The Role of Ventral Pallidal GABAergic neurons in cue-elicited reward seeking and consumption

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Dedication

This thesis is dedicated to the late Robert Knowlton and Wiggles Knowlton.

Wiggles, you have set a precedent of how to be an exceptional being, in youth and in old age. Our current kitties still follow your gospel.

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Abstract

Cues are powerful modulators of motivated behaviors. Environmental cues can invigorate reward-seeking by signaling to neuronal circuits whose output initiates reward-seeking behaviors. Understanding the contribution environmental cues have to elicit reward seeking behavior is an important area of focus in neuroscience research. Determining the neural circuitry responsible for encoding environmental reward-predictive signals can help determine what circuits are involved when cue reactivity becomes maladaptive. The ventral pallidum (VP) is a brain region implicated in contributing greatly to cue-elicited reward seeking. The predominate cell type in VP is gamma-Aminobutyric acidergic (GABA) neurons, many of which are projection neurons. These neurons are excited by rewards and reward-related cues, and project to other major brain regions involved in an array of motivated behaviors. However, VP GABA encoding of reward predictive cues, has yet to be explored. Additionally, it has been shown that VP signaling greatly impacts consumption and that impact varies between reward types. However, it has yet to be identified how VP GABA neurons contribute to routine consumption behaviors for different reward types. This proposal advances the understanding of the neural contributions to cue-elicited behaviors by 1) determining which cues VP GABA neurons encode and if this neuronal activity is predictive of reward seeking behavior and 2) if VP GABA functional modulation can impact consumption behavior of different value rewards. Our major findings were that VP GABA neuronal calcium activity developed in response to reward predictive cues as animals learned a cue-elicited reward seeking task. VP GABA neurons encoded a reward predictive auditory cue and the operant action required to obtain reward. Additionally, the reward predictive auditory cue elicited a VP GABA calcium response that was predictive of the incentive value of the cue. Finally, we found that activation of VP GABA neurons during routine consumption tasks can significantly decrease and increase chow intake, and increase sucrose intake, in a sex specific manner. These findings show that VP GABA neurons are an important component to the neural circuits that promote cue-elicited reward-

seeking and consumption behaviors. This work will guide future research interested in neuromodulation as a therapy for maladaptive reward-seeking or consumption behaviors.

Table of Contents

Contents

Acknowledgements i

Dedication ii

Abstract iii

Table of Contents v

List of Figures viii

List of Frequent Abbreviations ix

Chapter 1: Introduction 1

1.1 Cue-elicited reward seeking 1

1.2 Neural circuits involved in reward processing 3

1.3 Ventral Pallidum contribution to cue-elicited reward seeking 4

1.4 Ventral Pallidal cellular subtypes in reward seeking 7

1.5 Ventral Pallidal contribution to consumption of reward 9

1.6 Ventral Pallidal cell types in consumption 11

1.7 Objectives and Research Goals 12

Chapter 2: Ventral pallidal GABAergic neuron calcium activity encodes cue-driven reward-seeking and persists in the absence of reward delivery 14

2.1 Chapter Summary 14

2.2 Introduction 15

2.3 Materials and Methods 17

2.3.1 Subjects 17

2.3.2 Surgeries 18

2.3.3 Discriminative Stimulus (DS) Task 19

2.3.4 In vivo Calcium Recordings 21

2.3.5 Behavioral Analysis 23

2.3.6 Fiber Photometry Analysis....24
2.3.7 Histology....26
2.4 Results....28
2.4.1 Viral mixture and fiber implantation specifically targets ventral pallidal GABAergic neurons....28
2.4.2 Rats discriminate between reward-predictive and neutral cues....30
2.4.3 Ventral pallidal GABA neurons develop a calcium response following the onset of the reward-predictive cue but not the neutral cue....32
2.4.4 VP GABA neuronal response post reward-predictive cue is associated with cue onset, operant behavior, and initial reward consumption....35
2.4.5 VP GABA cue response predicts motivational value of the reward-predictive cue 37
2.4.6 VP GABA neurons have a response post reward-predictive cue even in the absence of reward....39
2.4.7 VP GABA neuronal response decreases with extinction of learned behavior 42
2.5 Discussion....43
2.5.1 Ventral pallidal GABA neurons develop calcium responses to cues across learning 44
2.5.2 Encoding of reward-seeking latency by VP GABA calcium activity....46
2.5.3 Elevation of calcium activity following port entry and during initial reward consumption....47
2.5.4 Caveats and Future Directions....48
2.6 Conclusions....49
Chapter 3: The Contribution of Ventral pallidal GABAergic neurons to consumption of chow and sucrose....51
3.1 Chapter Summary....51
3.2 Introduction....52
3.3 Materials and Methods....55
3.3.1 Subjects....55
3.3.2 Surgeries....55
3.3.3 DREADD Ligand and Administration....57
3.3.4 Behavioral Testing....57
3.3.5 Histology....60

3.3.6 Statistical Analysis....62
3.4 Results....64
3.4.1 Inactivation of VP GABA neurons has no significant effect on chow or sucrose consumption....64
3.4.2 Activation of VP GABA neurons causes a significant increase in chow and sucrose consumption/preference in a sex specific manner....69
3.4.3 Injection of ligand has no significant effect on chow or sucrose consumption in non-DREADD expressing animals, but may have off target effects on water consumption....74
3.4.4 Injection of ligand has no significant effect on animal movement in DREADD expressing or control animals....80
3.5 Discussion....83
3.5.1 Inactivation of VP GABA neurons has no significant effect on chow or sucrose consumption in non-food restricted animals....84
3.5.2 Activation of VP neurons impacts chow consumption in a sex specific manner 86
3.5.3 Non-selective effects of DREADD ligand on water consumption....89
3.5.4 Caveats and Future Directions....90
3.6 Conclusions....92
Chapter 4: Conclusions....94
4.1 Summary of Findings....95
4.1.1 VP GABA neurons encode reward predictive cues and this neural response in predictive of the vigor of reward-seeking behavior....95
4.1.2 VP GABA neuronal response to reward predictive cues is modulated by absence of reward, but persists even in the absence of reward....95
4.1.3 VP GABA neurons contribute to consumption of chow and sucrose reward in sated male rats....96
4.2 Future Directions....97
4.2.1 VP GABA neuronal subtypes in cue-elicited reward-seeking and consumption....97
4.2.2 Internal state effect on VP GABA encoding and contribution to cue-elicited reward seeking and consumption....100
4.2.3 VP GABA contribution to cue-elicited reward seeking with substances of abuse 101
References....104

List of Figures

Introduction

Figure 1. *Simplified circuit diagram of some reward seeking and consumption related brain regions and their connections to one another.*. 4

Chapter 2

Figure 1. *Anatomical and cellular characterization of GCaMP6f expression in ventral pallidum (VP)*. 28
Figure 2. *Behavioral task and training data*.. 30
Figure 3. *VP GABA Calcium activity during training*.. 33
Figure 4. *Event-related responses during performance of the DS task*. 37
Figure 5. *Effects of reward delivery and omission on DS calcium activity*. 40
Figure 6. *DS calcium activity during extinction learning*..... 41

Chapter 3

Figure 1. *DREADD virus is primarily expressed in VP.*..... 62
Figure 2. *VP GABA inhibition has no significant effects on consumption of chow, sucrose, and sucrose preference*.. 66
Figure 3. *VP GABA activation increases consumption of chow and sucrose at high ligand dose and decreases chow consumption at low ligand doses, specifically in male rats*..... 72
Figure 4. *DREADD ligand has no significant effects on consumption of chow or sucrose, however, does impact sucrose preference*..... 76
Figure 5. *DREADD ligand has off target effects on water consumption*. 78
Figure 6. *VP GABA inhibition or activation as well as DREADD ligand injections, have no significant effects on locomotion for reward-seeking*. 81

List of Frequent Abbreviations

VP	Ventral Pallidum
DS	Discriminative Stimulus
NS	Neutral Stimulus
PE	Port Entry
GABA	gamma-Aminobutyric acidergic
DREADD	Designer Receptor Exclusively Activated by Designer Drugs
Gi	AAV8-hSyn-DIO-hM4Di-mCherry
Gq	AAV8-hSyn-DIO-hM3Dq-mCherry
mCherry	AAV_8-hSyn-DIO-mCherry
NAc	Nucleus Accumbens
LH	Lateral Hypothalamus
VTA	Ventral Tegmental Area
GAD1	Glutamate decarboxylase 1 gene

Chapter 1: Introduction

1.1 Cue-elicited reward seeking

Our environment consistently guides our behaviors. Environmental stimuli activate animal sensory systems that relay information about the external world to the central nervous system. The integration of these signals can elicit biological and/or behavioral responses (Rehman et al., 2022). This stimulus-response pairing is necessary for animals to learn which behaviors lead to success and survival in their environments. Stimuli that elicit useful biological /behavioral responses are often learned and these responses are repeated by animals (Mehrkam, 2020). For example, primates rely heavily on visual stimuli to guide foraging for food and a common stimulus is the sight of a colorful fruit, possibly a yellow banana (Asensio et al., 2011; Teichroeb and Chapman, 2014). The response includes actions such as approaching and consuming the fruit. Yellow is now the cue that is associated with the consequence of finding ripe, nutritious, and tasty fruit. Conditioned cues that signal the availability of reward, like the color of ripe fruit, are useful predictive tools that allow animals to navigate their environment efficiently and successfully.

Reward predictive cues can obtain value that is associated with a positive affective state (reward) or a negative affective state (aversion) (Young, 1959). Cues associated with rewarding states can enhance reward seeking behavior, whereas cues associated with aversive states can constrain reward seeking behavior (White, 2011; Teichroeb and Chapman, 2014). In the example above,

the fruit color could be bright green or bright yellow, but those two cues could be associated with different affective states- yellow with ripe fruit and green unripe fruit. The yellow fruit will have a sweet, pleasurable taste, whereas the green fruit will have a bitter, unpleasant taste. The animal can use these cues to discriminate and make choices that best suit them based on their internal state. Thus, conditioned cues can modulate behavior based on their associated value.

How cues modulate behavior has become of particular interest in motivation research. Although frequently an advantageous learned behavior, cue-elicited reward seeking has the potential to become maladaptive leading to overconsumption of rewards, sometimes even despite negative consequences (Berridge, 2007; Smith et al., 2011; White, 2011). Cues associated with drugs of abuse and palatable food are known to elicit craving in human patients (Negrete and Emil, 1992; Boswell and Kober, 2016). This craving can be predictive of consumption or persistence of consumption of substances of abuse and palatable food (Jansen et al., 2003; Shiffman et al., 2013; Boswell and Kober, 2016). Additionally, cues associated with reward may be source of relapse or inability to moderate reward consumption. Cues can even reinstate reward seeking and relapse (Bossert et al., 2013; Pitchers et al., 2018).

