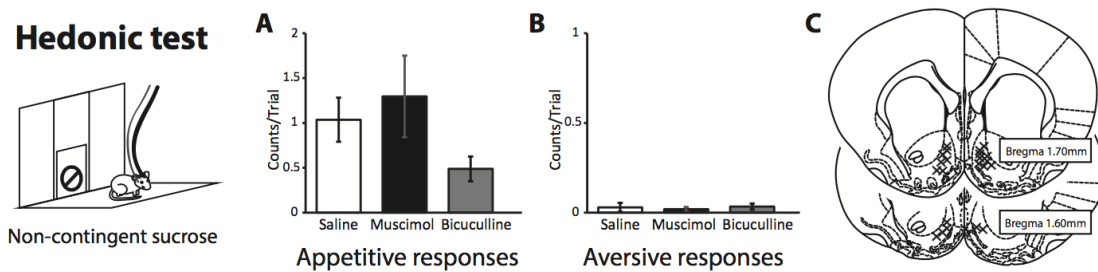
Figure 3.1. Pharmacological manipulations in the NAc did not affect the hedonic response to sucrose.
Muscimol (GABAA agonist), bicuculline (GABAA antagonist), and saline were microinfused into the NAc shell bilaterally. Subjects were connected to an infusion line for intraoral sucrose infusions (grey line) and a headstage cable for electrophysiology (black line). Twenty intraoral sucrose (20%) infusions were delivered (0.2 ml over 6 seconds each) passively and the corresponding taste reactivity was assessed. Pharmacological manipulations in the NAc did not alter the expression of (A) appetitive or (B) aversive taste reactivity (p values > 0.16). (C) Histological verification indicated that microinfusions were in the NAc shell.

Pharmacologically inhibiting NAc activity augmented the inhibitory neural encoding of sucrose in the VP

Electrode arrays were implanted into the VP to determine whether the neural encoding of sucrose was affected by pharmacological manipulations in the NAc. One hundred thirty-five, 140, and 113 VP neurons were recorded on saline, muscimol, and bicuculline test days, respectively. Locations of the recording units were determined histologically and broadly classified into anterior (Figure 3.2A) and posterior VP (Figure 3.2B). Analyses include both anterior and posterior regions, except where specified. Phasic firing rate changes of VP neurons were observed in response to intraoral sucrose infusions and examples are illustrated in Figures 3.2C-D.

Neuronal responses to sucrose
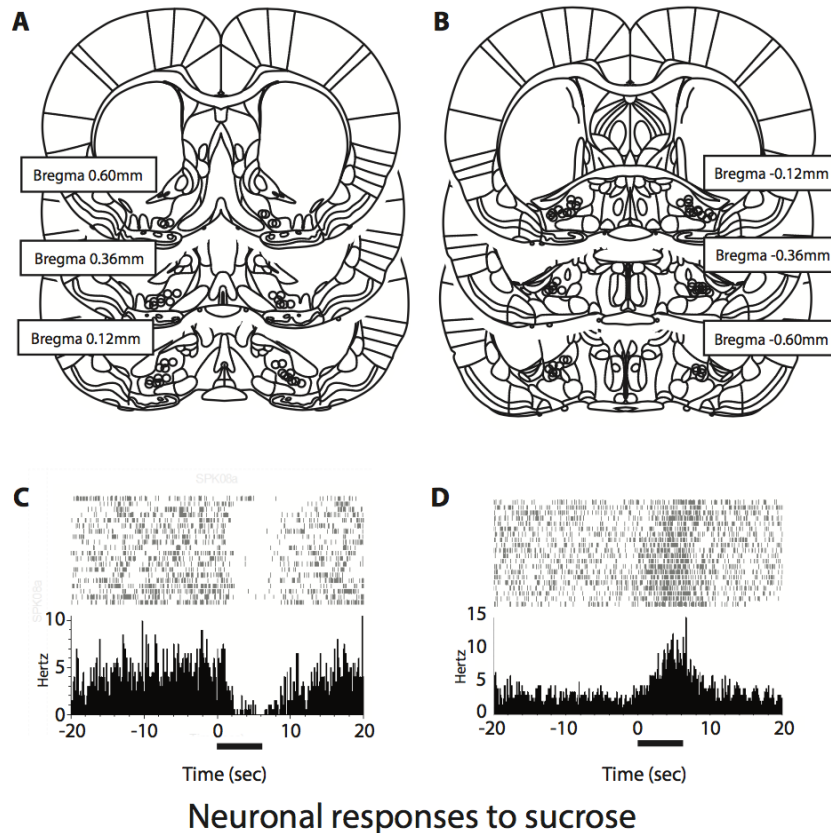
Figure 3.2. VP neurons encode passive infusions of sucrose.
Electrode arrays were implanted into the VP. Locations of units were classified into (A) anterior or (B) posterior VP for specific analyses. Phasic inhibitory (C) and excitatory (D) responses to sucrose infusion were observed, with neuronal event raster (top panel) and histogram of averaged firing rate (bottom panel) demonstrating changes of firing rate time-locked to sucrose infusions (horizontal black bar under histogram) in representative VP neurons.

In accordance with previous findings, the predominant phasic response following intraoral sucrose administration was phasic inhibition. Observed neuronal responses are summarized in Table 1. The sucrose-induced inhibitory response was enhanced following intra-NAc muscimol treatment. Repeated measures ANOVAs were used to analyze potential drug-induced changes in phasic response profiles. Significantly more phasic inhibitory responses were observed after muscimol microinfusion (Figure 3.3A: $F_{(2,22)} =$

4.52, $p = 0.023$) when compared to saline ($p = 0.035$) or bicuculline ($p = 0.035$). Drug treatment did not significantly alter excitatory responses ($F_{(2,22)} = 4.56, p = 0.64$). Interestingly, neither muscimol nor bicuculline microinfused into the NAc affected the baseline firing rates of VP neurons (Figure 3.3B: $F_{(2, 385)} = 1.00, p = 0.367$) or the response magnitude (Figure 3.3C: $F_{(2, 139)} = 0.71, p = 0.496$). This result indicates that pharmacological inhibition of the NAc selectively amplifies the VP phasic inhibitory encoding of sucrose.

We have previously observed a heterogeneity in the manner that reward information is processed in different areas of the VP (Chan et al., 2016). With the same approach, the possibility of a region-specific effect was examined by dividing the VP units into anterior and posterior regions (see Materials and Methods). Results of repeated measures ANOVAs (Region x Drug) for neural excitation and inhibition found only a significant main effect of Drug for inhibitory responses (Figures 3D-E: $F_{(2,22)} = 4.52, p = 0.023$) with no other effects (inhibitory responses: Region x Drug, $F_{(2,22)} = 0.15, p = 0.862$, ;excitatory responses: Region x Drug, $F_{(2,22)} = 0.45, p = 0.641$).

Consistent with this, results of chi-square tests indicated that muscimol and bicuculline treatments did not affect the proportion of phasic responses to sucrose infusion when compared to saline condition (Figures 3F-G, p values > 0.199). Therefore, muscimol microinfusion appeared to have broadly increased inhibitory encoding throughout the VP.
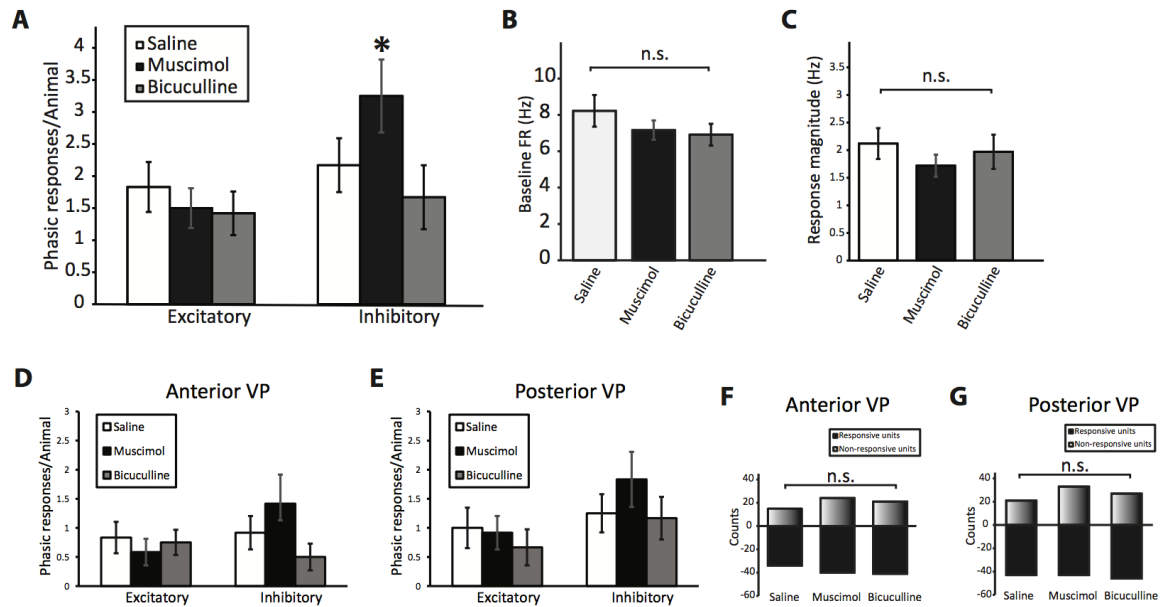
Figure 3.3. Administration of muscimol into the NAc altered sucrose encoding in the VP. Muscimol microinfused into the NAc resulted in significantly more phasic inhibitory responses for sucrose in the VP (*, $p = 0.035$) compared to saline control. Pharmacological manipulations in the NAc did not affect either the (B) baseline firing rate of VP neurons or the (C) magnitude of the firing rate change following sucrose administration. (D and E) Repeated measures ANOVAs revealed no region-specific effects when VP units were separated into anterior and posterior VP. (F and G) results of a chi-square analysis also indicated that muscimol and bicuculline treatments did not affect the proportion of phasic responses in a region-specific manner, muscimol (χ^2, $ps > 0.423$), bicuculline (χ^2, $ps > 0.609$).

Pharmacologically inhibiting the NAc enhanced the motivation to consume sucrose

To determine whether pharmacological manipulations of NAc activity affected motivation, animals were allowed to self-administer sucrose immediately after the Hedonic test. A repeated measures ANOVA revealed a significant main effect of Drug ($F_{(2, 22)} = 17.15$, $p = 0.00003$) indicating that muscimol administration significantly increased the voluntary consumption of sucrose when compared to saline control ($p = 0.002$) and bicuculline ($p = 0.0001$), Figure 3.4. However, bicuculline treatment had no effect on sucrose consumption when compared to saline ($p = 0.355$).
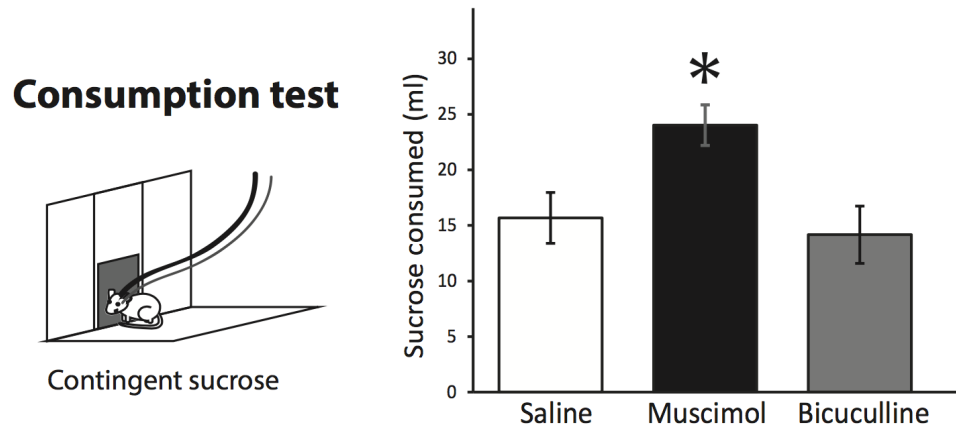
Figure 3.4. Muscimol increased consumption of sucrose in a self-administration test. Subjects were tested in an operant task in which each response yielded an intraoral sucrose (20%) infusion (0.2 ml over 6 seconds). Animals were allowed to self-administer sucrose for 90 minutes and the VP neuronal activity in this period was recorded. In this task, muscimol significantly increased sucrose consumption (*, $p = 0.00003$) when compared to saline ($p = 0.002$) and bicuculline ($p = 0.0001$).

Pharmacologically inhibiting NAc activity augmented the neural encoding of sucrose seeking

Coincident with the increase in behavioral responses, intra-NAc muscimol administration significantly enhanced the neural encoding of motivated behavior in the VP. In contrast to the patterned activity evoked by sucrose infusions, the pattern of activity surrounding reward-driven behavioral responses for sucrose were primarily excitatory. Phasic neural responses were typically initiated prior to the behavioral response for sucrose (Figures 3.5A-B). Ninety-nine, 109 and 86 VP neurons were recorded and analyzed on saline, muscimol and bicuculline test days, respectively. Repeated measures ANOVAs examining evoked phasic responses indicated no significant effect of Drug on excitatory responses (Figure 3.5C: $F_{(2,22)} = 2.47, p = 0.108$) but a significant effect on inhibitory responses (Figure 3.5C: $F_{(2,22)} = 4.18, p = 0.029$). An examination of this effect by VP region (Region x Drug repeated measures ANOVA)

again found significant drug effects only on inhibitory responses, and this effect did not

depend on region (Drug: $F_{(2,22)} = 4.52, p = 0.029$; Region: $F_{(1,11)} = 0.224, p = 0.646$;

Region x Drug: $F_{(2,22)} = 0.211, p = 0.811$). No significant Drug effects were observed on

excitatory responses (Figure 3.5D and E: Region: $F_{(1,22)} = 1.61, p = 0.231$; Drug: $F_{(2,22)} =$

$2.47, p = 0.108$; Region x Drug: $F_{(2,22)} = 1.41, p = 0.265$). However, muscimol caused a

significant increase in inhibitory responses (Figure 3.5E: $p = 0.032$) when compared to

bicuculline and a non-significant trend when compared to saline ($p = 0.085$). These data

indicate that coincident with the increase in sucrose consumption, intra-NAc muscimol

administration enhanced the inhibitory neural encoding of this task.

Intra-NAc muscimol infusions also caused a significant increase in the number of

neurons encoding response in the Consumption task from 43% (43/99) to 70% (76/109).

This effect was observed in both the anterior (Figure 3.5F: $\chi^2, p = 0.0185$) and posterior

VP (Figure 3.5G: $\chi^2, p = 0.0027$) as the proportion of phasic responses in both regions

increased significantly. Consistent with previous analyses, muscimol treatment did not

influence response magnitude ($F_{(2,165)} = 1.22, p = 0.297$).
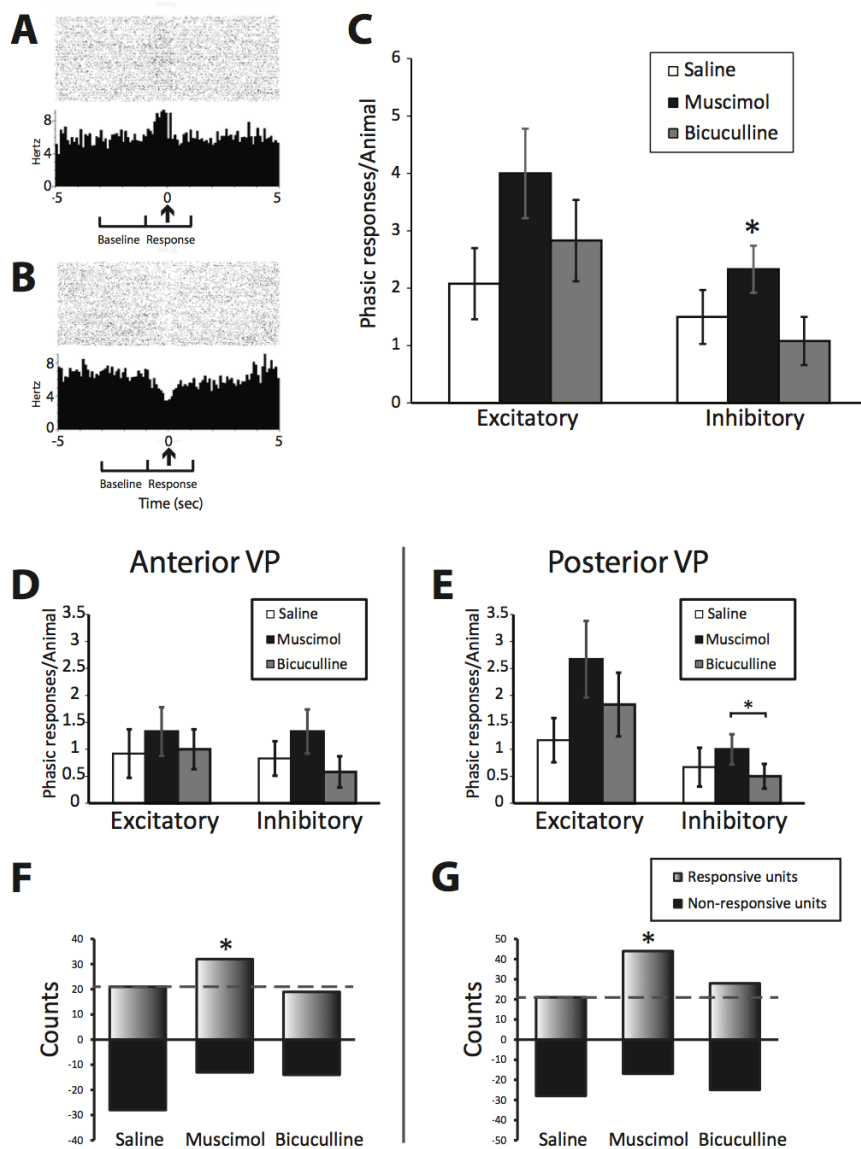
Figure 3.5. Muscimol in the NAc augmented the neural encoding of food-seeking behavior in the VP.

(A and B) Phasic excitatory and inhibitory responses were observed during the consumption test. Arrow at t = 0 indicates behavioral response for sucrose reward. The average firing rate during the response period (-1 to +1 sec) was compared to that of the baseline period (-3 to -1 sec) to identify phasic encoding of the task. I Results of a repeated measures ANOVA revealed that muscimol increased the occurrence of inhibitory responses encoding sucrose-seeking behavior ($p = 0.029$). (D and E) Separation of VP subregions to anterior and posterior revealed that muscimol administration significantly increased the occurrence of inhibitory responses, compared to bicuculline ($p = 0.032$). In addition, muscimol increased the number of VP neurons that encoded food-seeking behavior in both the (F) anterior VP ($\chi^2, p = 0.006$) and (G) posterior VP ($\chi^2, p = 0.002$).

Overlapping responses in the VP encoding sucrose and motivated behavior

Many units exhibited phasic responses in both the Hedonic and Consumption tests. 41.7% (20 out of 48 responses) of the sucrose-encoding VP units also encoded sucrose self-administration following saline administration. The populations of dual encoding were 59.6% (34 out of 57 responses) and 48.6% (18 out of 36) following muscimol and bicuculline testing, respectively. As described above, the predominant electrophysiological response to intraoral infusion was a decrease in firing rate, whereas behavioral response to obtain sucrose typically evoked an increased firing rate. Few units responded to both behaviors with the same type of response (30% for saline (6/20), 29% for muscimol (10/34), and 11% for bicuculline (2/18)). Finally, an additional saline control test day was conducted to determine the possible presence of off-target drug effects on behavior and neurophysiology. Subjects showed no difference in either taste reactivity, sucrose consumption, or any aspects of VP neuronal activity or encoding patterns between the two saline conditions (data not shown).

Discussion

Using pharmacological manipulations and *in vivo* electrophysiological recording techniques, the current study characterized functional NAc-VP connectivity that is involved in encoding hedonic information processing and motivated behavior. Although neither muscimol nor bicuculline had a significant effect on the expression of taste reactivity to non-contingent intraoral sucrose infusions in the Hedonic test, muscimol augmented the inhibitory neural encoding profile for sucrose. Additionally, muscimol dramatically increased sucrose self-administration by 53% in the subsequent

Consumption test. Consistent with previously reported electrophysiological data (Chan et al., 2016), the predominant VP neuronal response following a non-contingent intraoral sucrose infusion was a phasic decrease of firing rate. In contrast, sucrose-seeking behavior was generally associated with excitatory VP neuronal responses. While local muscimol infusions dramatically augmented the neural encoding of sucrose self-administration and increased sucrose consumption, there was an apparent lack of effect of bicuculline. This may be due to the fact that a lower dose of bicuculline was specifically chosen in order to avoid non-specific motoric effects and potential seizures that have been reported previously (Stratford & Kelley, 1997). Taken together, these results characterize a differential encoding of hedonic responses and motivated behavior by VP neurons, and demonstrate evidence that VP reward encoding is modulated by NAc neural activity.

Pharmacological inhibition of the NAc promoted motivation without affecting hedonic processing

The expression of neither appetitive nor aversive taste reactivity was significantly affected by the pharmacological manipulations used in these experiments. The NAc and VP are loci of hedonic and motivational regulation, two dissociable components of reward (Berridge & Robinson, 2003; Wassum, Ostlund, Maidment, & Balleine, 2009), and it is important to note that hedonic processing is more strongly associated with opioid and cannabinoid signaling (Peciña et al., 2000). Prior investigations have demonstrated that local NAc muscimol administration broadly invigorates motivation and food consumption, while only generating hedonic-enhancing effects in a spatially restricted

"hotspot" (Castro & Berridge, 2014; Faure, Richard, & Berridge, 2010). Since GABAergic signaling is more closely aligned with motivation, our data are consistent with prior pharmacological manipulations and contribute a corresponding characterization of downstream neuronal activity.

Previous studies have shown that VP encoding of reward tracks hedonic value (Chapter II). However, this encoding could reflect components of reward processing other than pure hedonic value (i.e., liking). In the present experiments, muscimol administration in the NAc significantly augmented the inhibitory encoding of non-contingent sucrose delivery by VP neurons despite the absence of an effect on hedonic processing. This dissociation demonstrates that the NAc has a contribution to VP activity that preferentially encodes a motivational signal. This specific contribution appears to be sufficient to promote motivated behavior and consumption when the context is appropriate for such action. Although this signal may be present during the non-contingent administration of sucrose, the situation does not allow for the expression of incentivized, goal directed action.

In these studies, muscimol administration profoundly augmented feeding behavior in the consumption test, an observation consistent with several prior reports. Microinfusion of GABA agonists into the NAc have been shown to profoundly increase food and sucrose consumption (Basso & Kelley, 1999; Hanlon, Baldo, Sadeghian, & Kelley, 2004; Stratford & Kelley, 1997). While the ratio of excitatory and inhibitory responses remained unaltered following muscimol administration, the total number of units found to encode aspects of this behavior significantly increased. In other words, more neuronal responses were observed during the consumption test following muscimol

administration. This effect was particularly strong in the posterior region of the VP, where there was a dramatic increase in the number of responsive units (44 compared to 22 following saline administration). Overall, pharmacological inhibition of the NAc augmented the VP encoding, and performance of, goal-directed action.

NAc-VP functional connectivity

      The observation that pharmacological inhibition of NAc activity amplifies the encoding of motivated behavior in the VP without altering the baseline firing rate or response magnitude demonstrates the nature of a functional connectivity between the two structures. Phasic reductions of NAc activity are linked with the processing of natural rewards and the engagement of goal directed action (Carlezon & Thomas, 2009; Roitman et al., 2005; Root et al., 2012; Taha & Fields, 2006; Wheeler et al., 2008). Consistent with this, pharmacological manipulations that reduce NAc activity (Baldo et al., 2005; Carlezon & Wise, 1996; Chartoff et al., 2006) promote motivated behavior. Given the strong connectivity between the NAc and the VP, it has been proposed that reductions in NAc GABAergic projection neuron activity act to disinhibit the VP to promote behavior (Nicola et al., 2004b; Smith, Tindell, Aldridge, & Berridge, 2009; Wheeler & Carelli, 2006). Indeed, the presence of natural rewards and their predictors have been shown to increase the firing rates of neurons in the VP (K. S. Smith et al., 2011; Tindell et al., 2009, 2006). However, studies characterizing phasic neuronal activity have shown that palatable gustatory stimuli cause predominantly inhibitory responses in both structures (Chan et al., 2016; Roitman et al., 2005; Wheeler et al., 2008). This is an unintuitive finding, suggesting anatomical complexity. In support, electrical stimulation of the NAc

induces mixed response types in the VP (Chrobak & Napier, 1993; G J Mogenson et al., 1983). Which may be due to the influence of NAc medium spiny neurons forming synapses on local interneurons in the VP (Záborszky & Cullinan, 1992), which could then modulate the threshold of excitation of discrete populations of VP neurons. Based on these observations, the nature of NAc-VP neuronal signaling appears to be a complex one, and NAc projection neurons could be modulating subpopulations of VP neurons that differentially encode aspects of the palatable stimulus or goal directed action. Despite these complexities, results of the current study demonstrate that NAc activity modulates both behavioral responses to rewarding stimuli and behaviorally-relevant phasic activity in the VP in a robust and predictable manner.

Insight into the neural representation of motivation and hedonic processing

Both the inherent hedonic value of a stimulus and the incentive generated by that stimulus guide motivated behavior. The NAc and VP are particularly important loci for processing this hedonic and motivational information to direct reward-driven behaviors. Pharmacological manipulations have demonstrated that the direct projection from the NAc to the VP is essential for the proper expression of learned approach (Leung & Balleine, 2013; McFarland & Kalivas, 2001). Although phasic neural encoding of hedonic and reward information has been observed in both the NAc and VP (Chan et al., 2016; M. F. Roitman et al., 2005; R. A. Wheeler et al., 2008), few experimental approaches have the ability to discern the two phenomena. Understanding how activity of an upstream structure modulates activity of downstream targets could provide important insights, not only on how behaviorally relevant information is being relayed selectively to

different brain circuitries, but also on the specific nature of the information that is being encoded. By manipulating NAc activity and recording VP neuronal activity in hedonic and motivation tasks, we present direct evidence demonstrating a functional connectivity between the NAc and the VP. This report details the influence of reduced NAc activity on VP neural encoding of reward information. Moreover, they support a role for NAc activity preferentially regulating the encoding of motivation by VP neurons.

Chapter IV

Dopaminergic Regulation of the Neural Encoding of Reward in the NAc

Abstract

Midbrain dopamine neurons regulate reward evaluation and motivated behavior, in part, by modulating the phasic firing rates of NAc neurons. Phasic changes of both NAc (R. A. Wheeler et al., 2008) and VP (Chapter II) neuronal firing rates encode various aspects of rewarding stimuli. While rewarding taste stimuli increase, and aversive stimuli reduce dopamine signaling, the causal relationship between dopaminergic signaling and the neural encoding of reward information in behaving animals is less clear. In this study, the effect of inhibiting dopaminergic neuronal activity in the ventral tegmental area (VTA) on accumbal neuronal activity and sucrose encoding was examined. The inhibitory DREADD, hM4Di, was virally expressed in the VTA of male Long Evans TH-Cre positive rats. Electrophysiological arrays recorded NAc neuronal firing rate changes in response to sucrose reward in normal and low dopamine environments. On each given test day, animals were exposed to blocks of 45 intraoral sucrose (20%) infusions (0.2 ml over 6 seconds) before and after IP injections of either CNO (2mg/kg) or saline vehicle (0.9%) in a counterbalanced design. Seven animals were tested in the current study, with 84 and 82 units recorded on CNO and saline conditions, respectively. Consistent with prior reports, sucrose infusions typically evoked inhibitory responses in NAc neurons during baseline recordings. Interestingly, neither saline or CNO affected the baseline firing rates of NAc neurons. However, CNO administration significantly altered the overall neural representation of sucrose, shifting the response profile from inhibitory to more excitatory. These observations suggest that reward and aversion-induced fluctuations in dopamine exert a pronounced influence over the way NAc neurons encode rewarding stimuli This study also examined the effects of dampening dopaminergic signaling on various reward-related tasks, including instrumental reward-seeking behavior (FR1), sucrose consumption and locomotion activity measurement, and found no significant effects. Finally, a progressive ratio (PR) test was employed and found a non-significant trend toward reduced responding in animals following chemogenetically suppressed dopamine signaling, suggesting a potential role for dopamine in effort-related behavior.

Introduction

The mesolimbic dopamine system has long been known as a central regulator of reward processing (Wise, 2004). Dopamine neurons in this pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) increase firing rate in response to primary rewarding stimuli and their predictors (Mirenowicz & Schultz, 1996; Mogenson et al., 1980; Oades & Halliday, 1987). Terminal measures of dopamine in the NAc using microdialysis confirm that both natural rewards and drugs of abuse elevate dopamine concentrations (Devine et al., 1993; Hernandez & Hoebel, 1988). Similarly, results from fast-scan cyclic voltammetry studies measuring subsecond changes in dopamine concentration uniformly reveal that a variety of rewarding stimuli, palatable tastes, cocaine, and their predictors all evoke rapid increases in dopamine concentration in the NAc (Roitman et al., 2008; Stuber et al., 2005; Wheeler et al., 2015). Interestingly, some of these studies also revealed that aversive stimuli can produced the opposite result, a phasic reduction of dopamine concentration, suggesting the possibility that dopamine is involved in the processing of both reward and aversion (Roitman et al., 2008; Twining et al., 2015; Wheeler et al., 2011).