Laboratory behavioral paradigms have been developed to understand how cues impact reward seeking in humans and animals. These paradigms rely on conditioning animals to associate cues, often auditory, to reward delivery (Pavlovian cues) or to reward availability, contingent on an intermediate action (operant cues) (Perry et al., 2014). These paradigms allow researchers to study

the acquisition, extinction, and even reinstatement of cue-elicited reward seeking. Animals readily learn that cues are paired with reward or reward-delivery, and initiate reward seeking in animals (Berridge, 2007; Smith et al., 2011). Additionally, animals are sensitive to changes in cue value, however, overtraining can lead to reward seeking in punishment or extinction contexts. This has been shown for both natural food rewards and for drugs of abuse (White, 2011; Morton et al., 2014; Perry et al., 2014). Some behavioral therapies have been adapted from these studies (McHugh et al., 2010), yet pharmacological targets could also help modulate maladaptive cue-elicited reward seeking. Therefore, research has begun to dissect neurobiology contributing to cue-elicited reward seeking behaviors and potential targets for pharmacological intervention.

1.2 Neural circuits involved in reward processing

Determining the neural circuitry contributing to cue-elicited reward seeking first started by establishing the neural correlates of reward. Dopamine related brain systems have been shown to contribute to reward and motivation of reward seeking (Robinson and Berridge, 1993). However, not surprisingly, nearly eliminating the existence of dopamine in the brain does not have an all or nothing effect on reward-seeking or reward preference (Cannon and Palmiter, 2003). Therefore, work has branched out to examine other brain regions that send and receive dopaminergic connections (Figure 1).

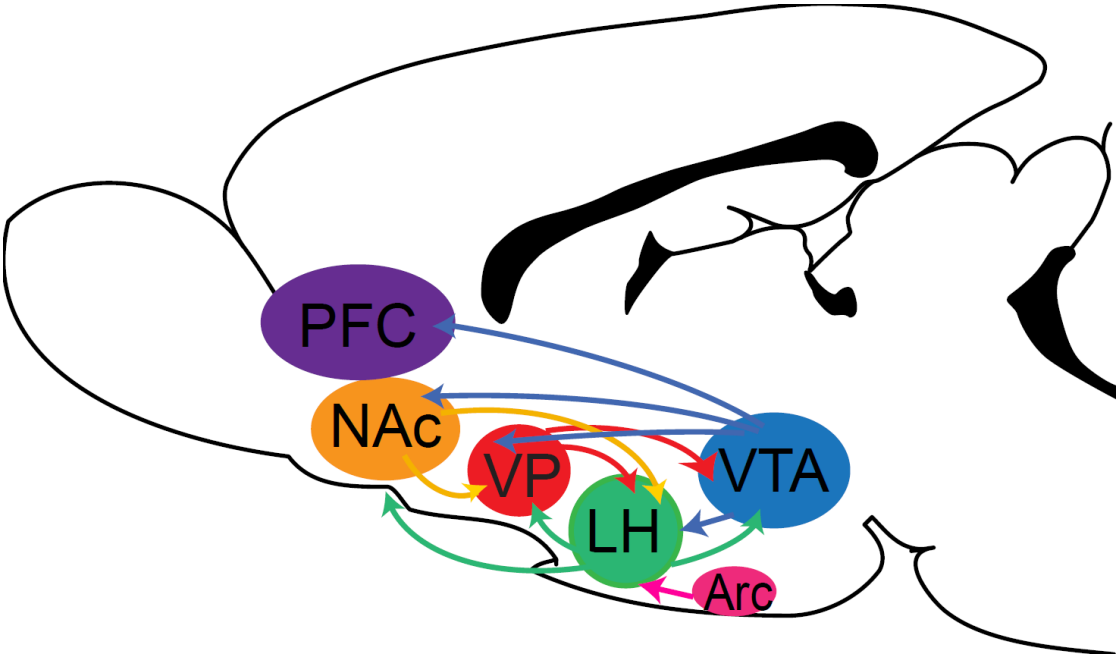

Figure 1. *Simplified circuit diagram of some reward seeking and consumption related brain regions and their connections to one another.* Prefrontal cortex (PFC), NAc (Nucleus Accumbens), Ventral Pallidum (VP), Lateral Hypothalamus (LH), Ventral Tegmental Area (VTA), Arcuate Nucleus of the Hypothalamus (Arc).

1.3 Ventral Pallidum contribution to cue-elicited reward seeking

The neural circuitry that contributes to reward seeking is still being dissected, but major brain regions that contribute to reward seeking have been identified (Figure 1). Amongst those brain regions is the ventral pallidum (VP). The VP was first identified as a functionally important structure involved in movement when Nissl stains showed it received dense inputs from the ventral striatum (including the nucleus accumbens; NAc) (Heimer et al., 1982). However, further examination of the functional contribution of the VP revealed it not only impacted motor output, but also contributed to a decrease in motivation to consume food and water (Morgane, 1961; Cromwell and Berridge, 1993).

Additionally, VP lesions increased the oral-facial reactions associated with an aversive state (Cromwell and Berridge, 1993; Robinson and Berridge, 1993). Therefore, the VP was established as a region that contributed to movement, the affective experience of reward, and the motivation to retrieve reward.

Following experiments revealed that ventral pallidum is crucial for learning and executing learned responses associated with reward. Pharmacological inhibition of VP neurons prevented reward induced reinstatement for cocaine and food-seeking behavior (McFarland and Kalivas, 2001). Additionally, once food-seeking tasks were learned, pharmacological inhibition of VP neurons decreased the amount of effort animals were willing to exert in the food-seeking task, even though animals still maintained motivation for food intake during the task (Farrar et al., 2008). VP inactivation also inhibits acquisition of reward-paired conditioned place preference (Hiroi and White, 1993; Dallimore et al., 2006).

The fact that VP is a brain region important in both reward impact and reward learning led researchers to the question whether VP was also important for processing the cues that predicted these rewards as this was seen in other associated reward structures such as the NAc (Tindell et al., 2004). When VP neurons were electrically recorded during a Pavlovian training task, several neuronal units were shown to increase firing rates in response to a reward-paired auditory tone and this number increased over training (Tindell et al., 2004; Smith et al., 2009). Later work found that VP neurons increased firing rate in response to both a reward predictive and a reward paired- auditory cue. Additionally the most temporally proximal reward paired cue response was amplified by opioid

agonist and amphetamine infusion in the NAc (Smith et al., 2011), suggesting a significant yet distinct role of cue responses in NAc and VP neurons. Later work showed that VP inhibition also decreases contextual cue inducted alcohol seeking (Perry et al., 2014). VP inactivation showed VP was necessary for optimal performance/ reward seeking in a discriminative stimulus task where an auditory cue was predictive of reward delivery and had to lever press to obtain reward during cue presentation (Richard et al., 2016).

Follow up studies suggest that VP neuronal activity drives reward seeking behavior. A subset of VP neurons encoded relative reward value of cues and this cue elicited response predicted the vigor of subsequent reward-seeking (Richard et al., 2016). Previous tests had observed similar roles for NAc neurons. Inactivation of the NAc core significantly decreased behavioral responding to a reward-predictive cues in the discriminative stimulus (DS) task (Ambroggi et al., 2011). Additionally, a subset of NAc neurons were shown to encode relative cue value in the DS task (Nicola et al., 2004a, 2004b; Ambroggi et al., 2011). Many models of VP's contributions to reward-seeking focus on its status as a major output for the NAc, indicating it may be the NAc that drives these cue response in the VP (Volkow et al., 2004). However, recent work had established that VP neurons respond to relevant stimuli like cues and rewards earlier than NAc neurons (Richard et al., 2016; Ottenheimer et al., 2018, 2020b). Additionally, VP neuronal cue responses more robustly predict reward seeking behavior when the cue is predictive of reward availability compared to when the cue is paired with reward delivery independently of the animal's behavior (Richard et al., 2018*)*,

indicating that VP neuronal encoding of reward-related cues is dependent on a reward-seeking action and is therefore encoding and integrated incentive value signal, one that incorporates both cue and response value.

1.4 Ventral Pallidal cellular subtypes in reward seeking

Richard et al. (2016, 2018) showed that VP neurons that are excited by reward predictive cues encode the incentive value of cues and predict reward seeking behavior. However, these studies also showed that VP neurons had a heterogeneous response to reward-predictive cues. Many VP neurons were excited by the reward-predictive cues, whereas a smaller subset were inhibited by reward-predictive cues. Additionally, the VP has proven to play a role in both appetitive and aversive behavior (Richard et al., 2016; Faget et al., 2018; Richard et al., 2018a; Tooley et al., 2018; Stephenson-Jones et al., 2020; Vachez et al., 2021). This data suggests that different subtypes of VP neurons encode and modulate motivated behaviors differently. The VP contains 3 major neuronal subtypes: cholinergic, glutamatergic, and gamma-Aminobutyric acidergic (GABA)(Root et al., 2015). Cholinergic neuronal numbers are small, and they also co-express markers of glutamate and GABA (Soares-Cunha and Heinsbroek, 2023). VP cholinergic neurons mainly project to the prefrontal cortex and basolateral amygdala, as well as having many collaterals to VP GABAergic neurons, suggesting they may be important in local neuronal communication. VP glutamatergic neurons make up about 15-20% of neurons in the VP (Geisler and Zahm, 2005; Hur and Zaborszky, 2005; Geisler et al., 2008; Faget et al., 2018; Heinsbroek et al., 2020; Soares-Cunha and Heinsbroek, 2023) and mainly

project to and influence the lateral habenula and other VP neurons, however they also project to and influence the ventral tegmental area (VTA) and mediodorsal thalamus (Levi et al., 2020). VP glutamatergic neurons primarily seem to contribute to aversive behaviors, are excited by aversive stimuli and constrain reward seeking behavior (Baker et al., 2016; Heinsbroek et al., 2017, 2020; Stephenson-Jones et al., 2020).

VP GABA neurons are the canonical cellular subtype in VP, making up about 75% of all cells in VP (Root et al., 2015; Soares-Cunha and Heinsbroek, 2023). They are mainly projection neurons that extend to many structures associated with reward seeking including the NAc, VTA, basolateral amygdala, subthalamic nucleus, lateral hypothalamus and prefrontal cortex (Root et al., 2015). VP GABA neurons also consist of subpopulations that co-express other neuropeptides such as neuropeptide Y, somatostatin and galanin (Johansson et al., 1984; Pérez et al., 2001; Zaborszky et al., 2012; Zhu et al., 2017).

Recordings and manipulations of VP GABA neurons are helping reveal their role in an array of behaviors. Electrophysiological properties of VP GABA neurons are altered under social stress paradigms (Morais-Silva et al., 2022). Fiber photometry recordings show that population calcium activity of VP GABAergic neurons was increased during physiological transitions from non-rapid eye movement sleep to either wakefulness or rapid eye movement sleep (Li et al., 2020). Furthermore, VP GABA neurons are shown to be intimately involved in cue-elicited reward seeking behavior. Electrophysiological recordings of VP GABA neurons during a Pavlovian reward-seeking tasks showed that

many neurons are excited by a reward-paired cue and reward delivery, and inhibited by aversive cues and stimuli (Stephenson-Jones et al., 2020). Activation of VP GABA neurons with optogenetics during a reward-seeking task promotes running to obtain reward (Mahler et al., 2014; Faget et al., 2018; Stephenson-Jones et al., 2020; Farrell et al., 2021, 2022). Additionally, chemogenic inhibition of VP GABA neurons causes rats to reward seek smaller, safer rewards as opposed to bigger, riskier rewards (Farrell et al., 2021). These studies provide evidence that VP GABA neurons are major contributors to appetitive, cue-elicited reward seeking behavior. However, it is unclear how VP GABA neurons develop responses to reward cues, encode instrumental reward cues, and if this cue evoked activity is predictive of reward seeking behavior.

1.5 Ventral Pallidal contribution to consumption of reward

Food intake involves multiple converging signals: the primary attributes of food (i.e. taste and smell), environmental cues that attract individuals to the food, and hormonal signals that provide information about energy homeostasis (Morton et al., 2014). Neural circuits integrate these cues and signals to initiate food-seeking and consumption. Research has been done to reveal which circuits are involved in food intake and food seeking behaviors. One major region defined as a center that integrates these homeostatic and motivational signals is the lateral hypothalamus (LH). Early lesions studies revealed that damage to the LH eliminates consumption all together (Anand and Brobeck, 1951). However,

follow-up review of the lesions revealed that LH was not the only region damaged, and that the ventral pallidum (directly anterior to LH) was also greatly damaged, implicating the VP as another node in food consumption (Cromwell and Berridge, 1993; Castro et al., 2015). Follow up studies revealed that lesions to LH or VP caused aphagia (Cromwell and Berridge, 1993; Castro et al., 2015). It was later found that blocking GABA-A receptors in VP increased food, but not water, intake in satiated rats (Stratford and Kelley, 1999). GABA-A antagonism selectively increased the intake of sucrose but not of water and quinine in water-deprived rats. In contrast, GABA-A agonist infusion in the VP suppressed the intake of saccharin, water and quinine (Shimura et al., 2006). VP receives orexin projections from LH, and orexin neurons are shown to be important in integrate homeostatic feeding signals to initiate feeding, whereas POMC neurons integrate homeostatic signals to decrease feeding (Inutsuka and Yamanaka, 2013). It has also been shown that increased consumption of chow in sated rats, caused by GABA antagonist infusion into VP, is attenuated when bilateral lesions of LH were paired with these infusions (Stratford and Wirtshafter, 2013). VP disinhibition with GABA-A antagonist in VP was also shown to cause a preferential increase in chow rich in fat compared to protein or carbohydrate rich chow, suggesting VP may also play a role in preferential consumption of specific macronutrients (Covelo et al., 2014). Additionally, a chronic high-fat and high-sugar diet hyperpolarized VP neurons and decreased their firing rate (Gendelis et al., 2020), suggesting VP neurons are also sensitive to the internal homeostatic and metabolic state of and animal. These experiments solidify that VP is a neural

node contributing to food intake, and that VP is integrated in neural circuitry that encodes homeostatic signals as well as motivational ones.

1.6 Ventral Pallidal cell types in consumption

Only recently has research started to examine which VP cell types are involved in consumption and how internal state may impact VP related consumption behaviors. VP GABA neurons are of utmost interest in food consumption because of their role in appetitive behaviors and reward-seeking (Mahler et al., 2014; Faget et al., 2018; Stephenson-Jones et al., 2020; Farrell et al., 2021)(Mahler, Stephen-Jones, etc.). Additionally, electrophysiological recordings of VP GABA neurons have revealed that their response to reward predictive cues is sensitive to internal-state of the animal (Stephenson-jones et al. 2020). Chemogenetic activation of VP GABA neurons in food-restricted mice caused no obvious increase in chow intake (Li et al., 2021). Whereas chemogenetic inhibition of VP GABA neurons decreased chow seeking and intake in hungry rats but not in sated rats (Farrell et al., 2022). VP GABA inhibition also caused decreased seeking and intake of palatable banana pellets in both hungry and sated rats (Farrell et al., 2022). These results show VP GABA neurons contribute to reward seeking and this contribution is sensitive to internal state. However, it is still unknown how VP GABA activation impacts intake in sated animals or how modulation of VP GABA neurons impacts consumption of specific macronutrient rewards.

1.7 Objectives and Research Goals

This dissertation addresses the need to characterize the role of VP GABA neurons in cue-elicited instrumental reward seeking and consumption. It explores the development of VP GABA calcium responses to reward related cues, the encoding of these cues and the predictive nature of this VP GABA activity on reward-seeking. It also determines the contribution of VP GABA neurons to consumption of chow and sucrose in sated male and female rats.

Chapter 2 describes how population level; VP GABA neuronal calcium activity develops while rats learn a discriminative stimulus task. Fiber photometry was used to show VP GABA population calcium activity encodes reward-predictive cues, instrumental actions, and initial reward consumption. This VP GABA response to reward predictive cues is significantly predictive of reward-seeking vigor and persists even in the absence of reward.

Chapter 3 describes how bidirectional modulation of VP GABA neuron activity impacts chow and sucrose consumption in sated rats. Chemogenetic DREADDs and a novel DREADD ligand were used to activate or inhibit VP GABA neurons during passive consumption tasks for chow and sucrose. Activation of VP GABA neurons bidirectionally impacted chow consumption depending on ligand concentration, decreasing consumption of chow at lower doses of ligand, and

increasing chow consumption at higher doses of ligand specifically in male rats. Activation of VP GABA neurons also increased sucrose consumption at both ligand concentrations specifically in male rats.

Chapter 2: Ventral pallidal GABAergic neuron calcium activity encodes cue-driven reward-seeking and persists in the absence of reward delivery

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2.1 Chapter Summary

Reward-seeking behavior is often initiated by environmental cues that signal reward availability. This is a necessary behavioral response; however, cue reactivity and reward-seeking behavior can become maladaptive. To better understand how cue elicited reward-seeking becomes maladaptive, it is important to understand the neural circuits involved in assigning appetitive value to rewarding cues and actions. Ventral pallidum (VP) neurons are known to contribute to cue elicited reward-seeking behavior and have heterogeneous responses in a discriminative stimulus (DS) task. The VP neuronal subtypes and output pathways that encode distinct aspects of the DS task remain unknown. Here, we used an intersectional viral approach with fiber photometry to record

bulk calcium activity in VP GABAergic (VP GABA) neurons in male and female rats as they learned and performed the DS task. We found that VP GABA neurons are excited by reward-predictive cues but not neutral cues, and that this response develops over time. We also found that this cue-evoked response predicts reward-seeking behavior. Additionally, we found increased VP GABA calcium activity at the time of expected reward delivery, which occurred even on trials when reward was omitted. Together, these findings suggest that VP GABA neurons encode reward expectation and calcium activity in these neurons is predictive of the vigor of cue-elicited reward-seeking.

2.2 Introduction

Environmental cues associated with rewards are powerful modulators of reward-seeking-behavior. Understanding how environmental cues can invigorate reward-seeking behavior is a necessary step in learning how cue reactivity, and subsequent reward-seeking, can become maladaptive. The ventral pallidum (VP) is a part of the ventral basal ganglia and an important node in the neural circuitry mediating cue-evoked reward-related behavior (Tindell et al., 2004, 2005; Smith et al., 2009; Richard et al., 2016; Ottenheimer et al., 2018; Richard et al., 2018a; Ottenheimer et al., 2020a). VP neurons have heterogeneous responses to both rewards and cues predictive of reward. Most VP neurons are excited by reward-predictive cues, while a smaller population is inhibited by the same cues. Additionally, the firing rates of some cue-excited VP neurons can predict the vigor of reward-seeking in response to that cue (Richard et al., 2016, 2018a). VP

neurons are also sensitive to reward identity and prior reward history (Lederman et al., 2021). Whether the VP neurons encoding reward-predictive cues, reward-seeking vigor, and reward identity are the same or distinct subsets of VP neurons remains unknown. Therefore, exploring the neurochemical and projection-specific identity of VP neurons is an important step to understanding the broader neural circuitry that impacts reward-seeking behavior.

Recent research has begun to probe which VP neuronal cell-types contribute to distinct aspects of reward processing and reward-seeking behavior (Kupchik and Prasad, 2021). The VP contains 3 major neuronal subtypes: cholinergic, glutamatergic, and gamma-aminobutyric acidergic (GABA) (Root et al., 2015). VP glutamatergic neurons constrain reward-seeking and promote aversion related behavior (Faget et al., 2018; Tooley et al., 2018; Stephenson-Jones et al., 2020). In contrast, VP GABA neurons are excited by rewards and Pavlovian reward-paired cues (Heinsbroek et al., 2020; Stephenson-Jones et al., 2020). Activation of VP GABA neurons drives positive reinforcement as well as cued drug-seeking and relapse (Faget et al., 2018; Heinsbroek et al., 2020; Prasad et al., 2020a; Farrell et al., 2022). Inhibition of VP GABA neurons can induce place avoidance and dampen risky decision making (Faget et al., 2018; Heinsbroek et al., 2020; Farrell et al., 2021). Yet, how VP GABA neurons develop responses to reward-predictive cues over time, or if their activity predicts the vigor of reward-seeking, remains unknown.

Here we examined whether the calcium activity of VP GABA neurons encodes cues predictive of reward availability and how this activity is related to

subsequent reward-seeking behavior. Rats were trained in a discriminative stimulus (DS) task with liquid sucrose reward while we recorded population-level calcium activity of VP GABA neurons using fiber photometry. We found that VP GABA neurons develop a calcium response to reward-predictive cues as rats learn the DS task. VP GABA calcium activity was found to respond following the reward-predictive cue, following the action required to obtain reward, and during reward consumption. Calcium increases post-cue and operant action persisted even on trials where reward was omitted. Finally, VP GABA cue-evoked calcium activity was shown to correlate with behavioral responses that measure the vigor of reward-seeking. These results suggest that VP GABA neurons encode environmental cues predictive of sucrose reward and this cued calcium response predicts the vigor of reward-seeking behavior.

2.3 Materials and Methods

2.3.1 Subjects

Male and female Long Evans rats (n=21, 9 male, 12 female; Envigo), weighing 250–275 grams at arrival, were individually housed in a temperature- and humidity-controlled colony room on a 12-hr. light/dark cycle. Rats were food restricted starting three days prior to the initiation of training in the DS task and until experiments were done. Rats received 5% of their body weight in food each day and the amount of food was adjusted daily to maintain rats at ~90% of their free-feeding body weight. All experimental procedures were approved by the

Institutional Animal Care and Use Committee at the University of Minnesota and were carried out in accordance with the guidelines on animal care and use of the National Institutes of Health of the United States.