In the NAc, *in vivo* electrophysiological recordings have characterized the neuronal responses to rewarding and aversive stimuli. NAc neurons differentially encode appetitive and aversive taste stimuli and their predictors (Roitman et al., 2005; Wheeler et al., 2008) as well as motivated behavior directed toward natural and drug rewards (Carelli, 2002; Day, Wheeler, Roitman, & Carelli, 2006; Nicola et al., 2004b). These studies describe that the predominant response to rewarding stimuli is a reduction in firing rate, and this reduction is also the predominant pattern of activity while an animal

is engaged in seeking behavior. Increased activity is associated with aversive stimuli. In fact there is evidence that this reward-encoding inhibitory response in the NAc is necessary for the generation of motivated behavior for food (Krause et al., 2010). Taken together, these findings suggest that phasic neuronal responses in the NAc encode reward valence and mediate reward-seeking behavior.

Since dopamine acts in the NAc to modulate sensitivity to glutamatergic inputs that regulate MSN activity, dopamine is well positioned to be a critical regulator of NAc neural encoding of rewarding and aversive stimuli (Surmeier et al., 2007). To examine the selective effect of dopamine on *in vivo* neuronal activity, this study examined the effects of suppressing the activity of VTA dopamine neurons using a chemogenetic DREADD approach. Expression of the inhibitory DREADD, hM4Di, was induced selectively in VTA dopaminergic neurons of rats selectively bred to express Cre-recombinase in tyrosine hydroxylase-expressing cells (TH-Cre rats). These inhibitory designer receptors can be activated by systemic injection of clozapine-N-oxide (CNO) which results in the suppression of midbrain dopaminergic neuronal activity and dampened dopamine signaling in the NAc (Liu et al., 2016). The effects of reduced dopamine signaling on NAc neuronal activity and reward encoding were monitored using in vivo electrophysiological recording techniques during sessions of sucrose administration, as well as tests of motivated behavior. The results indicate that inhibiting dopamine signaling shifted the neuronal response to sucrose in the NAc, abolishing the inhibitory encoding profile to sucrose observed in previous studies. Furthermore, CNO injection non-significantly reduced effort for sucrose reward in a progressive ratio task without having any observed effects on sucrose consumption or FR1 sucrose self-

administration. These data support a role of dopamine in regulating effortful responding for reward and identified a reliable underlying neurophysiological response that correlates with aspect of reduced motivational state.

Methods and Materials

Animals

A total of 7 male TH-Cre Long-Evans rats were used in this study. To create TH-Cre animals, homozygous female breeders, expressing Cre recombinase under the control of the TH promoter, were crossed with wild-type male Long-Evans rats. Tissue was collected from all offspring and genotyped (Transnetyx, TN). Male offspring heterogeneously expressing the Cre transgene received surgical procedures when they reached adulthood and weighed at least 300 grams. Following surgery, animals were single housed in AAALAC-accredited vivarium on a 12 h reversed light/dark cycle. All training and experimental procedures took place during the dark phase, the naturally active phase of the animals. Animals received *ad libitum* access to food and water throughout the study unless mentioned otherwise.

Surgeries

- Gi-DREADD

All rats received bilateral intra-VTA injections of the adeno-associated virus (AAV-hSyn-DIO-hM4D(Gi)-mCherry, UNC Vector Core, USA) containing the floxed inverted sequence of hM4Di(Gi)-mCherry, which is reoriented and expressed in the presence of Cre. Rats were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.)

and xylazine hydrochloride (10 mg/kg, i.p.), placed into a stereotaxic instrument (Kopf, Inc.) and 2 burr holes were drilled above the VTA on each side. Injection procedures followed those published previously for the expression of channelrhodopisin-2 in TH:Cre+ rats (A. J. McDonald, 1991). All target coordinates were measured relative to Bregma using the rat brain atlas of Paxinos and Watson (2006). The VTA was targeted using the following coordinates (in mm): -5.4 and-6.2 anteroposterior (AP), ±0.7 mediolateral (ML), -8.2 and -7.0 dorsoventral (DV) from skull surface for each AP placement. A custom made two-tipped injector (tips separated by 0.8 mm) permitted simultaneous virus infusion to bilateral AP sites at each of the two target DV depths. 1 µL of virus was bilaterally infused to each site (8 sites total, 4/hemisphere) at a rate of 0.1 µL/min. Following infusion, the injector remained in place for an additional 10 min. Rats received postoperative meloxicam suspension (1.0 mg/kg) for pain management and were returned to their home cages. All subjects recovered from the surgery and were given a minimum of 10 days before training and the second surgery. Recovered animals were trained to lever-press for food and sucrose before receiving the electrophysiology surgery.

- Electrophysiology array and intraoral catheter implantations

All subjects received a second surgery in which electrophysiology arrays and intraoral catheters (IO) were surgically implanted. Ketamine and xylazine were again used to deeply anesthetized animals.

The IO catheters were implanted bilaterally, lateral to the first maxillary molar and exteriorized on the top of the animal's head. Plastic washers were used to secure catheter placement. Microelectrode arrays (NB Labs, TX) for electrophysiological

recordings were stereotaxically lowered into the NAc bilaterally (Core: AP 0, ML ±2.0, DV -7.6; Shell AP -0.5, ML ±2.8, DV -7.5). The microelectrode arrays contained 8 microwires arranged in 2 columns (columns separated by 0.5 mm and 0.25 mm between rows). Ground wire for each microarray wrapped around a skull screw and was inserted into the brain approximately 1mm. Every animal had one array in NAc shell and the other in the core, counterbalanced between animals. Similar to treatments after the first surgery, animals received meloxicam daily starting the day of surgery and continuing for 4 consecutive days. Powdered food was given after IO surgery. Recovery of animals was monitored on daily basis for a minimum of 14 days. Intraoral catheters were flushed with water daily to maintain patency throughout the experiment. All subjects recovered from all surgeries and exhibited no health complications.

Apparatus

Training and electrophysiological recording sessions took place in a 43 x 43 x 53 cm Plexiglass chamber (Med. Associates, VT) inside a faraday cage insulated with sound-attenuating foam. An infusion line was installed and connected to a syringe pump outside of the faraday cage for solution delivery. The pump was connected to a computer and was controlled by a MedPC computer program. A fluid swivel for intraoral infusion and an electrical commutator for the electrophysiology cable were positioned above the chamber, set up in a fashion that allowed animals to move without restraint. A separate computer with commercially available programs PlexControl and NeuroExplorer was used to collect data from electrophysiological recordings. In addition, a camera was

placed under the chamber to record the orofacial movements during tastant delivery for taste reactivity assessment.

Behavioral procedures

- Operant responding for sucrose

Animals were trained to lever press for sucrose after recovery from the first surgery. Food was removed from the homecage 24 hours prior to the initial training session. During training, in which animals were free to explore the operant chamber, each successful lever press resulted in the delivery of 1 sucrose pellet (FR1) was dispensed into the food receptacle. Additionally, one sucrose pellet was dispensed into the food receptacle every 2 minutes when no successful lever press registered. The program terminated when 50 sucrose pellets were rewarded or 60 minutes had passed. During this initial training, animals were maintained at 90% body weight. All animals reached stable operant responding behavior for at least 3 days before returning to *ad libitum* feeding conditions and receiving the second surgery.

After recovery from the second surgery, animals were restricted to 90% of body weight and returned to operant training sessions in which responding was reinforced by intraoral infusion of a sucrose solution (0.2 ml delivered over 6 seconds). All animals were returned to *ad libitum* feeding conditions and continued to exhibit stable self-administration behavior.

-Effect of reduced dopamine on locomotor activity

To test if the CNO-induced reduction in dopamine signaling had any effect on general motor activity, locomotor behavior was tested after animals fully recovered from

the electrophysiology surgery. Animals were acclimated to the locomotion testing chamber prior to testing. Ambulatory behavior was recorded in an automated manner using through with motion sensor data relayed to a computer using commercial software. Animals were administered either saline or CNO (i.p., 2.0 mg/kg) 30 minutes prior to the testing session, which lasted 4 hours. Total activity (all movements detected) and ambulatory activities (horizontal movements/distance traveled) were used as measures for overall activity level and locomotor activity, respectively.

- Effect of reduced dopamine on instrumental reward-seeking behavior

The effect of midbrain dopamine neuron inhibition on instrumental lever-pressing behavior for sucrose reward was examined in all rats. As described above, all animals were trained to lever press (FR = 1) for sucrose infusions (0.2 ml over 6 sec). On the test day, animals received either a 2.0 mg/kg CNO injection or saline injection of equivalent volume. 30 minutes later, animals had the opportunity to respond for sucrose in a 60-minute session.

- Effect of reduced dopamine in effort-related behavior

A progressive ratio (PR) requirement for reinforcement can be used to assess motivational state (Randall et al., 2012). Subjects trained to lever press for sucrose reward (20%, 0.2 ml over 6 sec.) received either CNO, 2.0 mg/kg or saline (i.p.). Thirty minutes later, animals were provided the opportunity to self-administer sucrose under a PR requirement of reinforcement. Under this requirement, the number of responses required to receive a sucrose reward increases (ratio schedule: 1, 2, 4, 8, 16, 32) after every 10 rewards received. The schedule requirement at which each subject terminated self-administration behavior was identified as the break-point.

- Effect of reduced dopamine on sucrose consumption

To evaluate if CNO had any effect on sucrose consumption, subjects were injected with either CNO (2.0 mg/kg) or saline (i.p.) and returned to their home cages for 30 minutes where they had access to a bottle of 20% sucrose. Consumption of the palatable sucrose solution was recorded.

- The effect of reduced dopamine on taste reactivity

The effect of CNO on taste reactivity was tested by presenting subjects with 45 intraoral infusions of sucrose (0.2 ml, over 6 sec.) before, and 45 infusions after, injection of CNO (2.0 mg/kg, i.p.) or saline. Baseline taste reactivity responses to sucrose were recorded, then tastant infusions resumed 30 min following CNO administration. Rats were tested in multiple sessions (minimum of 48 hours between tests) in which animals received either CNO or saline during test. Appetitive and aversive taste reactivity responses were assessed in a frame-by-frame analysis in the manner described in Chapter II and III. A mixed-design ANOVA was used to examine the within subject effects of injection (before vs. after injection) and between treatments (saline vs. CNO).

Voltammetry recordings

In order to evaluate whether CNO administration can reduce NAc dopamine signaling in TH-Cre rats that express the Gi DREADD in VTA dopamine neurons, recordings of stimulated dopamine release in the NAc were conducted in a manner similar to previously reported methods (Graf et al., 2013). The VTA stimulating electrode was connected to a rotating commutator (Crist Instruments). Briefly, glass-encased, cylindrical carbon-fiber microelectrodes with an exposed length of 75–100 μm and

Ag/AgCl reference electrodes were prepared. Subjects were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) before lowering the carbon-fiber microelectrode into the NAc. The fiber was held at –0.4 V against Ag/AgCl between scans and then driven to +1.3 V and back in a triangular fashion at 400 V/s for each voltammetric measurement. Application of this triangle waveform causes oxidation and reduction of chemical species that are electroactive within this potential range, producing a change in current at the carbon fiber. Dopamine was identified by plotting changes in current against applied potential to produce a cyclic voltammogram. Current arising from electrode surface processes was subtracted as background. Background-subtracted cyclic voltammograms were obtained by digitally subtracting voltammograms collected during stimulation from those collected during baseline recording. Voltammetric responses were viewed as color plots with the abscissa as voltage, the ordinate as acquisition time, and the current encoded in color (Heien et al., 2005; Heien, Johnson, & Wightman, 2004). Temporal responses were determined by monitoring the current at the peak oxidation potential for dopamine by principal component regression. Measurements were made every 100 ms and, after driving the electrode into the NAc core, the electrode equilibrated for 40 min before any data were collected. Analyte identification and quantification were achieved using principal component regression analysis described previously in detail (Heien et al., 2004). All data presented here fit the resulting model at the 95% confidence level. Briefly, training sets were generated from background-subtracted cyclic voltammograms collected during and after electrical stimulations. At least 10 voltammograms were obtained for dopamine and pH. The resulting current amplitude was converted to dopamine concentration based on calibration of the electrode. Ten 60-

sec. trials with stimulation onset at 5 sec. were recorded. Another 60, 60-sec. trials were recorded immediately after CNO injection (2.0mg/kg, i.p.).

Effect of reduced dopamine on the NAc encoding of a rewarding stimulus

Electrophysiological recording techniques used in this study were identical to those detailed in Chapter II and III. A commercially available neurophysiological system (OmniPlex, Plexon Inc., TX) was used to carry out online unit isolation and recording. The neurophysiological system provides a continuous digital output of neuronal events to a computer. At the same time, behavioral events (i.e. sucrose pump initiation) were registered by a different computer (Med Associates, VT) and corresponding transistor-transistor logic signals (TTLs) were communicated with the electrophysiological system. Discrimination of patterned waveform activity for neuronal units was accomplished using a principle component analysis (PCA) procedure. Sample of waveforms from a channel were manually selected and the waveform properties were used to as a template to identify and record any subsequent waveforms matching the template. This provides the capability to record neuronal activity from distinct units at each channel. Following recording procedures, additional PCA processing was conducted using Offline Sorter (Plexon Inc., TX) to ensure proper unit isolation. Unit activity was aligned with TTL timestamps of sucrose infusions (pump initiation) to analyze changes of neuronal activity in response to tastant presentation.

To determine the effect of reduced dopamine signaling on the encoding of sucrose by NAc neurons, subjects received 45 infusions of sucrose before and after CNO administration. Changes in neuronal activity in response to sucrose infusions were

examined in 100 ms bins using NeuroExplorer (Nex Technologies, MA). Due to the

limited number of phasic units recorded in the NAc, the effect of CNO administration on

the overall activity of the NAc, the activity patterns of all neurons were averaged in the

manner of Roitman et al. (2010). The effect of reduced dopamine signaling on the

activity of NAc neurons was tested by comparing the average baseline firing rates of all

recorded units before and after injection of CNO.

Phasic changes in firing rate in response to sucrose infusions (increases or

decreases in rate) were characterized by comparing the average firing rate during sucrose

infusion (6 sec.) to that of the baseline period (6 sec. before sucrose infusion) using

paired t-tests. A repeated-measures ANOVA was used to determine if CNO or saline

affected sucrose encoding by NAc neurons.

Drugs

Clozapine-n-oxide (CNO) was purchased from Tocris (Bristol, UK). D-

Amphetamine sulfate was purchased from Sigma Aldrich (St. Louis, MO). All drugs

were prepared in sterile saline (0.9% NaCl) and administered by systemic intraperitoneal

(i.p.) injection.

Tissue Preparation and Immunohistochemistry

All rats were examined for reporter expression in the VTA. Following

experimental procedures, subjects were anesthetized and perfused. Before perfusion, a 10

μA current was passed through each electrode. Animals were then perfused with 10%

formaldehyde and 4% potassium ferrocyanide. Brains were removed and incubated in the

same formaldehyde/potassium ferrocyanide solution before DREADD expression and electrode placement verification.