2.3.2 Surgeries

During surgery, rats were anesthetized with isoflurane (5%) and placed in a stereotaxic apparatus, after which surgical anesthesia was maintained with isoflurane (0.5–2.0%). Rats received preoperative injections of carprofen (5 mg/kg) for analgesia and cefazolin (75 mg/kg) to prevent infection. To achieve cell-type specific calcium indicator expression we used a dual viral approach combining a glutamate decarboxylase 1 (GAD1) promoter virus for the expression of Cre recombinase with a virus for expression of GCaMP6f in a cre-dependent manner (Liu et al., 2013; Wakabayashi et al., 2019; Shields et al., 2021). Rats (n=19, 9 male, 10 female) received 0.8 uL of a 1:1 mixture of AAV9-Syn-Flex-GCaMP6f (2.1 * 10^13 GC/mL; Addgene) and AAV8-GAD1-cre (8.29x10^13 GC/mL; University of Minnesota Viral Vector Core) was injected unilaterally into VP. Syringes for viral delivery and optical fiber implants were aimed at the VP using the following coordinates relative to bregma: +0.3 mm AP, +/- 2.3 mm ML, - 8.3 mm DV. A subset of these rats (n=4, 2 male, 2 female) also received, contralateral to the primary recording location, an infusion of AAV9-Syn-Flex-GCaMP6f and fiber implant into VP and a retrograde AAV8-GAD1-cre (AVVrg-GAD1-Cre;1.03x10^14 GC/mL; University of Minnesota Viral Vector Core) into LH (-2.9 mm AP, 1.8 mm ML, -8.4 mm DV). This combination of injections did not lead to GCaMP6f expression. Thus, these recordings are

included in the “no expression” control data. For external controls (n=2, 2 female) 0.8 uL of a 1:1 mixture of EGFP (AAV9-Hsyn-DIO-EGFP;4.6*10^13 GC/mL; Addgene) and AAV8-GAD1-cre was injected unilaterally into VP. Virus was delivered through 28-gauge injectors at a rate of 0.1 µl per min. Injectors were left in place for 10 min following the infusion to allow virus to diffuse away from the infusion site. Following viral injections, rats used for fiber photometry measurements and behavioral testing (n=15; 6 males and 9 females) received fiber implants (.48 NA, 400 micron, 9mm; Doric) in VP for optical measurement of calcium activity. Implants were secured to the skull with bone screws and dental acrylic. Rats recovered for at least one week before beginning handling. To allow for sufficient viral expression, rats recovered for at least 4 weeks before behavioral training or brain extraction for RNAscope.

2.3.3 Discriminative Stimulus (DS) Task

Four to six weeks after surgery, rats were trained in a discriminative stimulus (DS) task (Ottenheimer et al., 2018; Richard et al., 2018a; Gómez-A et al., 2022). The DS task is an instrumental task where rats learn to discriminate between reward-predictive (DS) and neutral cues (NS). Auditory cues consisted of a siren or white noise, with cue assignments counterbalanced across subjects. Rats received reward if they entered the port during the DS cue but not the NS cue. Over time rats learn to discriminate between the cues, responding almost exclusively to the DS.

Initial Training (Port Training and Stages 1-4)

Prior to training with cues, rats underwent port training in the operant boxes while tethered to the fiber optic patch cables to acclimate them to both the cables and operant boxes. In port training sessions, animals received 30 trials of 10% sucrose delivery. For each trial, once sucrose reward was retrieved from the port, another trial was initiated. Once rats met the criteria of 30 reward retrievals in 1 hour, they transitioned to Stage 1 of DS task training. In Stages 1-4 of the DS task, each session consisted of 30 DS trials. Each trial consisted of a window in which an auditory cue (the DS; siren or white noise) signaled 10% sucrose availability for up to 60 seconds (Stage 1), 30 seconds (Stage 2), 20 seconds (Stage 3), or 10 seconds (Stage 4). The DS cue played until the rat entered the port or the availability window was over. Inter-trial intervals (ITI) were pseudo-random and adjusted so that the average time between the start of each DS presentation was 120 seconds (i.e. as the DS length decreased, the ITI lengthened from 60-110 seconds). Rats were required to respond during at least 70% of DS presentations (0.70 response probability) to pass each stage. After meeting these criteria for Stage 4, rats moved onto the full version of the DS task.

Full DS Task Training (Stage 5)

The full version of the DS task (Stage 5) consisted of 60 trials, 30 of which were DS and 30 of which were NS. The DS was presented for a maximum of 10 seconds, or until the animal entered the port. The NS played for 10 seconds

independent of animal behavior. DS or NS trial order was determined pseudo-randomly. Inter-trial intervals were determined pseudo-randomly with an average interval time of 50 seconds (30-70 seconds). A criterion of 0.70 DS response probability and 0.30 NS response probability was required for animals to complete Stage 5.

Probe Trials and Extinction

Following completion of Stage 5 testing, we added in a 1 second delay between the port entry and reward delivery, as well as probe trials for 50% of DS presentations. During probe trials, port entries during the DS were not followed by reward delivery. This allowed us to better determine whether we saw distinct calcium responses to the cue presentation, port entry, and initial lick bouts. Rats underwent 2 days of testing under these conditions. Finally, animals underwent 3 days of extinction in which sucrose was no longer delivered.

2.3.4 In vivo Calcium Recordings

Fiber photometry recordings were done throughout training in the DS task. Recordings were conducted according to methods described previously (Saunders et al., 2018). Rats were first acclimated to the tethering cable (0.48, 400-micron core optical fiber) and the operant box. During recording sessions, the tethering optical fiber is connected to a fluorescent mini cube (Doric Lenses, Canada) which transmits excitation light from both a 465 nm LED and a 405 nm LED. 465 nm excitation stimulates calcium-dependent GCaMP6f fluorescence,

and 405 nm excitation stimulates GCaMP6f fluorescence in a calcium-independent fashion. GCaMP6f fluorescence travels to the mini cube via the tethering cable, passes through a GFP emission filter and is amplified and focused onto a high sensitivity photodetector. A real-time signal processor (RZ5P, Tucker-Davis Technologies) running Synapse software was used to modulate the output of each LED and record photometry signals. Before each session, the power level for each LED was measured and adjusted to a range of approximately 10-25 microwatts (Meng et al., 2018). The offset for each animal was kept constant, between 20-30 mA (Tucker-Davis Technologies user manual), and the level was adjusted to obtain the appropriate power. The 465 nm LED driver frequency was set to 211 Hz while the 405 nm LED driver frequency was set to 531 Hz. This allowed for demodulation of the separate LED signals, yielding a calcium-dependent signal and a calcium-independent signal (isosbestic signal; (Tian et al., 2009; Dana et al., 2018; Patel et al., 2019) to use as an internal control for each animal. Task events (cue presentations, port entries and lick bouts) were time stamped in the photometry data file via a TTL signal generated by the behavioral equipment (Med Associates Super Port and TTL panel). Video recordings were simultaneously collected from each operant box (Amcrest).

Calcium-dependent fluorescent changes were detected in the calcium dependent channel (465nm), whereas calcium-independent fluorescent changes were detected in both the dependent and independent (405 nm, isosbestic) calcium channels. VP GABA bulk activity is represented as a change in

fluorescence over time. Calcium dependent and independent channels were fit and combined to control for any artifacts not associated with neuronal activity. This combined signal (delta F/F) was normalized in peri-event time windows for each trial, where baseline activity 5 seconds before cue presentation was used to calculate a z-score for each delta F/F data point in the peri-event window.

2.3.5 Behavioral Analysis

DS and NS response probabilities were calculated for each cue type as the ratio between the number of trials where the animal entered the port within 10 seconds of cue onset to the number of overall trials, for each specific cue type. We used port entry latency as a behavioral proxy for reward-seeking vigor and motivation (Richard et al., 2018a; Lederman et al., 2021). Port entry latency was calculated as the difference between port entry time points and cue onset time points. Port entry latency was only calculated on trials where animals entered the port within 10 seconds of cue presentation, allowing for a more consistent measure of latency across stages. One animal was excluded for lack of cue discrimination after 12 days of Stage 5 training. To examine how the port entry probability developed across training, two linear mixed-effects models were run. For training sessions between Stages 1-4, the model was fit with fixed effects for day and sex, and a random effect of subject. For Stage 5 sessions, the model was fit with fixed effects for day, sex, and cue type, and a random effect of subject. A paired t-test was run for criteria day port entry probability and latency, between cue types.

2.3.6 Fiber Photometry Analysis

Post-cue response across training

Fluorescent values and behavioral timestamps were exported to MATLAB. Each training session was analyzed in a custom MATLAB script (available upon request). Signals driven by 465 nm and 405 nm LEDs were down sampled to 40 Hz, fit to one another, and combined (465 nm - fitted 405 nm/fitted 405 nm; delta F/F) to control for any artifacts not associated with neuronal activity. Z-scores of the delta F/F photometry signals for each trial were calculated based on the mean and standard deviation of fluorescent values 5 seconds before the cue. We used an area under the curve analysis on z-scored trial traces to collect a single value between 0- and 5-seconds post-cue (when the bulk of the DS calcium event occurred) to run linear mixed effects models. To examine how the calcium signal developed across training, two linear mixed effects models were run. For training sessions between Stages 1-4, the model was fit with fixed effects for day and sex, and a random effect of subject. For Stage 5 sessions, the model was fit with fixed effects for day, sex, and cue type and random effect of subject. Depending on the best-fitting covariance model (Verbeke, 1997), the degrees of freedom may be a non-integer value. Any significant interactions were further assessed with pairwise comparisons with Sidak corrections for multiple comparisons.

Isolating post-cue responses to behavioral events

To isolate calcium signals associated with the cue from that of the port entry and reward delivery, we ran an encoding model on Z-scored calcium peri-event time windows (Parker et al., 2016). The analysis isolates each timestamped event (cue presentation, port-entry, initial lick onset) and relates that to each sampling point recorded from the calcium signal. To do this, event onset timestamps were transformed into binary indicators for each sampling point. Then, linear regression was run using the z-scored, stage 5, GCaMP6f recordings as the response variable and the transformation of the timestamps of the cue presentation, port entry, and initial licks as the predictor variables. This regression model output provided response kernels for each distinct event. AUC values were then acquired from regression kernels and student's t-tests were run defining the AUC's significance from null.

Post-cue correlation to reward-seeking motivation

To determine if DS-evoked neuronal activity predicts the latency of reward-seeking, our behavioral proxy of reward-seeking motivation, we ran a linear correlation analysis relating the peri-DS Z-scored delta F/F calcium signal from Stage 5 trials to trial-by-trial port entry latency (Pearson's correlation coefficients) for each rat. Because the encoding model described above revealed significant port-entry related increases in calcium activity after, but not before, port entry, for our correlation analysis we excluded fluorescence recorded from any post-port entry time points. We also shuffled these data to create random control data for comparison to the true calcium and latency relations. Comparison

of the shuffled and unshuffled correlations were done in 0.10 second time bins with multiple t-tests with Sidak corrections for multiple comparisons.