A subset of TH:Cre+ rats (n = 4) were tested for mCherry (the hM4D-Gi-reporter) and tyrosine hydroxylase (TH) co-expression to verify dopamine neuron-specific expression. Subjects were deeply anesthetized with ketamine/xylazine, and transcardially perfused with 0.05 M phosphate buffered saline (PBS) and then 4% paraformaldehyde. Brains were removed and after post-fixing and freezing, sliced through the VTA in 40 μm sections in a cryostat. To label TH-expressing cells in the VTA, brain slices were washed three times (0.05 M PBS), and then blocked and permeabilized in solution with 0.3 M glycine (Invitrogen), 5% donkey serum (Sigma), and 0.3% PBST (0.3% Triton X-100 (Sigma) in 0.05 M PBS) for 20 min. at room temperature. Following three 0.05 M PBS washes, tissue was incubated overnight with mouse anti-TH primary antibody (1:1600 dilution, Millipore) in 0.1% PBST and 5% donkey serum solution at 4°C. The following day, tissue was washed three times (0.05 M PBS) and incubated in the secondary antibody solution (0.1% PBST and secondary antibody: Alexa Fluor 488 donkey anti-mouse (1:2000 dilution, Invitrogen)) at room temperature for 2 hours. Tissue was washed three more times with 0.05 M PBS before being mounted onto glass slides and coverslipped with Vectashield mounting medium with DAPI (Vector Laboratories) for imaging.

All imaging procedures for the characterization of mCherry and TH co-expression were conducted using an Eclipse Ti-S inverted microscope (Nikon). Images were acquired using a CoolSNAP ES2 camera (Photometrics) and NIS-Element Microscope Imaging Software (Version 4.00.03, Nikon). Colocalization of TH immunofluorescence

and mCHerry expression was carried out in multiple randomly selected fields (100x magnification) within the VTA of each subject. An identical analysis was conducted in a nearby TH-negative control region, the interpeduncular nucleus (IPN). In each of these areas, mCherry expressing cells were first identified, and each mCherry positive cell was examined for TH labeling. In this manner, the selectivity of targeting dopamine neurons in the VTA with viral particles was determined.

Results

Selective expression of Gi DREADD receptors on midbrain dopaminergic neurons

Gi DREADD receptors were virally transfected in dopamine neurons in the VTA in TH-Cre rats. These animals express cre-recombinase under the control of a TH promoter, which is produced in dopaminergic neurons. The expression of the Gi DREADD is dependent on the presence of cre-recombinase and is therefore selective to dopamine neurons. To validate the selectivity of DREADD receptor expression in the VTA, a separate group of animals (n = 4) injected with the Gi DREADD virus was examined immunohistochemically for TH expression using a selective TH antibody. 205 VTA neurons expressing the mCherry reporter of the DREADD receptor were examined and 92% (189/205) were found to also express TH (Figure 4.1A-F). It is important to note that a very small number of mCherry-expressing neurons (n = 4 neurons) was observed in the IPN (Figure 4.1G), an area where significant non-specific expression has been reported in the TH-Cre mouse strain (Lammel et al., 2015). These findings demonstrate that this chemogenetic approach provides the ability to selectively modulate the activity of dopamine neurons.
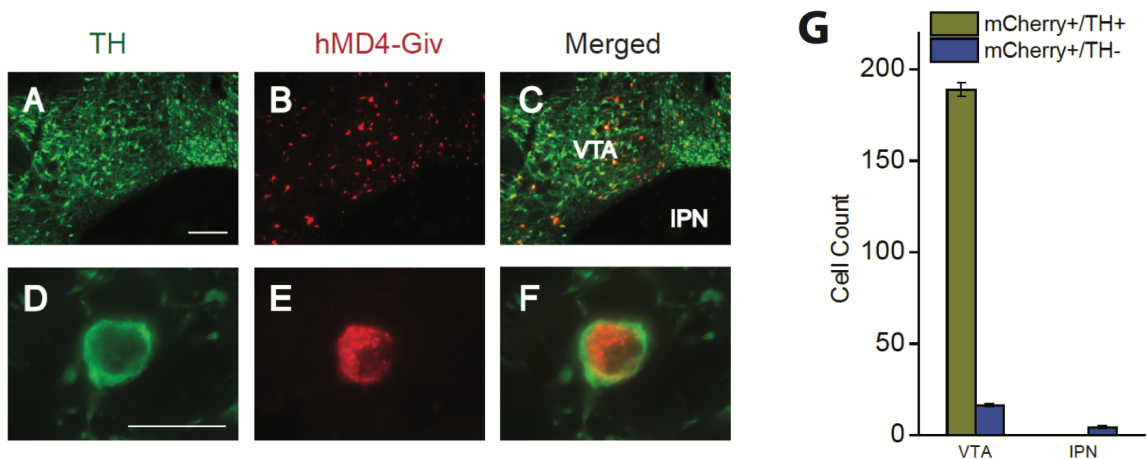
Figure 4.1. Selective expression of Gi DREADD by VTA dopamine neurons.
VTA sections were (A) immunolabelled with TH antibody to examine colocalization
with (B) Gi DREADD reporter mCherry. (C) Merged image showing that expression of
DREADD receptors was restricted within the VTA. Bottom figures (D-F) are 100x
images of a TH+ dopamine neuron in the VTA that expresses Gi DREADD mCherry
reporter. Scale bars are 100μm. (G) Quantified cell counts of mCherry positive cells that
are TH+ or TH- in the VTA and the IPN.

The effect of chemogenetic suppression of dopamine neuron activity on striatal dopamine release and the NAc encoding of reward

Voltammetric recordings were conducted in a separate group of anesthetized

animals to examine the impact of CNO administration on dopaminergic signaling in the

NAc. A carbon fiber electrode was lowered into the NAc shell to record changes in

dopamine concentration elicited by electrical stimulation of the VTA (60 Hz, 24 pulses,

4V). Following several periods of electrical stimulation to establish a stable baseline

response, CNO was administered and stimulation continued at regular intervals (1

stimulation/2 min). It was observed that CNO injection dramatically suppressed

electrically-evoked dopamine release in the NAc (Figure 4.2 Post-CNO). This proof of

concept experiment demonstrates that activation of the Gi-coupled DREADD receptor

effectively inhibits NAc dopamine signaling.
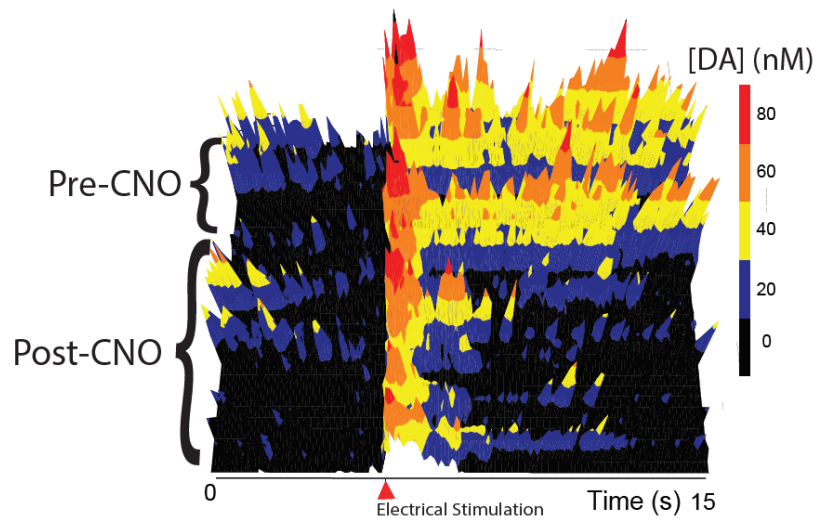
Figure 4.2. CNO administration suppressed stimulated dopamine release in the NAc.
Heat map showing that stimulated release was dampened after CNO injection, with warm
colors (red and orange) indicating higher levels of dopamine concentration. y-axis
represents trials and x-axis represents time during each trial. Electrical stimulation of the
VTA is indicated by the red triangle (x-axis).
Data collected by Mitchell Spring

To examine the effect of a selective reduction in dopamine release on the activity

of NAc neurons, electrode arrays were implanted into the NAc core and shell of the NAc

for single-unit recordings. The phasic activity of NAc neurons was measured in response

to sucrose infusions both before and after CNO administration to investigate the potential

effect on hedonic responses to sucrose. In each sucrose session, 45 passive infusions of

20% sucrose were delivered intraorally. 84 NAc units were recorded on the CNO test day

and 82 on the saline test day from all 7 subjects. Interestingly, repeated-measures

ANOVAs (Pre vs. Post injections of CNO and saline) revealed that CNO had no effect on

baseline activity of NAc neurons (Figure 4.3A: $F_{(1,155)} = 0.008$, $p = 0.928$), but

significantly altered the encoding pattern of sucrose infusions. The predominant response

to sucrose infusions under baseline conditions was a decrease in firing rate (Figure 4.3

B). However, following CNO-administration, this profile shifted as more cells exhibited

an excitatory response profile (Figure 4.3B: $F_{(1,165)} = 5.14$, $p = 0.025$). Contributing to this

effect was a population of neurons that exhibited non-phasic responses to sucrose

administration, but excitatory responses following CNO administration (Figure 4.3C).

These data demonstrate that selective reductions in dopamine signaling altered the

neuronal encoding of a sucrose reward by NAc neurons.
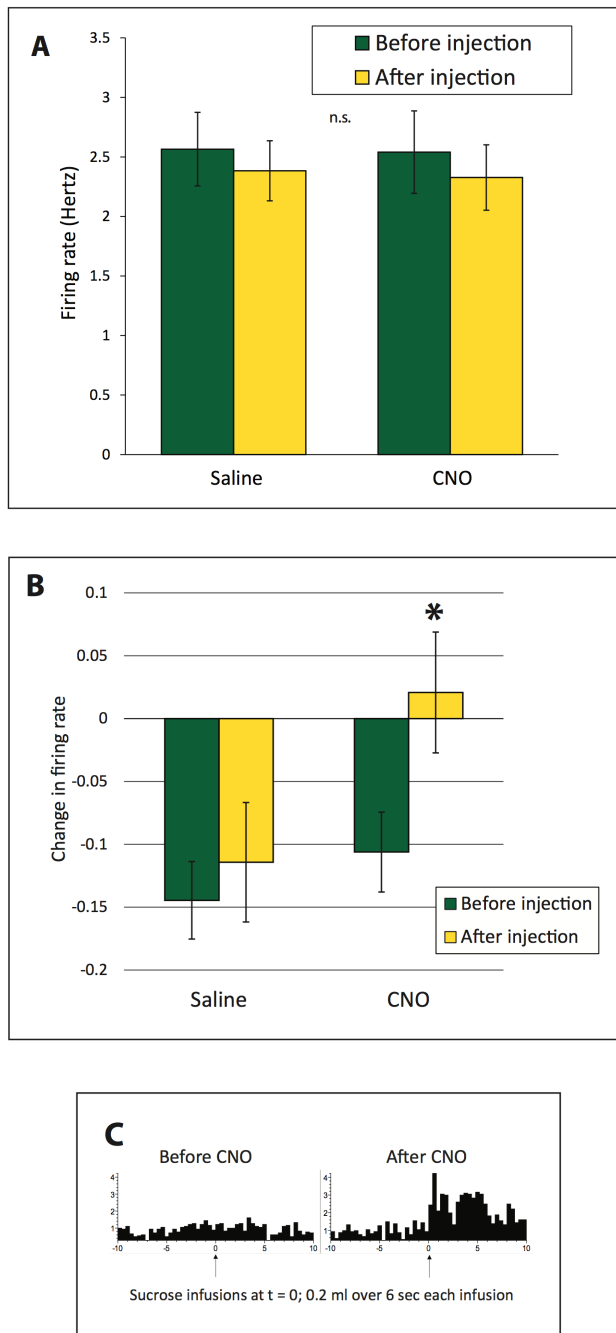
Figure 4.3. CNO injection altered reward encoding- neuronal responses in the NAc without affecting baseline activity.
(A) Electrophysiological recordings were conducted before and after injections of CNO and saline. Baseline firing rates (Hz) of NAc neurons recorded were not significantly different between conditions. (B) Sucrose responses observed in the NAc remained primarily inhibitory after saline injection. In contrast, CNO injection abolished the inhibitory response profile. (C) Representative NAc neuron that responded to sucrose with a phasic excitatory response after CNO injection.

Reduced dopamine signaling in the NAc did not affect taste reactivity

Suppression of NAc dopamine signaling did not affect taste reactivity. Subjects (n = 7) expressed no difference in the expression of appetitive taste responses following either saline or CNO administration (Figure 4.4: $F_{(1,6)} = 0.643, p = 0.808$). These data indicate that reduced dopamine signaling in the ventral striatum did not influence the taste reactivity response.
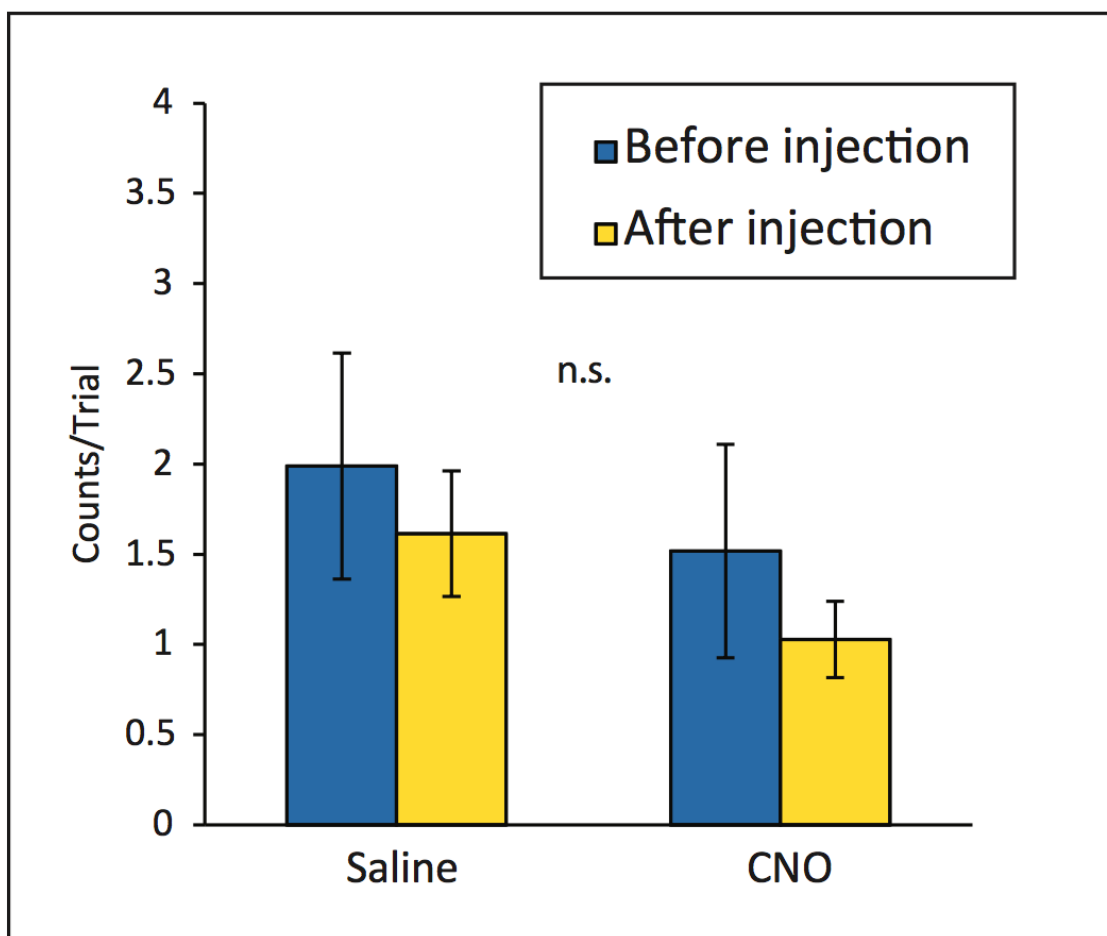
Figure 4.4. CNO administration did not affect the expression of taste reactivity. Appetitive taste reactivity was measured before and after CNO and saline injections. Neither treatment affected the expression of appetitive taste responses.
Reduced dopamine signaling did not affect locomotor activity