2.3.7 Histology

Validation of fiber placement and region-specific expression

Following completion of behavioral testing with fiber photometry recordings, animals (n=15, 6 males and 9 females) were deeply anesthetized with pentobarbital and were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed, post-fixed in 4% paraformaldehyde for 4–24 hr., cryoprotected in 30% sucrose for >48 hours, and sectioned at 40 um on a microtome. Sections were then washed with PBS, wet-mounted on coated glass slides in PBS, air-dried, cover slipped with Vectashield mounting medium with DAPI, and imaged on a fluorescent microscope. We verified the location of injection or optical fiber recording sites using anatomical markers of VP (anterior commissure, lateral ventricle size). Additionally, fiber implants in the contralateral VP were originally implanted in a subset of subjects to record from lateral hypothalamus projecting VP GABA neurons, however, viral expression was not obtained, and no fluorescent protein was expressed in the cells. We used recordings from these animals (n=4, 2 male, 2 female) as “no expression” control sessions. Placement of fiber was assessed for these control recordings as well and confirmed to fall within VP.

Validation of cell-type specific GCaMP6f expression

Animals designated for RNAscope analysis (n=6, 3 females, 3 males) were deeply anesthetized with pentobarbital and decapitated. Fresh tissue was extracted and flash frozen in dry-ice-cooled isopentane. Brains were stored in a -80-degree Celsius freezer for up to 1 year. Tissue was stored until sectioning of tissue was done on the cryostat (coronal sections, 16 um) and jump mounted for RNAScope processing. Tissue was processed to determine the location and extent of viral expression and neural identity of the neurons recorded. To validate our viral approach, we used RNAscope® *in situ* hybridization with probes for *Gad1*, *Slc17a6 (Vglut2)* and *GCaMP6* (Fig. 1D-E). The channels were respectively: GCaMP6 probe (488 nm excitation), glutamatergic probe (Slc17a6, 555 nm excitation), GABAergic probe (Gad1, 647 nm excitation). Imaging of the tissue was done on a confocal (Nikon upright c2, UIC-UMN) at 20x with 2x digital zoom, and settings were optimized with the help of the imaging core. The exact same acquisition settings were used on all tissue samples. HALO software (Indica Labs) quantified the amount of cell specificity achieved from viral injections. Briefly, total cell number and location within an image was determined by nuclear staining (DAPI). Probes (Gad1, vGlut, GCaMP6) are assigned their respective fluorophores within the software and the software compares the location of each fluorophore to the location of the DAPI labeled cells to confirm fluorophore expression is associated with a self-defined nuclei and cell size. HALO analysis software and software settings were optimized for the tissue from all animals and then settings were used to batch analyze cell counts of GCaMP6f positive cells. No difference was seen in expression between different subregions

of VP. The percentage and number of each defined cell identity of interest were determined for 3 sections per animal to determine cell specificity within and between all subjects.

2.4 Results

2.4.1 Viral mixture and fiber implantation specifically targets ventral pallidal GABAergic neurons

A mixture of DIO-GCaMP6f and GAD1-Cre viruses were infused into VP to express the genetically encoded calcium indicator (GCaMP6f) specifically in VP GABA neurons. For measurement of calcium-related fluorescence, an optical fiber was implanted into VP (Fig. 1A -1B). All rats that underwent calcium activity recording had viral expression and fiber placements within VP (Fig. 1C). To validate the cell-type specificity of our viral approach, we used RNAScope *in situ* hybridization with probes for *Gad1*, *Slc17a6 (Vglut2)* and GCaMP in rats that did not receive fiber implants (Fig. 1D). Because no difference was seen in expression or selectively in different subregions of VP, these data were pooled for analysis. Overall, GCaMP6f (GFP +) and Gad1 positive cells made up 90.12 % of GFP + cells (82.09% +/- 3.14% Gad1/GFP, 8.03% +/- 1.63% Gad1/Vglut2/GFP; Fig. 1E). Therefore, the cells we were recording with fiber photometry during the DS task were primarily and selectively GABAergic. In contrast, only 0.12% +/- 0.05% of GFP + cells were exclusively glutamatergic.

Finally, 9.75 % +/- 4.12% of cells were GFP+ with no glutamatergic or GABAergic probe expression.

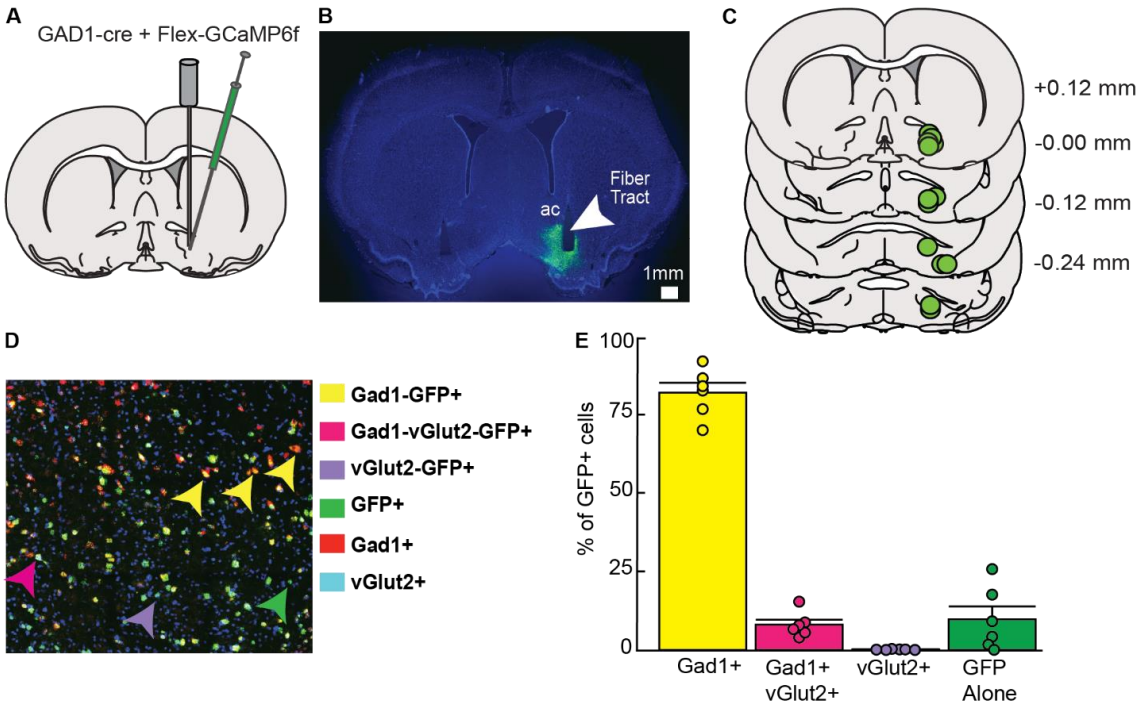

Figure 1. *Anatomical and cellular characterization of GCaMP6f expression in ventral pallidum (VP)*. (A) Diagram of intersectional viral approach and fiber optic implant used for cell type-specific calcium recordings in the VP. (B) Representative image of virally expressed GCaMP6f and optical fiber tract placement in VP for calcium activity recordings (right side), and for no-expression control recordings (left side). (C) Verification of fiber and expression at calcium activity recording sites in VP-containing coronal sections. Values listed indicated distance (mm) from bregma. (D) Representative image of RNAscope processed tissue with arrows denoting location of each defined GCaMP6f expressing (GFP+) cell type. (E) Quantification of the % GFP+ cells expressing Gad1, vGlut2 or both cell-type specific markers, n=6; 3 VP sections utilized for each subject's average (82.09% +/- 3.14% Gad1, 0.12% +/- 0.05% of glutamatergic, 8.03% +/- 1.63% Gad1/Vglut2/GFP, 9.75 % +/- 4.12% of just GFP+. Data are mean +/- SEM. Dots represent individual rats.

2.4.2 Rats discriminate between reward-predictive and neutral cues

Rats (n=12; 6M, 6F) were trained on an operant discriminative stimulus task where reward availability was signaled by a reward-predictive cue (DS). Rats received a 10% sucrose reward if they entered the operant chamber port (port entry) during DS presentations, but not if they entered during the control cue (NS) or non-cue periods (Fig. 2A). During Stages 1-4 (DS alone) of the task, rats received presentations of just the DS, which decreased in length over sessions from 60 to 10 seconds incrementally. As expected, port entry probability during the DS increased significantly across sessions during this phase of training (Fig. 2B; session effect: $F(1,98.36) = 93.01$, $p < 0.001$).

Once rats entered the port during at least 70% of DS presentations in Stage 4, they progressed to the full version of the task (Stage 5), in which the NS control cue was introduced (Fig. 2A). Rats were deemed capable of discriminating between the DS and NS when they met criteria of a minimum of 70% percent DS response probability and a maximum of 30 % NS response probability. One animal was excluded for lack of cue discrimination after 12 days of stage 5 training.

As expected, rats learned to discriminate between the DS and NS cues over time (Fig. 2C; interaction of session and cue, $F(1,218.46) = 25.32$, $p < 0.001$), increasing their probability of entering the port during the DS, and decreasing their probability of entering the port during the NS. In addition to responding more frequently to the DS than the NS when they met criteria (Fig.

2D; $p < 0.001$), rats also entered the port more quickly following the DS compared to the NS on trials when they made a port entry (Fig. 2E; $p < 0.001$).

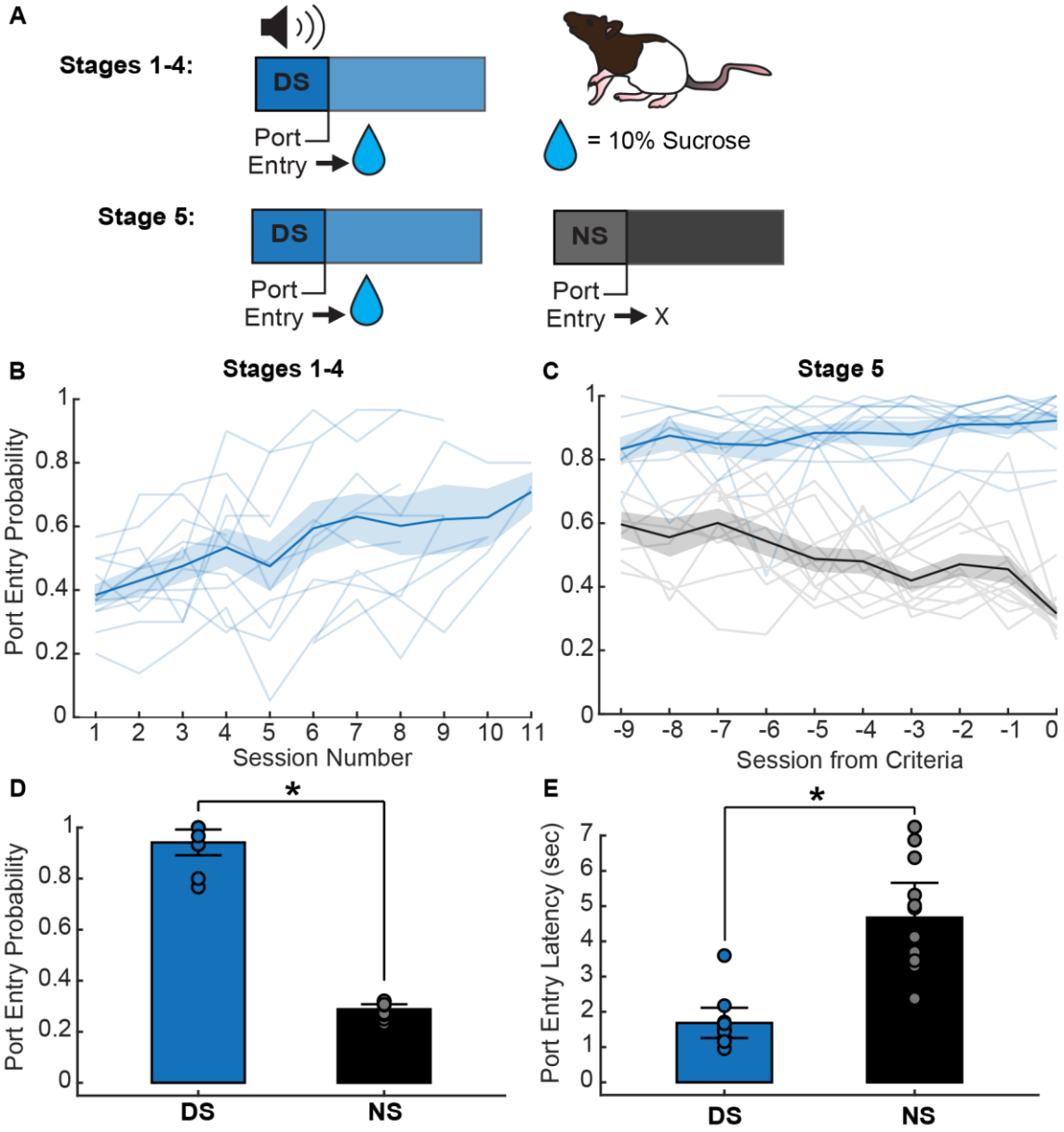

***Figure 2**. Behavioral task and training data*. (A) DS behavioral task trial design using 10% sucrose reward. (B) DS response probability (blue) during stages 1-4 of DS task (session effect: $F(1,98.36) = 93.01$, $p < 0.001$). The line with shading indicates mean +/- SEM. Lines alone indicate individual subjects. (C) Average DS (blue) and NS (black) response probability across the last 10 days of stage 5 training (9 days before criteria is

reached; session*cue effect: $F(1,218.46) = 25.32$, $p < 0.001$). The line with shading indicates mean +/- SEM. Lines alone indicate individual subjects. (D) Average DS and NS response probability on the day discrimination criteria was reached (paired t-test, *$p < 0.001$). Data are mean +/- SEM. Dots represent individual rats. (E) Average DS and NS port entry latency on day discrimination criteria reached (paired t-test, *$p < 0.001$). Data are mean +/- SEM. Dots represent individual rats.

2.4.3 Ventral pallidal GABA neurons develop a calcium response following the onset of the reward-predictive cue but not the neutral cue

Fiber photometry measurements of calcium activity were conducted throughout training to assess the development of responses across learning of the DS task. Using these methods, as rats learned the task, we observed VP GABA neurons develop a calcium response following the onset of the DS (Fig. 3A) but not the NS (Fig. 3B). The VP GABA neuronal calcium response is not apparent in response to the DS during the first session of training (Fig. 3C). Area under the curve (AUC) was calculated for each individual rat's average Z-scored calcium trace during each session of training. For initial training with just the DS, we observed a significant effect of training session on the average AUC prior to the introduction of the NS (Fig. 3F; main effect of session, $F(8,63.53) = 5.16$, $p < 0.001$). By the first day of Stage 5, when the NS is introduced, an increase in calcium activity occurs soon after DS onset and persists for nearly 10 seconds (Fig. 3D, blue line). Cue-evoked calcium activity in response to the NS is reduced relative to the DS (Fig. 3D, black line). On the day subjects met behavioral criteria for cue discrimination (70% or greater response to the DS, 30% or less for the NS) the VP GABA calcium response post-DS is robust and persists for

closer to 5 seconds, whereas the post-NS response is minimal in amplitude and duration. AUC analysis for the last 10 sessions of Stage 5 training shows a significant difference between DS and NS calcium responses (Fig. 3G; main effect of cue, $F(1,194.18) = 46.64$, $p < 0.001$). No effect of cue was seen in control recordings (GFP and no-expression controls; main effect of cue, $F(1,48.37) = 0.18$, $p = .68$). These findings suggest VP GABA neurons develop a response to reward-predictive cues while learning the DS task, but do not respond to neutral cues never paired with reward.

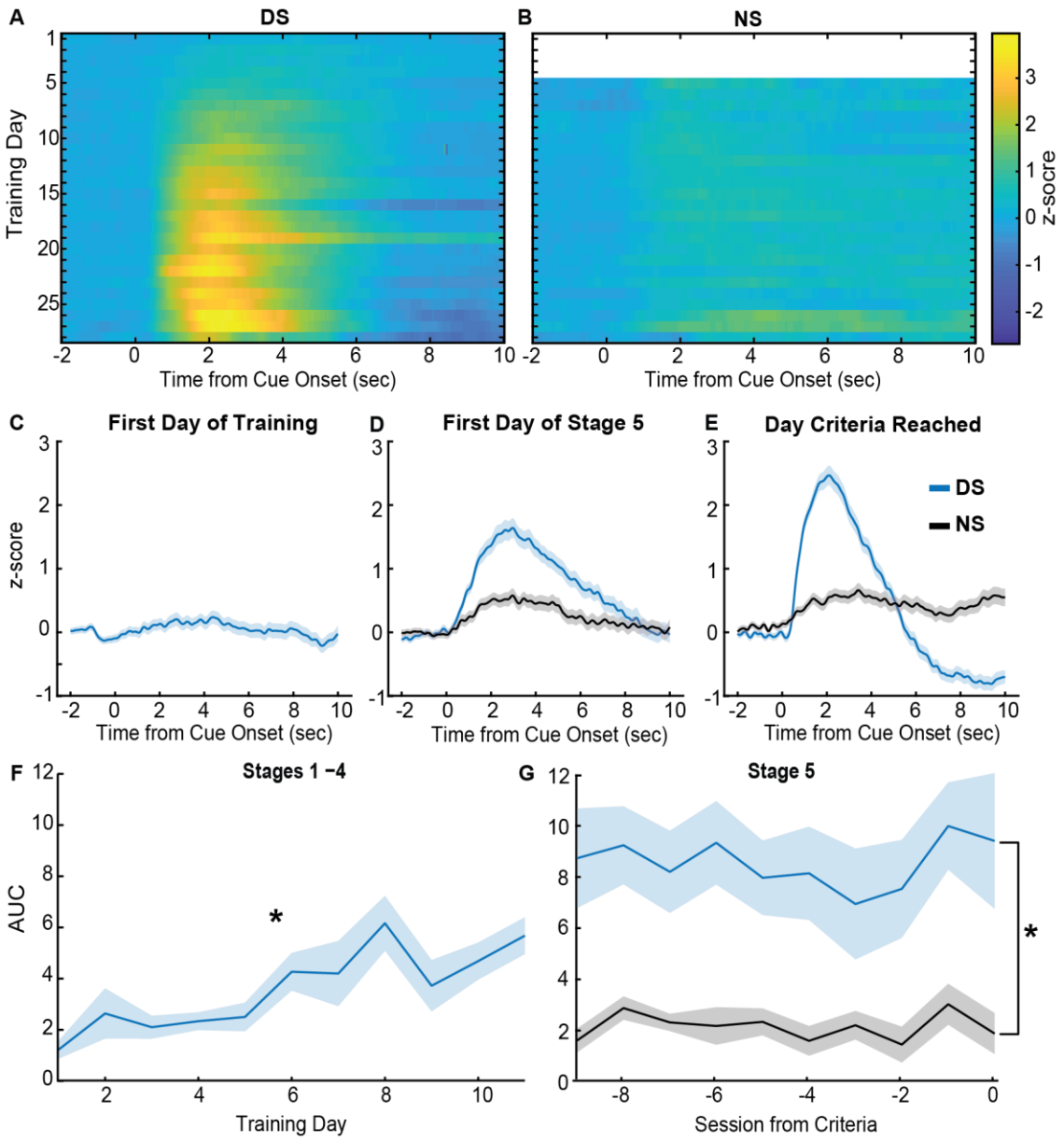

Figure 3. *VP GABA Calcium activity during training*. (A) Heatmap of average Z-scored delta F/F response from VP GCaMP6f during the peri-DS cue time window (x-axis) across training days (y-axis) of all stages (n=12 rats) (B) Heatmap of z-scored delta F/F response during the peri-NS cue time window across all Stage 5 training days (n=12 rats). (C) Average peri-DS calcium trace on day 1 of training (n=12 rats; +/-SEM). (D) Average peri-cue (DS=blue, NS=black) calcium trace on the first day of Stage 5 (n=12 rats; +/-SEM). (E) Average peri-cue calcium trace on the day each animal met criteria (n=12; +/- SEM). (F) Average AUC (0-5 seconds) of post-DS calcium traces across animals (n=12; +/- SEM) during Stages 1-4 (main effect of session, effect:

F(8,63.53)=5.16, *p < 0.001). (G) Average AUC (0-5 seconds) of post-cue calcium traces for the DS (blue) and NS (black) across animals (n=12; +/- SEM), during the last 10 days of Stage 5 training (main effect of cue, F(1,194.18)=46.6437, *p < 0.001).

2.4.4 VP GABA neuronal response post reward-predictive cue is associated with cue onset, operant behavior, and initial reward consumption

Prior work from electrophysiological recordings in VP has found that DS-excited single units increase their firing at a median time of 90 milliseconds after cue onset and return to baseline within 1 second, even for units with the longest duration responses (Richard et al., 2016, 2018a). In contrast, we observed changes in fluorescence from population-level calcium activity in VP GABA neurons, starting approximately 1 second after cue onset and persisting in the average traces for multiple seconds. While GCaMP6f is a fast calcium indicator (Chen et al., 2013), in terms of both rise and decay kinetics, it still introduces slower dynamics compared to electrophysiological recordings (Wei et al., 2020). Prior work from electrophysiological recordings in the DS task has also found that VP single units are responsive to reward-seeking actions and at the time of reward consumption (Richard et al., 2016, 2018a) and may encode the relative value of primary rewards or reward prediction errors (Ottenheimer et al., 2018, 2020a, 2020b) in addition to the incentive motivational value of cues. Therefore, it is likely that the calcium activity of VP GABA neurons is sensitive to multiple behavioral epochs following cue onset. That is, multiple calcium responses may contribute to the prolonged calcium trace seen when trials are averaged. When we sorted individual trials by the animals' latency to enter the port, we observed

both increased fluorescence that was time-locked to the cue, as well as an increase that appeared to be time locked to the port entry after the DS presentation (Fig. 4A). We observed a similar pattern when we plotted the trials time-locked to port entry (PE) and sorted by port entry latency (Fig. 4B). Moreover, when trials that were time-locked to initial lick responses were sorted by port entry latency, we observed a distinct calcium response that occurs after initial lick onset, at least on trials where latency between reward delivery (time of PE) and licking behavior is longer (Fig. 4C). Therefore, the extended calcium responses we see following DS onset could be related to all three behavioral epochs: sensing the cue, the operant action, and the initial reward consumption.