To examine whether dopamine suppression causes non-specific motoric effects, animals (n = 7) were tested in a locomotion test. In this test, both total movement and ambulatory locomotor activity were measured for 4 hours after injection of either CNO or saline. Results show that CNO did not have any effect on activity level or locomotion (Figure 4.5, $F_{(1, 6)} = 3.90$, $p = 0.096$).
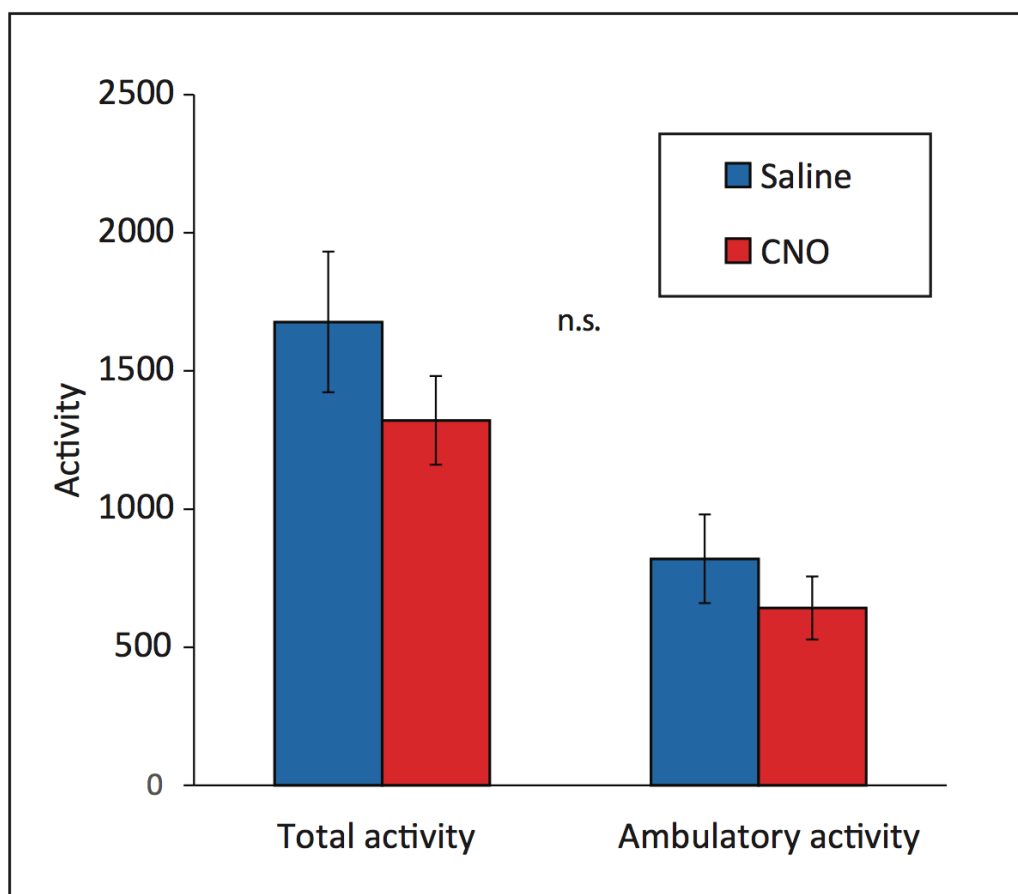
Figure 4.5. CNO administration did not result in non-specific motoric effects. The overall activity level (total activity) of animals did not differ between CNO and saline conditions. CNO also did not affect locomotor activity (ambulatory activity or distance walked) in any subjects.

Reduced dopamine signaling did not affect sucrose consumption

To evaluate consumption, subjects were allowed to drink 20% sucrose *ad libitum*

from a bottle in their home cage after CNO or saline administration. In this test, no

difference was observed (Figure 4.6, $F_{(1, 3)} = 0.038$, $p = 0.866$) between groups.
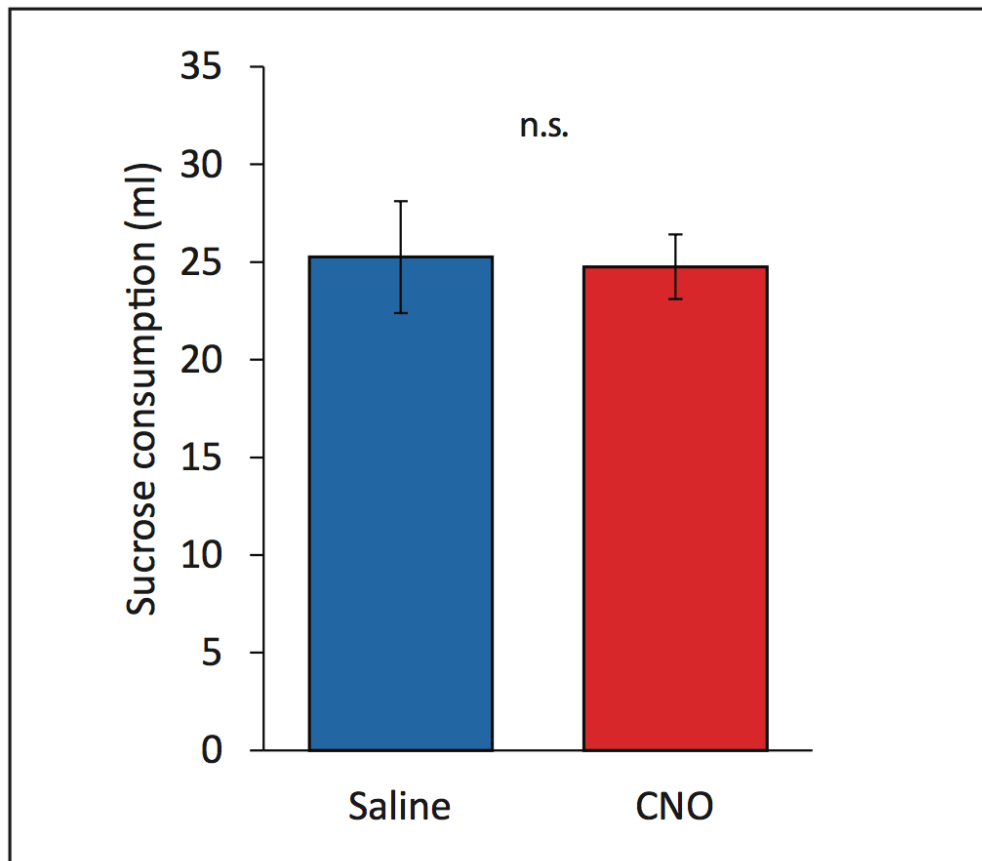
Figure 4.6. Chemogenetic suppression of dopamine signaling did not affect sucrose consumption.
The amounts of sucrose consumed 30 minutes after CNO or saline injection were measured and no differences were observed between conditions.

Reduced dopamine signaling reduces motivation to seek higher-effort sucrose rewards

To investigate the effect of suppressed dopamine on motivated behavior, subjects

were tested in a reward-directed instrumental task (FR1) and a progressive ratio (PR)

task. CNO did not affect lever press behavior for a sucrose reward at a low FR1 schedule (Figure 4.7A, $F_{(1, 6)} = 4.88$, $p = 0.069$). These data suggest that reduced dopamine signaling does not disrupt low-effort instrumental behavior.

To further examine the role of dopamine in more effortful responding, a progressive ratio (PR) test was employed. This test assesses the threshold at which the requirement for reinforcement reaches a level that disrupts reward-seeking behavior (breakpoint). The current results (n = 3) reveal a non-significant trend (Figure 4.7B, $F_{(1,2)}$ = 2.286, $p = 0.270$) toward a lowered the breakpoint in the CNO-treated animals (average = 8 ± 4) when compared with the saline condition (average = 13.33 ± 2.67). It should be noted that this analysis is severely underpowered due to the low n, and the trend is interesting as it highlights a potential role for dopamine in mediating effortful reward-seeking behavior (PR8+) but not low-cost reward-seeking behavior (FR1).
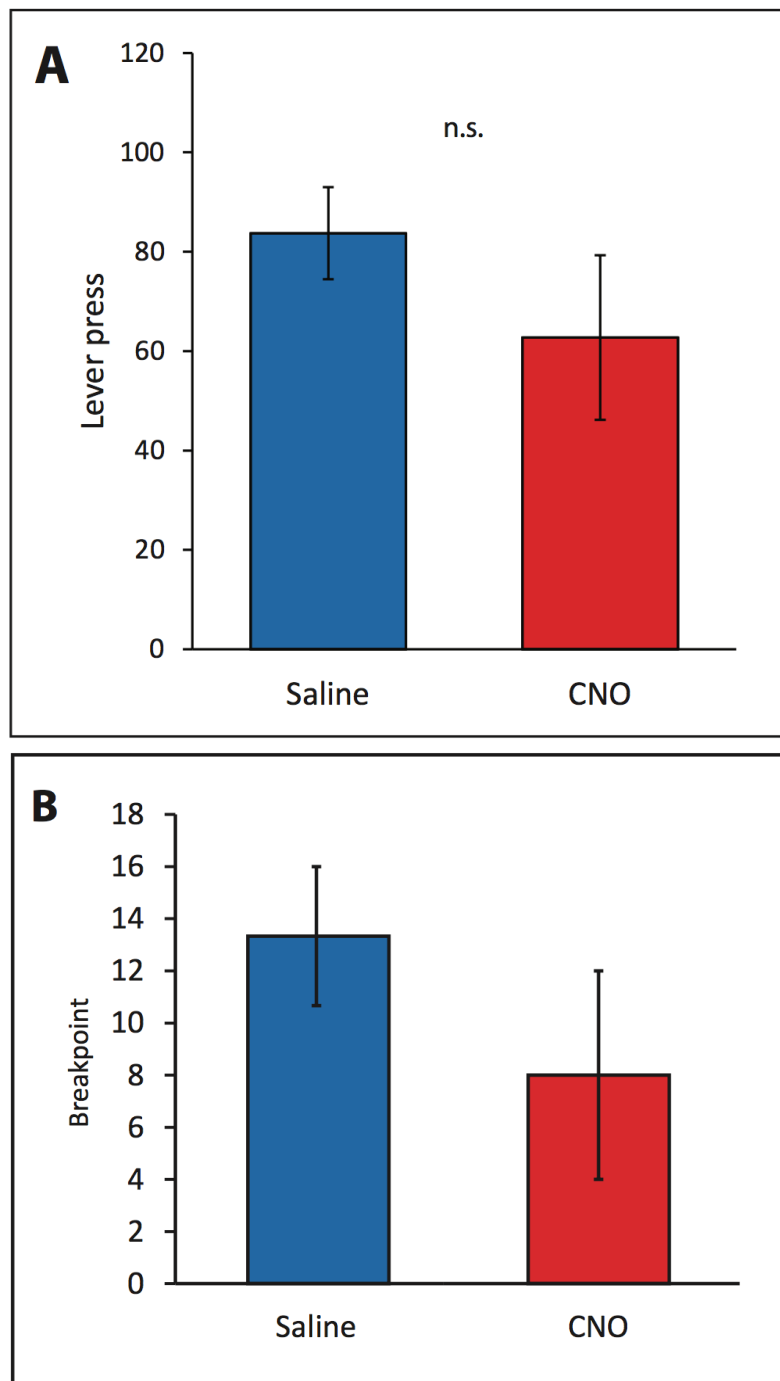
Figure 4.7. Reduced dopamine signaling may reduce the motivation to self-administer sucrose when effort is required.
Inhibition of dopamine signaling in the NAc did not affect (A) fixed ratio 1 (FR1) sucrose self-administration but (B) induced a trend toward lowered breakpoints in a progressive ratio (PR) test.

Discussion

Dopamine signaling has an essential role in the regulation of motivated behavior. This is especially true for the mesolimbic projection of VTA dopamine neurons terminating at the NAc. This study was designed to investigate the causal relationship between striatal dopamine signaling and the neural encoding of reward. The preliminary data presented here provide interesting insights into how NAc neuronal responses are modulated by dopamine as well as the role of dopamine in motivation. Chemogenetic inhibition of midbrain dopamine neuronal activity suppressed the dopamine signaling in the NAc, as demonstrated by fast-scan cyclic voltammetry data, providing the foundation of the experiments in this study. CNO-induced suppression of dopamine signaling also selectively affected phasic neuronal responses to sucrose without affecting the baseline activity of NAc neurons overall. Behavioral tests found that selective suppression of dopamine signaling did not alter taste reactivity or low cost reward-seeking behaviors, such as sucrose consumption or FR1 sucrose self-administration. However, data from the PR test reveal an interesting trend that suggest that dopamine signaling plays an important role in meeting the requirements needed to obtain reward when this requirement requires significant effort.

This study generated results consistent with current research describing a role for dopamine in regulating effort-based motivated behavior but not in hedonic processing (Berridge & Kringelbach, 2015; Salamone, Yohn, López-Cruz, San Miguel, & Correa, 2016; Wise, 2008). Pharmacology studies have found that dopamine concentration in the NAc measured with microdialysis is strongly correlated to the expression of reward-driven instrumental responses (Salamone, Cousins, McCullough, Carriero, & Berkowitz,

1994), and inhibition of dopamine signaling by lesion or antagonists significantly reduces lever pressing for food without affecting free consumption (Cousins, Wei, & Salamone, 1994; Salamone, 1994). In addition, lesioning the mesolimbic dopamine projection and pharmacologically inhibiting NAc dopamine signaling appear to have no effect on taste reactivity (Berridge et al., 1989; Peciña et al., 1997). Together with the data collected in this study, dopamine appears to play a critical role in making effortful responding. In other words, reduced dopamine signaling leads to a behavioral phenotype that reflects either hypersensitivity to effort requirement or reduced reward-driven motivation.

Several studies indicate that while rewarding stimuli are increasing dopamine signaling in the NAc, the predominant response of NAc neurons is a reduction in firing rate (Carelli, 2002; Carlezon & Wise, 1996; Day et al., 2006; Nicola et al., 2004b; Roitman et al., 2005; Wheeler et al., 2008). These findings have led to the hypothesis that rewarding stimuli are encoded by broadly reduced striatal activity (Carlezon & Thomas, 2009), while broadly excitatory activity reflects aversive stimuli (Roitman et al., 2005; Wheeler et al., 2008). These current studies are generally consistent with this interpretation. For example, here we observe that selective suppression of dopamine activity, as would be observed in response to an aversive stimulus (Roitman et al., 2008; Wheeler et al., 2011) promotes a shift in the NAc encoding of a palatable tastant to become more excitatory. This shift in encoding was not associated with a change in taste reactivity, but may be associated with a reduced motivation to obtain a sucrose reward, as demonstrated by a trend toward lowered motivation to work for sucrose reward when requirement required became higher. Of course, due to the few subjects tested in this design, additional studies will need to be conducted to determine if this trend is reliable

and significant. Perhaps most interestingly, is the discordance between the altered sucrose encoding by NAc neurons and the lack of effect on taste reactivity. This finding may indicate that hedonic information might not be encoded by phasic responses observed in the NAc at the time of tastant exposure. Intraoral tastant delivery has been widely used in studies to dissociate hedonics from motivation (Berridge et al., 1989; Peciña et al., 2000, 2003; Shimura et al., 2006; Wyvell & Berridge, 2000). Electrophysiological recordings of neuronal activity in the reward circuitry have characterized phasic neuronal responses time-locked to intraoral infusions and identified them as hedonic encoding responses (Chan et al., 2016; Roitman et al., 2005, 2010; Smith et al., 2011; Tindell, Berridge, Zhang, Peciña, & Aldridge, 2005; Wheeler et al., 2008). However, the results of this study and in the previous study (Chapter III) both revealed that phasic neuronal responses can be altered without changes in the expression of taste reactivity. The changes of neural encoding of taste stimulus appears to correlate better with behavioral measures in subsequent behavioral tests when animals are allowed to obtain reward through self-administration. GABAergic manipulation in the NAc significantly affected sucrose consumption when animals gained access to food receptacle (Chapter III), and inhibition of VTA dopamine neuronal activity had a tendency to dampen motivation (data presented in this chapter). Both treatments also altered the neural encoding to sucrose without affecting taste reactivity. This suggests the possibility that neural encoding of taste stimuli delivered intraorally could be encoding the motivational value carried by the stimulus despite the fact that goal-directed behavior is prevented due to experimental restrictions. Therefore, caution must be exercised when interpreting the neural responses to taste infusions as hedonic encoding at the current stage. Indeed, it is the broad goal of

this body of work to better discern the nature of the information that is carried through these reward circuits that modify behavior. It will be important for future studies to include a battery of behavioral measures that can effectively separate hedonic processing from motivation.

Overall, this study provides a promising starting point for future experiments to investigate how dopamine signaling and neuronal responses to appetitive and aversive stimuli interact in the NAc by identifying and characterizing the neurophysiological processes during various affective states. These studies suggest that dopamine is a potent regulator of the patterned encoding of rewarding stimuli by NAc neurons, and aversion-induced reductions in dopamine signaling change this encoding in a predictable manner that translates to reduced motivation for reward. Additional studies will better define the nature of this specific signal and the role it has in guiding behavior. Further studies of this sort can provide important insights into the causes of mood and motivational disorders. Additionally, such studies can tease apart the contributions of NAc output pathways to the progression of this signal to downstream structures, like the ventral pallidum.

Chapter V

DISCUSSION

Summary

The studies described in this dissertation present electrophysiological evidence that various aspects of reward processing are encoded by neuronal activity in the NAc and the VP. The appropriate expression of behaviors essential for survival is based on the ability to evaluate reward value. Therefore the neurobiology of reward processing in the brain is a critical piece to the puzzle of understanding fundamental brain processes. Equally important, our knowledge of these physiological processes directly contributes to our understanding of many motivational and affective disorders, which may facilitate the development of effective treatments.

Since the NAc is a neural structure that has been heavily implicated in reward processing, these studies investigated how reward is encoded by the activity in the NAc, and how the NAc relays reward information to the VP. The first two studies focus on characterizing the functional relationship between the NAc and VP in reward encoding. The first study revealed that the VP, similar to the NAc, encodes appetitive and aversive taste stimuli differentially. The observed neuronal responses are sensitive to changes in hedonic valence, supporting the conclusion that neuronal activity in the VP encodes reward information. The second study demonstrated that enhanced GABAergic signaling in the NAc modulates both reward encoding in the VP and motivated behavior for sucrose, revealing evidence of functional connectivity between the NAc and the VP. Finally, the last study was designed to examine the role of midbrain dopamine signaling

in NAc reward encoding using a chemogenetic approach. Preliminary data indicate that suppression of NAc dopamine signaling alters reward encoding and may reduce the motivation to seek reward when increased effort is required. The following sections discuss the findings from each study with the goal of generating a conceptual framework for understanding how reward and aversion signals are represented in the brain.

1.1 Reward Encoding by VP Neuronal Activity

The VP is a major output target of the NAc. Previous studies have shown that NAc neurons respond to associative reward cues that promote behavior and encode appetitive and aversive taste valences differentially (Ambroggi, Ghazizadeh, Nicola, & Fields, 2011; Carelli, 2002; Day et al., 2006; Loriaux et al., 2011; Nicola et al., 2004b; Roitman et al., 2005; Wheeler et al., 2008). The first study tested the hypothesis that the VP encodes reward-related information by characterizing neuronal responses in the VP following exposure to appetitive and aversive taste stimuli. Results of this experiment indicate that a sweet saccharin solution elicits primarily phasic inhibitory responses in the VP while a bitter taste causes predominantly excitatory responses (Chapter II) (Chan et al., 2016). Furthermore, this study provides evidence that the VP neuronal encoding of saccharin changes as the tastant becomes aversive. In brief, as the saccharin taste became predictive of cocaine administration, behavioral devaluation was observed and neural encoding in the VP also shifted drastically. The data also demonstrate that the phasic encoding pattern of gustatory stimuli VP resembles that observed in the NAc. The observation that neuronal responses to taste stimuli in the VP correspond and track changes in hedonic taste reactivity suggest that the VP encodes some aspect of reward

processing. This observed function of the VP is in agreement with the current literature (Berridge, 2009; Ho & Berridge, 2013; Ho & Berridge, 2014; Shimura et al., 2006; Simmons et al., 2014; Smith & Berridge, 2005; Tindell et al., 2006; Wheeler & Carelli, 2006). The taste reactivity test was employed in this study in an attempt to isolate hedonics from motivation since non-contingent taste stimuli were delivered to the subjects intraorally. The experimental separation of hedonic responses from motivated behavior is important, since the two have been suggested to be distinct components of reward (Berridge, 2009; Berridge & Robinson, 1998). However, while the neuronal encoding of taste infusion was found to track changes of taste reactivity in a cocaine-induced taste aversion paradigm, results from the following study (Chapter III) suggest that the nature of this neural encoding is more complicated. Specifically, these results indicate that the neuronal response to sucrose in the VP can be altered without altering the expression of taste reactivity. This disconnect suggests that the encoding of sucrose by VP neurons may better reflect a different process. In fact, data from the second (Chapter III) and third (Chapter IV) study suggest that the neural encoding observed during taste infusions serves as a better predictor of subsequent motivated behavior. This raises the possibility that NAc and VP neuronal responses observed during intraoral solution delivery might be encoding a reward-elicited motivational state that may only be expressed in a context that allows for goal-directed action. Further experimentation will be necessary to better understand this phenomenon.

While the data support the overarching hypothesis that the VP encodes aspects of reward and is functionally connected to the NAc, some observations also raised questions regarding the connectivity between the two structures. The neuronal response profile for

appetitive taste stimuli in the NAc is characterized by predominantly phasic inhibition (Nicola et al., 2004b; Roitman et al., 2005). Based on the GABAergic nature of the NAc-projecting MSNs to the VP, we hypothesized that the observed encoding profiles for appetitive and aversive taste stimuli would be the opposite in the VP. Contrary to our expectations, the neural encoding of appetitive tastants was also predominantly inhibitory. The same was observed for aversive tastant encoding, with quinine infusions evoking predominantly phasic increases of firing rates in both the VP (Chapter II) and the NAc (Roitman et al., 2005). This conundrum suggests either multi-synaptic connectivity between the NAc and the VP, or the overriding involvement of other sources mediating the nature of neuronal responses in both the NAc and the VP. Both explanations are plausible as indicated by findings from other research groups. The first possibility suggests a functional multi-synaptic connectivity between the NAc and the VP, which likely involves regulation by interneurons and/or local collaterals. In support of the hypothesis that reward processing involves direct communication between the two structures, disconnection studies demonstrate clear evidence that the NAc-VP circuitry is functional and needed for reward learning and cocaine-primed reinstatement (Leung & Balleine, 2013; McFarland & Kalivas, 2001). In addition, opioid stimulation in either the hedonic hotspot in the NAc or the VP can augment Fos expression in the other site (Smith & Berridge, 2007).

On the other hand, recent electrophysiological data collected from behaving animals have demonstrated that the overall onset of VP cue-elicited responses has a shorter latency than those in the NAc, suggesting VP encoding of reward might not be entirely generated from activity of NAc inputs (Richard et al., 2016). Together, these data

indicate that while NAc-VP connectivity is critical for certain types of reward-related

learning, there is a complexity to the connectivity between the structures.

In order to further examine reward encoding in the VP, the first study (Chapter II)

featured a cocaine-induced CTA paradigm and tested whether the neuronal responses in

the VP follow changes in hedonic perception. Davis and Riley (2010) proposed that this

procedure is capable of capturing the aversive effects caused by rewarding drugs such as

cocaine (also alcohol, morphine, amphetamine, and Δ9-tetrahydrocannabinol (THC) and

can therefore be used to examine the paradoxical affective effects of addiction. However,

the interpretation of drug-induced CTA has been a subject of debate for multiple decades.

Drug-induced CTA does not usually evoke the same disgust reaction observed in CTA

induced by sickness (e.g. LiCl, radiation), therefore it is unlikely that the drug-induced

aversion is a consequence of visceral sickness (Davis & Riley, 2010). While the actual

neural substrate of CTA is yet to be determined, evidence strongly indicates that drugs of

abuse have both rewarding and aversive properties (Cappell, LeBlanc, & Endrenyi, 1973;

Coussens, Crowder, & Davis, 1973; Elsmore & Fletcher, 1972; Goudie, Dickins, &

Thornton, 1978; Nachman, Lester, & Le Magnen, 1970). In many of these studies, the

aversive effect of drugs of abuse was tested by presenting the associated cues alone while

subjects had no access to the drugs. This suggests the possibility that the animals learned

that drug-associated cues predict drug availability, as well as the delayed access to drug

which can be aversive (Wheeler et al., 2011).

This also implies that drug-induced CTA could be modeling the negative affective

state that accompanies drug-craving. This idea is supported by the observation that drug

associated cues elicit self-reports of craving and a negative affective state in human drug

addicts, a factor that is closely associated with relapse (Paliwal et al., 2008; Robbins, Ehrman, Childress, Cornish, & O'Brien, 2000; Rajita Sinha et al., 1999). If this phenomenon is involved in the development of a cocaine CTA, the likely physiological mechanisms that mediate this process is a conditioned compensatory response (CCR). This process describes a conditioned physiological shift that occurs in opposition to the acute effects of the drug of abuse and offsets the acute effects of the drug. For example, if opiate administration causes breathing depression and a reduction in gastrointestinal motility, a context that predicts opiate administration can elicit hyperventilation and gastrointestinal motility. This process has been shown to play an important role in conditioned drug tolerance (Siegel & Ramos, 2002) and has been observed in response to a variety of drugs of abuse (Eckardt, 1976; McDonald et al., 1997; Nyland & Grigson, 2013; Siegel, 2005; Tandon et al., 2016; Turenne et al., 1996; Wheeler et al., 2008). This type of Pavlovian conditioning entails an unusual association formed between a neutral conditional stimulus (CS) and a biologically significant unconditional stimulus (UCS). In this case, the CS comes to elicit a preparatory state in anticipation of the US, instead of directly substituting for the UCS. Rodent studies have provided data supporting the hypothesis that CCR mediates tolerance by demonstrating the ability of drug-associated cues in reducing mortality from ethanol (Melchior, 1990), pentobarbital (Vila, 1989) and heroin overdosing (Siegel, Hinson, Krank, & McCully, 1982). These findings suggest that the observed taste devaluation is a result of a CCR from saccharin-cocaine pairing (Nyland & Grigson, 2013; Wheeler et al., 2008). The data presented in Chapter II therefore also provide insights into the neurophysiological events that mediate conditioned drug tolerance.

Another interesting observation from this study is that while the VP strongly encodes the devalued saccharin taste (n = 23, paired group), fewer VP units responded to the innately aversive quinine taste stimulus (n = 5, paired group). This suggests that while qualities of rewarding stimuli are being encoded in the VP, naturally aversive stimuli are likely being preferentially encoded in other structures or pathways. One possible candidate for aversion encoding is the LH, which receives NAc projections and also appears to encode aversive taste stimuli (Li, Yoshida, Monk, & Katz, 2013). It is also possible that the encoding of an innately aversive gustatory stimulus involves projections to a wider network of neural structures, engaging both the VP and the LH.

2.1. Neuronal Activity in the NAc Modulates Reward Encoding in the VP

The results of the first study indicated that VP neuronal activity encodes aspects of gustatory reward. However, it is unclear what inputs into the VP are mediating these responses. Likely candidates include structures in the reward circuitry such as the NAc, prefrontal cortex, amygdala and VTA. Anatomical, electrophysiological and pharmacological evidence all suggest that the NAc is a prime candidate for modulating reward encoding in the VP (Chapter I). However, the results described in Chapter II suggested that if the NAc is the principle driver of reward-related VP activity, the influence is complex. The experiments described in Chapter III therefore tested NAc-VP functional connectivity by pharmacologically manipulating activity in the NAc and recording the activity of VP neurons.