To test this, we ran an encoding model on the peri-event calcium data for each rat, for each trial, on the day the animal reached final behavioral criteria in Stage 5 of the DS task. This encoding model was adapted from Parker et al. 2016. Here, a binary of each behavioral epoch was correlated to each time point of the calcium signal, and separate kernels of regression coefficients were acquired for each behavioral epoch (Fig. 4D-F). A time bin analysis was run on each kernel, comparing the average kernel value at each 0.1 second time bin post event onset to a null value, corrected for multiple comparisons. This analysis found that the DS kernel was significantly greater than zero during 0.90-3.0 second time bins post-cue (Fig. 4D; $ps<0.05$), the PE kernel was significantly greater than zero during 1.40-2.40 second time bins (Fig. 4E; $ps<0.05$) and the initial lick kernel was significantly increased during 1.30-3.60 second time bins

(Fig. 4F; ps<0.05). AUC analysis of the average kernels for each events indicated each kernel is significantly greater than zero (Fig. 4G; DS=1.91+/-0.72, PE=2.10+/-0.94, lox=1.90+/-0.66; p's<0.05) Therefore, all three task events (cue onset, reward-seeking, and consumption) are contributing to the VP GABA calcium response.

2.4.5 VP GABA cue response predicts motivational value of the reward-predictive cue

We next sought to determine whether DS cue-evoked VP GABA calcium activity is correlated with port entry latency on a trial-by-trial basis. Previous work found that the DS-evoked spiking activity of many single units in VP is predictive of the latency it takes the animal to perform the behavior needed to obtain reward, whether that behavior is a lever press or a port entry (Richard et al., 2016, 2018a). Because the encoding model described above revealed significant port-entry related increases in calcium activity after, but not before, port entry, for our correlation analysis we only used calcium signals between cue onset and the port entry to examine how cue-elicited VP GABA activity predicted the animal's latency to enter the port. Latency correlations were calculated for each rat individually in 0.10 second time bins and compared to shuffled controls (Fig. 4H). When we examined the mean correlation coefficients over time, we found that post-DS VP GABA calcium activity is initially positively correlated with port entry latency when compared to shuffled data in the 0-0.50 second time bins

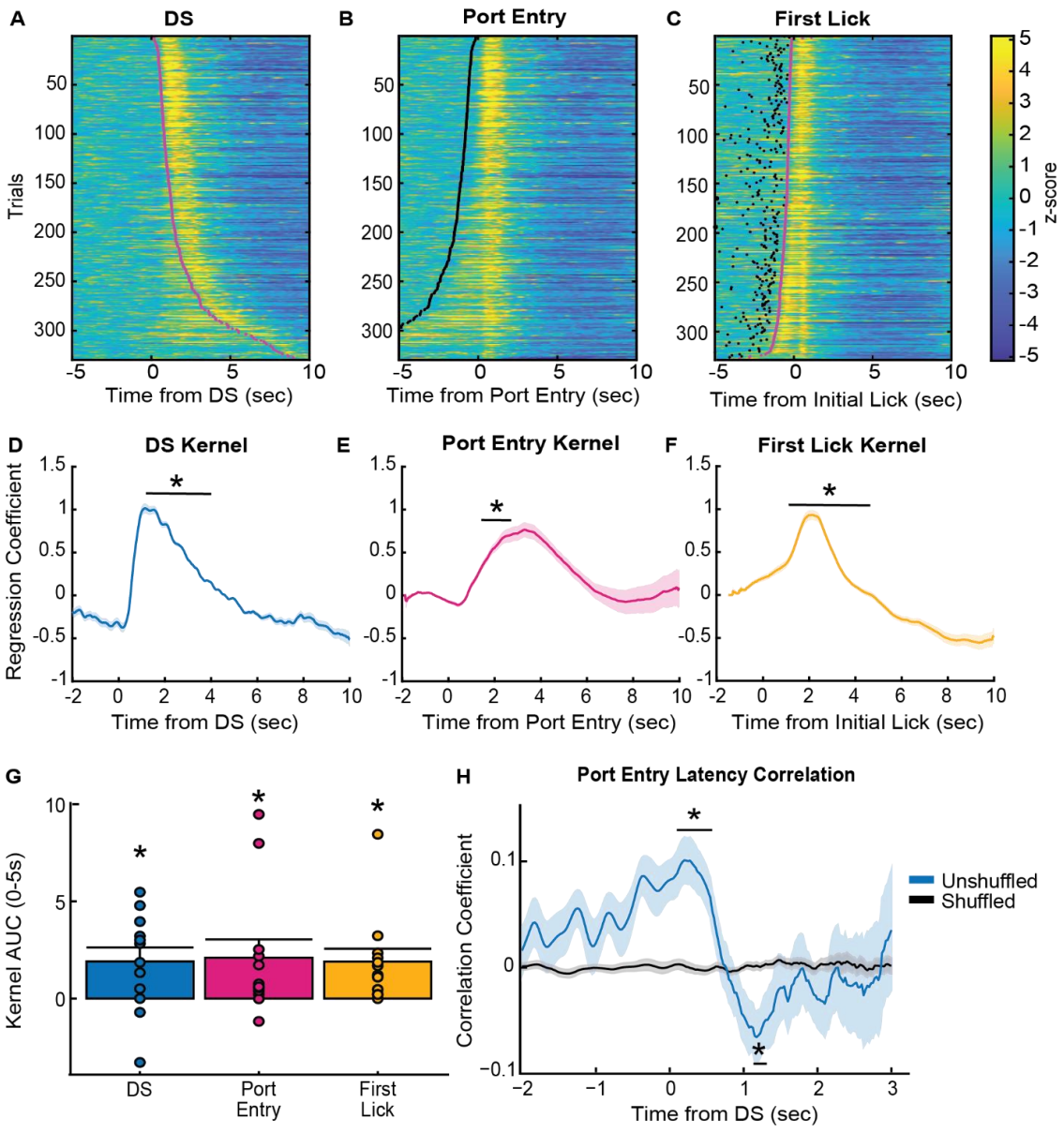

Figure 4*. Event-related responses during performance of the DS task*. (A) Heatmap of Z-scored responses from Stage 5 of a representative animal, trials sorted by PE latency, time-locked to the DS cue onset. Magenta dots represent the time of port entry. (B) Heatmap of Z-scored responses from Stage 5 of a representative animal, trials sorted by PE latency, time-locked to port entry. Black dots represent cue onset. (C) Heatmap of Z-scored responses from Stage 5 of a representative animal, sorted by PE latency, time-locked to initial lick onset. (D) Average DS cue onset kernel of lasso regression coefficients across all animals (n=12), significant difference from zero between 0.90-3.40

sec time bins (*ps<0.05) (E) Average port entry onset kernel of lasso regression coefficients across all animals (n=12), significant difference from zero between 1.40-2.40 sec time bins (*ps<0.05) (F) Average initial lick onset kernel of lasso regression coefficients across all animals (n=12), significant difference from zero between 1.30-3.60 second time bins (*ps<0.05). (G) Average AUC of each regression kernel across animals (n=12), t-test comparing each AUC to zero. Mean + SEM, dots indicate individual subjects. (H) Linear Pearson's correlation coefficients for animal's trial latency to DS evoked VP GABA calcium activity correlation (unshuffled= blue, shuffled=black). Time bin analysis indicated that true (unshuffled) coefficients were significantly different from zero between 0.00 to 0.50 sec time bins (*p<.006), and 1.10-1.20 sec time bins (*p<.03). Lines with shading indicate mean +/- SEM.

(p<.006). This ramping positive correlation is initiated pre-cue onset, during the baseline period. This suggests that baseline activity may predict port entry latency, such that greater baseline VP GABA activity pre-cue leads to a longer post-cue latency to enter the port. However, the 1.10-1.20 second time bins after cue onset are negatively correlated to the latency to enter the port (p<.03). This significant time of negative correlation roughly aligns with kernel time bins in which we observed significant DS-evoked calcium activity (0.90-1.30 second bins). This suggests that VP GABA calcium activity in response to DS cue onset is predictive of port entry latency, such that higher VP GABA calcium activity is associated with shorter port entry latencies, thus greater encoding of the motivational value of the cue.

2.4.6 VP GABA neurons have a response post reward-predictive cue even in the absence of reward

Because calcium imaging is a proxy for neural activity with slow kinetics and VP neurons have been shown to encode relative reward value, as well as prediction errors (Ottenheimer et al., 2018, 2020a, 2020a), we next aimed to further dissect the nature of the VP GABA neuron calcium activity we observed in response to the port entry and initial lick epochs. First, we introduced a 1 second delay between port entry and reward delivery to further separate the timing of reward-seeking and initial reward consumption. We also included probe trials where the reward-predictive cue was presented but reward delivery did not occur after port entry (Fig. 5A). This allowed us to determine whether calcium activity is elevated post port entry even in the absence of reward, thus allowing us to see if the port entry responses are related to reward consumption or evaluation. Rats were tested for 2 days in sessions in which 50% of DS trials were probe trials, to prevent behavioral extinction. Reward-seeking behavior persisted through both probe days, where the DS port entry probability remained above our response criteria (port entry behavior during at least 70% of DS trials; Fig. 5B). Average VP GABA calcium traces during trials where reward was delivered at a 1 second delay had two distinct peaks, suggesting that the port entry response was delayed somewhat to match the new reward timing (Fig. 5C). Interestingly, this VP GABA response was present even on probe trials, where no reward was delivered. Comparison of the AUC of VP GABA response between the rewarded trials and probe trials revealed no significant difference between the two signals (Fig. 5D; rewarded: 5.38 +/- 0.92, probe: 6.38+/-0.73; $F(1,33.99)=0.10$, $p=0.75$; Bayes factor of 4.17 in favor of the null) Therefore, VP GABA neurons still

respond to the cue and at the time that reward delivery is expected in the absence of reward. This suggests that VP GABA calcium activity responses after the port entry may be more related to reward anticipation or expectation, than to actual reward consumption or evaluation.