To examine the behavioral relevance of VP activity, pharmacological stimulation of GABA activity in the anterior NAc shell was chosen based on the role of this area in

regulating feeding behavior (Basso & Kelley, 1999; Reynolds & Berridge, 2002; Stratford & Kelley, 1997, 1999). The observation that administration of a GABA agonist in the VP increases feeding behavior suggests that reduction of activity in the NAc promotes reward-driven behavior by disinhibiting the NAc projection to the VP (Stratford, Kelley, & Simansky, 1999). This is further corroborated by findings that the predominant response of NAc neurons during consumption of a palatable solution is a reduction in firing rate (Nicola et al., 2004b; Roitman et al., 2005).

2.2. Evidence of NAc-VP functional connectivity

The projections from the NAc to the VP are GABAergic (H J Groenewegen & Russchen, 1984) and the NAc is therefore believed to exert an inhibitory influence on VP neuronal activity. The NAc shell subregion projects to the vmVP subregion while the NAc core projects to the dlVP (Groenewegen & Russchen, 1984; Zahm et al., 1996). It has been hypothesized that projections from the VP to the interpeduncular nucleus (IPN) and brainstem may serve as an output pathway that translates reward information to taste-guided and other motivated behaviors (Groenewegen et al., 1999; Mogenson et al., 1980). This proposed pathway is supported by the observation that VP neuronal activity corresponds to locomotor events during goal-directed tasks (Root et al., 2013). The VP has also been implicated in other behaviors such as guiding saccadic eye movements that track reward value (Tachibana & Hikosaka, 2012) and amygdala-evoked prepulse inhibition (Forcelli, West, Murnen, & Malkova, 2012). Thus, the VP appears to have a complex function, translating sensory- and reward-related information to motivated behavioral output.

A disconnection study is a classic experimental approach used to determine the role of a given projection between two structures. In cases in which projections are mostly ipsilateral, inhibition or lesion of the two structures of interest on contralateral sides should disrupt the projections bilaterally, thus hampering any behavior regulated by the pathway. With this approach, communication between the NAc and VP has been shown to be necessary for some forms of reward learning. In one such study, a retrograde tracer was first injected into the VP demonstrating that strong imnmunoreactivity was present in the ipsilateral NAc but not on the contralateral side (Leung & Balleine, 2013). After establishing that the NAc-VP projection is ipsilateral, the authors showed that appetitive Pavlovian to Instrumental Transfer (PIT) learning involving a palatable sucrose reward was disrupted when the NAc-VP pathway was pharmacologically disconnected. Specifically, the ability of a Pavlovian cue associated with sucrose reward to increase instrumental responding (lever press) for sucrose was abolished. In addition, stimulation with the mu-receptor agonist (DAMGO) in the NAc shell hedonic hotspot can enhance appetitive taste reactivity, increase food consumption, and augment the neuronal firing to sucrose in the VP hedonic hotspot (Smith & Berridge, 2005; Smith et al., 2011). In another disconnection study, the information flow necessary for cocaine-induced reinstatement was investigated from the VTA to the dPFC, NAc core, and then to the VP (McFarland & Kalivas, 2001). Pharmacological blockade of either the dPFC, NAc core or the VP alone all resulted in lowered cocaine-primed reinstatement. Contralateral inactivation of the dPFC and VP suppressed the ability of cocaine to induce reinstatement after extinction of cocaine self-administration, whereas ipsilateral inactivation had no effect. The authors of this study concluded that the dPFC-NAcCore-VP circuitry is

engaged and is required during cocaine-induced reinstatement. More recently, an optogenetic manipulation of NAc-VP projections determined that the NAcCore-VP projection plays an important role for both cocaine- and drug cue- induced reinstatement of cocaine seeking (Stefanik et al., 2013). Taken together, evidence is overwhelming that there are projections from the NAc to the VP, and they function to modulate various aspects of reward processing and motivated behaviors. Recordings conducted in the current studies targeted the vmVP and investigated its role in reward processing. Future studies can explore whether and how aspects of motivated behaviors are neuronally represented in the NAcCore-dlVP projection.

2.3. Dissociating the Neural Encoding of Hedonics and Motivation

In addition to investigating the roles of NAc activity in VP reward encoding, the study described in Chapter III was also designed to better discern the encoding of hedonic and motivational information in the VP. Consequently, subjects were tested in a hedonic and a motivation task while neuronal activity was recorded in the VP. Microinfusion of muscimol into the NAc shell did not alter the expression of taste reactivity, but did increase the inhibitory encoding of sucrose. Similar to results obtained in the first study (using saccharin), the palatable sucrose solution evoked predominantly inhibitory activity, suggesting a common neural response in the VP that encodes reward value. In contrast to findings from the first study, the disconnection between the behavioral (no effect on taste reactivity) and neuronal responses (altered sucrose encoding) observed here suggest that the VP encodes more than simply hedonic responses. The next test in this study examined sucrose consumption and found supporting evidence that VP

neuronal responses correlate better with motivated behavior than taste reactivity responses.

Immediately after the hedonic test, animals were given 90 minutes to self-administer a sucrose solution as a test of motivation. NAc muscimol administration significantly increased voluntary sucrose consumption by over 70% in this test, despite not affecting hedonic taste reactivity. More interestingly, the number of VP neurons encoding sucrose-seeking behavior significantly increased (43 to 76 in the same VP neuronal population). These findings demonstrate that inhibiting NAc activity by enhancing GABAergic signaling results in increased motivation for sucrose and augmented neural responsiveness in the VP both before and during the sucrose self-administration task. Since the enhanced encoding of sucrose is not associated with increased hedonic perception, but is associated with enhanced motivation, the parsimonious explanation is that VP activity is reflecting, at least in part, reward-related motivation. The increased observations of inhibitory responses during the hedonic task may encode an increased incentive for sucrose that becomes expressed when the animal has the opportunity to respond for sucrose in the second part of the experiment.

Evidently, the NAc-VP connectivity is anatomically and functionally complex as demonstrated by the current studies and prior pharmacological studies showing loci-specific effects in reward and aversion (Castro & Berridge, 2014; Reynolds & Berridge, 2008; Richard & Berridge, 2011). In these studies, administration of opioid agonists created opposite effects in the NAc depending on the location of microinfusion. The NAc hedonic hotspot appears to be located in the anterior portion of the medial NAc shell, and stimulation with opioid agonists can increase both consumption and the expression of

appetitive taste reactivity. On the other hand, the posterior portion of the medial NAc

shell generated fearful and defensive behaviors when microinfused with GABAergic

agonists or glutamatergic antagonists (Faure et al., 2010; Reynolds & Berridge, 2001).

These observations strongly suggest that the NAc contains functionally diverse loci

within the shell subregion. Further experimentation is necessary to better characterize

how reward information is encoded in the NAc-VP circuitry in a region-specific fashion.

The results of the previous studies indicate that while the NAc and VP are

functionally connected to regulate reward processes and motivated behavior, this

relationship is complex. The complex pattern of results could reflect several factors. First,

while pharmacological manipulation such as the one used in this study have been widely

used to produce robust behavioral effects, this approach does not excel in circuit-specific

dissection, or replicating temporally-precise physiological processes. Also, the

GABAergic manipulations employed in this study do not differentiate the potential

sources of an inhibitory effect, which could originate from either or both local

GABAergic interneurons and D2 MSN axonal collaterals (Dobbs et al., 2016).

Nevertheless, perhaps the most interesting finding in this study is that inhibition of NAc

activity does not result in simply phasic excitation or increased response magnitude in the

VP, but rather an augmentation of the overall responsiveness of neurons in the VP and an

increased ability of these neurons to generate phasic responses to taste stimuli. The drive

on these neurons likely is not limited to input from the NAc. While these data suggest

that NAc activity modulates VP encoding of reward information, the influence from the

NAc is not always the critical determinant of behavior, and the influence from other

structures is certainly involved. For example, augmentation of feeding behavior by

pharmacologically stimulating mu-opioid signaling in the NAc is not impaired by lesion or opioid antagonists administered into the VP (Smith & Berridge, 2007; Taha, Katsuura, Noorvash, Seroussi, & Fields, 2009). Also, stimulation of glutamatergic inputs from the amygdala can modulate the activity of VP neurons (Mitrovic & Napier, 1998). Hence, the VP should be viewed as an integration center where reward signals converge to mediate phasic changes of neuronal activity observed during affective experiences and motivated behaviors. The nature of specific information conveyed by these inputs is of great interest if the scientific community is to continue dissecting the specific processes that are integrated in the VP to produce sophisticated reward-related behavior. These findings contribute to our understanding of affective neuroscience by providing a better characterization of the type of reward information conveyed from the NAc to the VP.

3.1. Midbrain Dopaminergic Signaling Regulates Reward Encoding in the NAc

In order to better discern the specific influence of one neurotransmitter system on reward and motivational processing, the relationship between dopamine signaling and neural encoding of reward in the NAc was examined by combining chemogenetic and electrophysiological techniques. Inhibitory designer receptors (Gi DREADD) that are selectively activated by CNO were virally expressed in midbrain dopaminergic neurons. The NAc neuronal activity in response to a rewarding stimulus was then recorded when the activity of dopamine neurons was suppressed by activating the inhibitory DREADD. To verify that this preparation effectively modulated dopamine signaling in the NAc, the effect of CNO on stimulated release of dopamine was measured in the NAc using voltammetry. As expected, systemic injection of CNO reduced the stimulated release of

dopamine in the NAc, validating the chemogenetic approach to generate a striatal environment in which dopaminergic signaling is suppressed.

This approach yielded several interesting preliminary results. First, there was no effect of suppressed dopamine signaling on taste reactivity. This is consistent with existing literature, as pharmacological manipulations of dopamine signaling have been shown to not alter hedonic processing (Berridge et al., 1989; Berridge & Robinson, 1998; Liggins, Pihl, Benkelfat, & Leyton, 2012; S Peciña et al., 1997; Peciña & Berridge, 2013; Peciña et al., 2003; Treit & Berridge, 1990; Wyvell & Berridge, 2000). In addition, reducing dopamine signaling had no effects on voluntary sucrose consumption, or locomotor activity. These observations demonstrate that this manipulation of dopamine does not produce non-specific motoric effects that could confound the interpretation of results from other behavioral tasks.

To investigate the role of dopamine in reward encoding in the NAc, neuronal responses to sucrose infusions were recorded before and after CNO administration. Prior to CNO injection, a net inhibitory response to sucrose was observed. This finding is consistent with the observed phasic encoding profile for appetitive taste stimuli reported in other studies (Roitman et al., 2010; Wheeler et al., 2008), again supporting the hypothesis that reward processing is associated with inhibited activity in the NAc (Carlezon & Thomas, 2009). Interestingly, suppression of dopamine signaling significantly changed the net response profile from inhibitory to excitatory, the response profile observed for aversive stimuli (Roitman et al., 2010; Wheeler et al., 2008). A progressive ratio test was used to evaluate the effects on motivation. Although still preliminary, current data show a trend toward reduced motivation after CNO injection

specifically when the effort requirement for reward is high. CNO administration appears to lower breakpoint during the PR test, while FR1 sucrose-seeking behavior was not affected. Future studies will more thoroughly characterize the effects of dopamine suppression on effort-related behavior and NAc neuronal activity.

Dopamine modulates the activity of many structures of the reward circuitry and can exert different effects depending on where and what type of dopamine receptors are activated. MSNs in the NAc generally express either D1Rs or D2Rs, with a small population expressing both types of dopamine receptors (Bertran-Gonzalez et al., 2008). Both D1 and D2 receptors in the NAc have been implicated in modulating activity in the VP, as indicated by Fos-immunoreactivity (Robertson & Jian, 1995). Even within the NAc, the effect of dopamine is complicated because D2R-expressing MSN collaterals exert a potent inhibitory effects on D1R expressing MSNs (Dobbs et al., 2016). In addition, dopamine receptors are found on both MSNs and interneurons within the NAc (Clarke & Adermark, 2015; Soares-Cunha et al., 2016). While cholinergic and GABAergic interneurons only comprise of a small portion of the NAc neuronal population, they exhibit vast arborization, synapsing on many NAc neurons and can modulate reward behavior (Brown et al., 2012; Mark, Shabani, Dobbs, & Hansen, 2011; Warner-Schmidt et al., 2012; Witten et al., 2010). Furthermore, discoveries of the hedonic hotspots in the NAc and the VP clearly indicate that these structures are anatomically and functionally heterogeneous. This observation of topographical differences was observed for both hedonic processing and also consummatory behavior (Kelley & Swanson, 1997; Peciña, 2008).

3.2. The Complex Nature of Mesolimbic Dopamine Signaling

The influence of dopamine on NAc neurons depends on the type of dopamine receptor they express, which is an important point to consider when interpreting the effects of suppressed dopamine signaling on NAc neuronal activity. D1 and D2 MSNs both contain multiple members of receptors that belong in the same subfamily. D1 and D5 receptors belong to D1-like receptors (D1Rs) whereas D2, D3, and D4 belong to the D2-like receptor family (D2Rs). D2Rs have a much higher affinity for dopamine compared to D1Rs (Richfield et al., 1989). It has been estimated that D2Rs exhibit affinity 10 to 100 times higher than that of D1Rs (Beaulieu & Gainetdinov, 2011), consistent with the interpretation that most D2Rs are bound by dopamine under basal signaling conditions (Dreyer, Herrik, Berg, & Hounsgaard, 2010). Neurons projecting to the VP express both D1 and D2Rs (Kupchik et al., 2015; Lu, Ghasemzadeh, & Kalivas, 1998). D1 neurons in the NAc are evenly distributed throughout the structure. D2Rs are evenly distributed in the NAc core but appear to be more densely located in the ventral and medial part of the NAc shell (Gangarossa et al., 2013). In general, the activity of D1 neurons involves Gs/olf and Gq, and is stimulatory in nature. D1R activity can increase the surface expression of ionotropic glutamate receptors and modulate ion channel functions to maintain the striatal neurons at an "up state" that is more responsive to glutamate. On the other hand, D2 neurons couple to Gi/o and therefore exert an inhibitory effect by suppressing PKA activity, reducing surface expression of AMPA receptors, modulating the function of ion channels (Neve, Seamans, & Trantham-Davidson, 2004). D2Rs can also be found presynaptically in the NAc, providing negative feedback on dopamine release by VTA neurons (Maura, Giardi, & Raiteri, 1988; Yamamoto & Davy,

1992). Approximately 5-15% of striatal neurons in the NAc express both D1Rs and D2Rs (Gagnon et al., 2017; Hasbi, O'Dowd, & George, 2011; Lee et al., 2004; Perreault, Hasbi, O'Dowd, & George, 2011).

The D1 signaling pathway has been suggested to be particularly important for reward and reward-associated learning (Richard & Berridge, 2011; Touzani, Bodnar, & Sclafani, 2008; Xiu et al., 2014). Transgenic D1R knockout mice show lower motivation to work for sucrose (El-Ghundi et al., 2003; Gallardo et al., 2014). The lack of D1R in animals also impairs Pavlovian conditioning (Parker et al., 2010) and prevented the learning of taste aversion (Cannon, Scannell, & Palmiter, 2005). A similar lack of motivation is observed for cocaine (Caine et al., 2007) and ethanol in self-administration studies (El-Ghundi et al., 1998). Optogenetic stimulation of the D1R signaling pathway increases the motivation to work for food reward (Soares-Cunha et al., 2016). Since the D1 projection is known to be present in both the direct and indirect pathways, reward and motivated behavior is likely regulated through sophisticated interaction of both signaling pathways.

While D2R-expressing MSNs are selective in their projections, the role of NAc D2R activity in the regulation of behavior is less clear. D2Rs in the indirect pathway have been implicated in both reward and aversion-related behaviors (Al-Hasani et al., 2015; Soares-Cunha et al., 2016). Blockade of D2 receptor with raclopride reduces sucrose motivation and shorter lick bursts as measured by automated lickometer (Higgs & Cooper, 2000). Knockout of D2R revealed a similar effect, reducing motivation for ethanol (Cunningham et al., 2000), cocaine (Welter et al., 2007), and morphine (Elmer et al., 2002; Maldonado et al., 1997). Such findings are difficult to interpret, as dopamine

would affect both presynaptic and postsynaptic D2R. Genetic knockout of postsynaptic D2Rs (D2L isoform) demonstrated that activity of these receptors is required for avoidance learning for electric footshock (J. W. Smith, Fetsko, Xu, & Wang, 2002). Optogenetic stimulation of D2 MSNs attenuated cocaine-induced conditioned place preference (Lobo et al., 2010) and suppressed self-administration of cocaine (Bock et al., 2013), supporting an anti-reward role for the activation of this pathway. In sum, while it is clear that while D2 signaling modulates reward-related behaviors, understanding its specific roles in motivated behavior require further examination.

D1 and D2 signaling pathways in the reward circuit are anatomically and functionally complex. Recent data suggest that the D1 and D2 systems are more likely to be two interacting systems, especially in the indirect pathway (Calabresi, Picconi, Tozzi, Ghiglieri, & Di Filippo, 2014; Dobbs et al., 2016). Similar complexity arises when looking at the functions of D1Rs and D2Rs in regulating behavior. The reward-augmenting effect of disrupting glutamatergic signaling through administration of the glutamate receptor antagonist (DNQX) in the NAc hedonic hotspot requires D1R activity, whereas the fear behavior generated from infusing DNQX in NAc area outside the hedonic hotspot requires both D1R and D2R activity (Richard & Berridge, 2011). In addition, recent work indicates that dopamine receptors are also present on interneurons in the NAc, and are likely to also modulate the activity of D1R and D2R MSNS (Tritsch et al., 2012). In other words, it is important to note that, while it is tempting to attribute selective D1 and D2 signaling to specific brain processes, further experimentation is required to better understand how a single neurotransmitter can be modulating multiple, and sometimes opposite functions in the NAc. Possible explanations for the

multifunctional role of dopamine include temporally different responses (differential activation of D1Rs and D2Rs by tonic and phasic dopamine responses) in the NAc, and region-specific dopamine signaling that is engaged by specific sensory stimuli. It is also possible that a phasic increase of dopamine preferentially activates D1Rs, while D2Rs are preferentially disinhibited by reduced dopamine, allowing different physiological responses to be generated. The cellular and circuit heterogeneity in the NAc generates a complex network that offers high flexibility and capacity in information encoding. The observations of diverse neuronal responses to stimuli (phasic excitation and inhibition) could also be attributed to this complex nature of the NAc network (Roitman et al., 2005; Wheeler et al., 2008). Taken together, this study provides preliminary data suggesting that dopamine signaling in the NAc modulates neuronal responses to a stimulus that encode motivational processes.

4. Neuronal Encoding of Reward and Aversion

Putting all of the accumulated data together, it is clear that the neurophysiological events encoding an affective experience can be characterized, from the VTA dopamine projection to the NAc mediating motivated behavior (Chapter IV), to the NAc response to rewarding and aversive stimuli and the neuronal responses in the VP (Chapter II and III). In this model, rewarding stimuli evoke dopamine release and phasic inhibitory responses in the NAc (Figure 5.1). The increase in dopamine may reflect increased dopamine binding to D2Rs, creating an inhibitory effect on neuronal activity. On the other hand, aversive or devalued taste stimuli generate physiological effects in the opposite direction, eliciting phasic decreases of dopamine and excitatory neuronal responses (Figure 5.2).

This phasic drop of dopamine in the NAc may result in the release of the tonic inhibitory D2R activity that, in turn, increases MSN excitability.

The electrophysiological results from recordings in the NAc and the VP clearly demonstrate the encoding of aspects of reward information. Both excitatory and inhibitory phasic responses were observed in these two structures. These neuronal responses also appear to directly mediate reward-driven behavior. In fact, not only has reduced NAc activity been associated with feeding behavior, it was also shown that local electrical stimulation preventing inhibitory responses but local electrical stimulation prevents inhibitory responses and disrupts sucrose consummatory behavior (Krause et al., 2010). Unfortunately, while *in vivo* electrophysiological recording techniques can capture behaviorally-relevant neuronal responses, the technique does not provide insights into the identity of neurons being recorded. In the NAc, MSNs can be generally identified as they exhibit much slower firing rates compared to fast spiking interneurons. Nonetheless, it is not possible to discriminate D1R- from D2R- expressing MSNs. In structures that are morphologically diverse with complicated connectivity patterns such as the VP, firing rate is not a reliable factor for characterizing cell type. Future studies in which transgenic rats expressing D1R-Cre and D2R-Cre neurons will be needed to test some of the predictions of this model; for example if aversion-induced reductions in dopamine signaling selectively increase the activity of D2R MSNs.

Neuronal encoding for natural rewards such as sucrose and water recruit significantly overlapping neuronal populations in the NAc (Carelli, 2002). However, it has been observed that drugs of abuse engage different populations of NAc neurons during conditioned behavioral response from natural rewards (Carelli, 2002; Chang et al.,

1998). This suggests that NAc contains separate functional circuits for each drug of abuse and a general neuronal ensemble that encode naturally appetitive stimuli. Further, this may indicate that additional top-down glutamatergic control plays a large role in determining the populations of neurons that are activated during the experience of a specific reward.
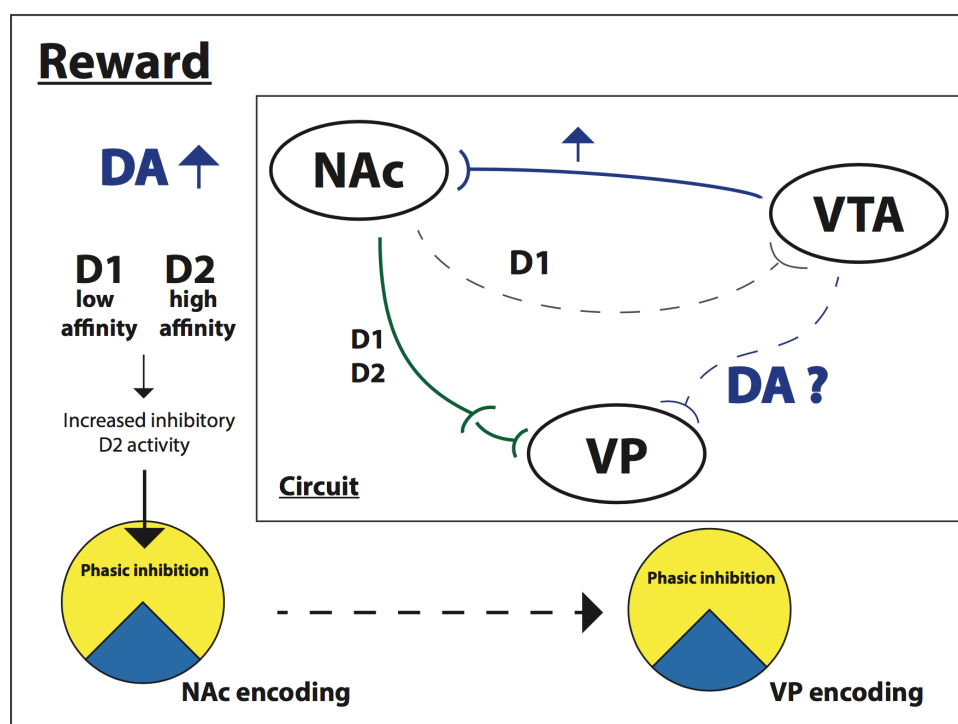
Figure 5.1. Model of neuronal responses to reward in the VTA-NAc-VP circuit. The experience of a rewarding stimulus causes the VTA projection that terminates in the NAc to release dopamine. Increased dopamine tone in the NAc may result in increased D2R activity and a stronger inhibitory tone. This could promote phasic inhibitory responses encoding appetitive gustatory stimuli. Multi-synaptic projections from the NAc to the VP modulate VP neuronal activity. VP neurons also respond to the experience of an appetitive stimulus with predominantly phasic inhibitory activity.
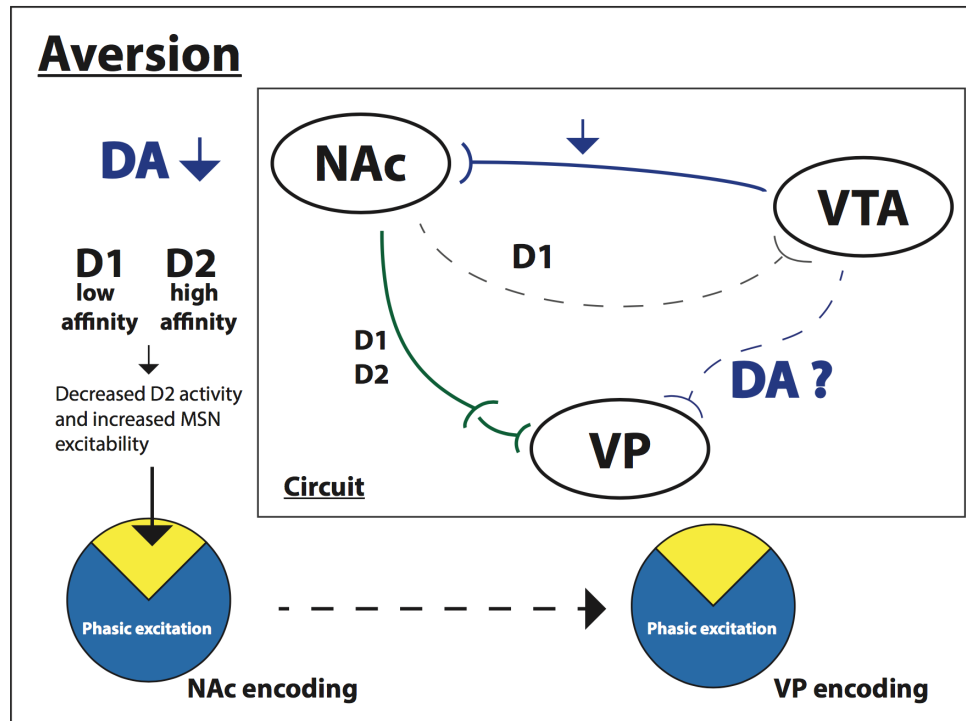
Figure 5.2. Model of neuronal responses to aversion in the VTA-NAc-VP circuit. The experience of an aversive stimulus reduces dopamine signaling in the NAc. Reduced dopamine signaling in the NAc preferentially affects the high affinity D2Rs in the NAc, resulting in disinhibition and increased excitability of D2R MSNs. This is reflected in the pattern of phasic excitatory responses in the NAc. Multi-synaptic NAc-VP projections modulate VP neuron excitability. VP neurons encode the experience of an aversive stimulus with predominantly phasic excitatory activity.

Overall, this dissertation provides insights into how the striatal-pallidal circuitry functions to process and relay reward information. Some data suggest a direct, predictable regulation of VP activity based on strong GABAergic input from the NAc (Tindell et al., 2006). Results from the first study demonstrated that neuronal activity in the VP encodes reward information carried by taste stimuli. However, the patterns of responses do not support the hypothesis that phasic responses in the VP are directly and monosynaptically mediated by changes of neuronal activity in the NAc. An alternative explanation is that the NAc regulates the activity of the VP in a multisynaptic manner

within the VP, and this influence may also include regulation through other structures. The second study further characterized the complexity of the NAc-regulation of the VP by directly examining the effects of manipulating activity in the NAc while simultaneously monitoring neuronal responses in the VP. Following pharmacologically increased GABAergic signaling in the NAc, the VP exhibited more phasic responses (both excitatory and inhibitory) encoding sucrose infusions as well as reward-seeking behavior. These electrophysiological data confirm that NAc activity modulates VP responses that encode reward information by increasing responsiveness in the VP. However, these data further demonstrate that NAc activity is not simply broadly disinhibiting the VP. In addition, the possibility of a third structure, such as the VTA or the LH, contributing to the neural encoding of reward in the VP cannot be excluded. In fact, since manipulating activity of the NAc did not alter baseline firing rates, and instead preferentially affected the responsiveness in the VP during a reward experience, VP activity is likely heavily regulated by other inputs (e.g. PFC, amygdala, hippocampus, LH). Finally, the last study tested the hypothesis that midbrain dopaminergic signaling affects neuronal responses to reward in the NAc. By chemogenetically suppressing the activity of VTA dopamine neurons, the neuronal response to sucrose in the NAc was significantly disrupted, and animals experienced a lowered motivational state in a PR test. These data support our hypothesis that striatal dopaminergic signaling plays an important role in modulating the patterned neuronal responses that encode reward in the NAc. Taken together, these data indicate that exposure to an appetitive or aversive taste stimulus evokes dopaminergic responses that modulate the phasic neuronal responses in the NAc. Changes in the neuronal activity in the NAc then exert a modulatory influence

on neuronal activity in the VP. This provides a more complete picture of a circuitry that propagates reward information carried by a stimulus from the midbrain to the striatum, then to the pallidum, and together these structures regulate physiological reward responses (i.e. appropriate "liking" and "wanting" behaviors).

Conclusion

Reward information processing is a fundamental brain function necessary for guiding appropriate behavioral responses. Here we report that neurons in the VP encode reward information and describe how NAc input modulates VP responses. Also, we presented preliminary data directly linking dopamine signaling to predictable neuronal responses in the NAc that reflect motivated behavior. The results obtained in these experiments are consistent with models suggesting a directional flow of information from the VTA to the NAc, to the VP before returning to the VTA. While these studies describe the function of structures that regulate reward processing and motivated behavior, they also reveal additional complexities in the system. These studies indicate that using advanced techniques for circuit dissection along with sophisticated behavioral approaches can better characterize the specific types of information being integrated at the level of the VP. Future directions to investigate how reward is being encoded and represented neuronally will include experiments that examine neuronal populations or circuits that are activated during specific behavioral tasks. With the steadfast development of advanced tools that provide increasingly better temporal and spatial resolutions, as well as phenotypic control, the future of affective neuroscience research is unquestionably heading in a direction that will eventually allow the characterization of the neurophysiological events that mediate some of the most interesting aspects of the human condition.

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