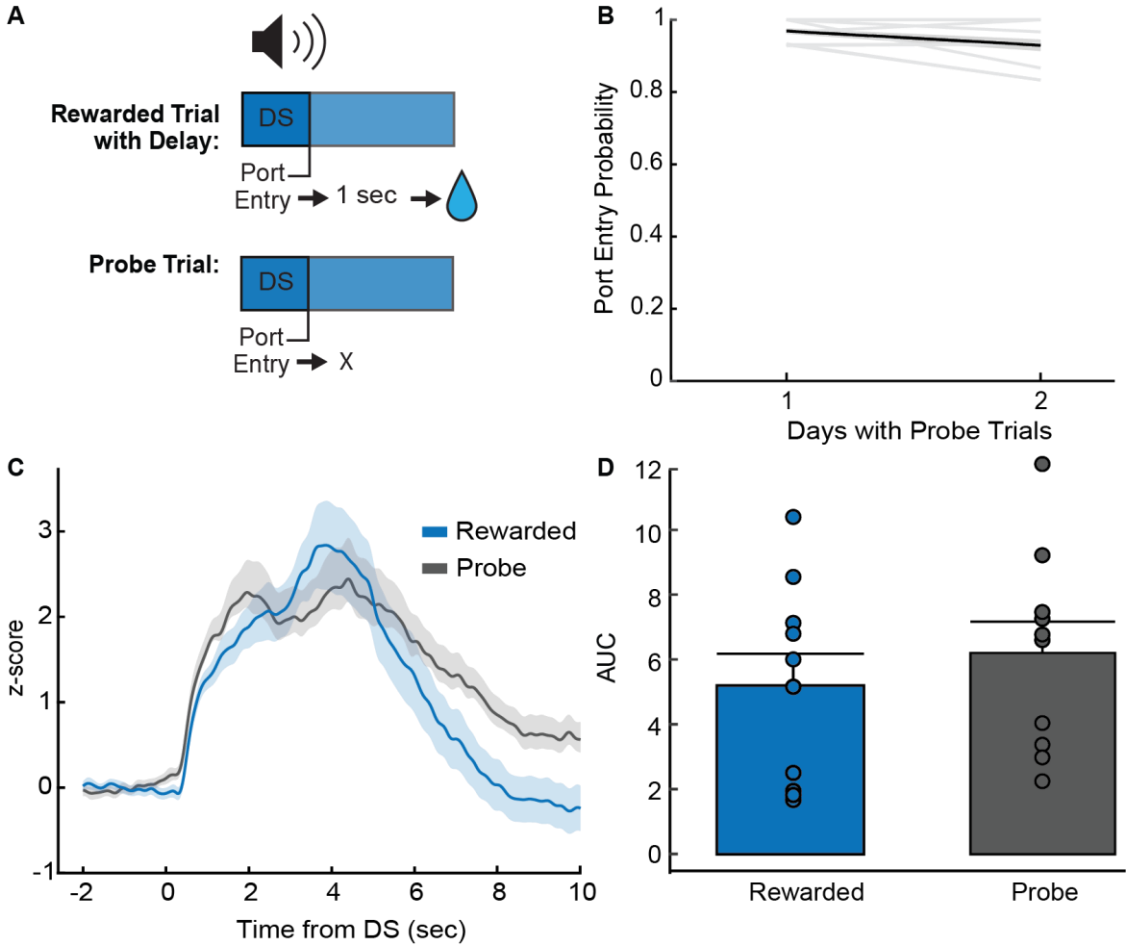

Figure 5*. Effects of reward delivery and omission on DS calcium activity*. (A) Diagram of delayed reward DS trials and probe DS trials. (B) Average DS port entry probability (n=10 rats) across delayed reward and probe trial sessions (2 days). Behavior remains above criteria of 0.70. (C) Average Z-score peri-DS calcium traces of delayed reward trials and probe trials averaged across animals (n=10) for both days (blue=delayed reward trials, black=probe trials, +/- SEM). (D) Average AUC of post-DS calcium traces (0-5 seconds) for delayed reward (blue) and probe trials (black) across animals (n=10; +/- SEM). We

observed no significant difference (sucrose: 5.38 +/- 0.92, probe: 6.38+/-0.73; p = 0.78; Bayes factor of 4.17 in favor of the null). Mean + SEM. Dots indicate individual subjects.

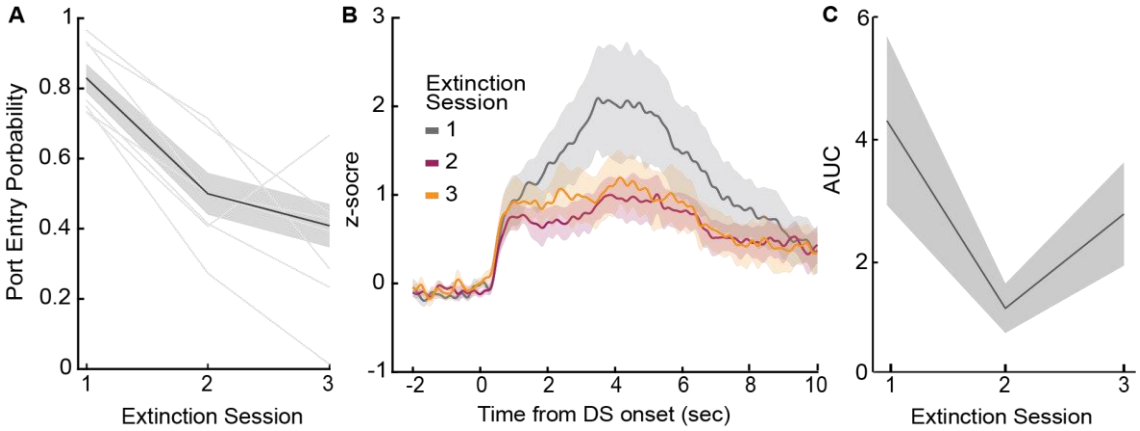

Figure 6. *DS calcium activity during extinction learning*. (A) Average DS port entry probability across animals (n=8) across extinction sessions (main effect of day: F(2,17)=16.85, p<0.002). (B) Average VP GABA calcium traces across animals (n=8) for each day of training (+/- SEM). (C) Average AUC of post-DS calcium traces (0-5 seconds) across animals (n=8) for each session of extinction training (main effect of day: F(2,18)=2.77, p=0.11). Lines with shading indicate mean +/- SEM. Lines alone indicate individual subjects.

2.4.7 VP GABA neuronal response decreases with extinction of learned behavior

Finally, we examined how consistent omission of reward affected reward-seeking behavior and VP GABA calcium activity. Rats received extinction training for 3 days immediately after probe trial days, where the DS was played but no reward was delivered after port entry. The DS port entry probability of the rats decreased significantly across extinction training days (Fig. 6A; main effect of day: F(2,17)=16.85, p<0.002). VP GABA calcium activity followed a similar

pattern, where higher calcium activity in response to DS cue is seen on day 1 of extinction compared to day 2 and 3 of extinction (Fig. 6B). Analysis of the AUC of the traces found a trend toward a significant decrease across extinction days (Fig. 6C; main effect of day effect: $F(2,18)=2.77$, $p=0.10$) and planned follow-up comparisons reveal that VP GABA activity on day 2 of extinction training was significantly reduced relative to day 1 (Fig. 6D; $p<0.05$). This indicates that VP GABA calcium activity is sensitive to extinction learning and changes in the learned value of cues and cue-elicited reward-seeking. Interestingly, both DS-elicited behavior and calcium activity remained above zero, potentially indicating some persistent motivational value of the cue, even after 3 days of extinction.

2.5 Discussion

Here we assessed population-level calcium activity in VP GABA neurons while rats learned and performed a cue-elicited reward-seeking task. As rats learned the task, calcium responses developed to the reward-predictive cue but not the neutral cue. An encoding model revealed encoding of the reward-predictive cue, as well as the operant behavior and initial reward consumption. Calcium activity evoked by the reward-predictive cue also predicted the latency of reward-seeking behavior. Calcium responses to the cues and operant behavior persisted even on trials with reward omission. However, when reward was completely omitted from all trials, rats' behavior, and calcium responses to the reward-predictive cue, decreased. Therefore, VP GABA neurons encode both reward-seeking behavior and the motivational value of reward-predictive cues.

2.5.1 Ventral pallidal GABA neurons develop calcium responses to cues across learning

Consistent with our overall hypothesis we observed increased calcium activity in VP GABA neurons in response to a cue predicting reward availability, relative to a neutral control cue. Increased firing by these neurons in response to a reward-paired cue has previously been observed in a Pavlovian paradigm (Stephenson-Jones et al., 2020). Specifically, spiking activity of optogenetically-identified VP GABA neurons is elevated in response to a cue predicting water reward in thirsty mice. Additionally, cue-evoked excitatory responses are blunted as mice become satiated on water. These findings suggest that VP GABA neurons may encode the motivational value of cues, but it remained unclear whether this would extend to instrumental reward-seeking in freely moving rats, or to trial-by-trial variations in motivated behavior that are independent of gradual changes in the learned value of the cue due to satiation. Additionally, we wanted to assess the development of these responses across training.

Previous work using *in vivo* electrophysiology to record the activity of VP units in a non-cell-type specific manner found excitatory responses to rewards and reward-related cues in both Pavlovian and instrumental paradigms (Tindell et al., 2004, 2005; Smith et al., 2009, 2011; Ahrens et al., 2016; Richard et al., 2016; Ottenheimer et al., 2018, 2018, 2020a). In a Pavlovian paradigm, the proportion of VP neurons that are excited by a reward-paired cue increases from early to late training as behavioral responses to the cue develop (Tindell et al., 2004). To our knowledge, the development of VP cue responses over time has

not previously been reported in an instrumental setting with DS cues. Here DS-evoked calcium activity increases as rats learn the task. Our population level measurements make it unclear whether this finding is due to rate or population level cue encoding, but it is consistent with VP encoding of the learned value of cues in an instrumental task.

Importantly, the observed peri-cue calcium response persisted for multiple seconds after cue onset. The kinetics of even the relatively fast GCaMP6f calcium sensor used here limit our ability to separate out responses to individual behavioral events that occur in close succession relative to *in vivo* electrophysiology (Wei et al., 2020). This is especially true when assessing responses averaged across trials. To more accurately isolate responses to specific behavioral events, and to determine whether peri-DS calcium activity is attributable to the DS specifically, we used linear regression to evaluate how associated each behavioral epoch was with the calcium signal at each timepoint of the task (Parker et al., 2016). With this encoding model, we demonstrate that VP GABA calcium activity encodes the reward-predictive cue. This is consistent with previous work showing increases in VP spiking activity immediately after cue onset in instrumental and Pavlovian paradigms (Tindell et al., 2004; Richard et al., 2016; Ottenheimer et al., 2018; Richard et al., 2018a; Ottenheimer et al., 2020a; Stephenson-Jones et al., 2020).

2.5.2 Encoding of reward-seeking latency by VP GABA calcium activity

We also demonstrate that while pre-cue VP GABA calcium activity is positively correlated with trial-by-trial latency, this relationship switches to a negative correlation around the time of DS-evoked calcium activity. We observed this relationship even after excluding calcium activity after port entry. This negative correlation suggests that greater DS-evoked VP GABA calcium activity predicts reward-seeking latency or vigor. Prior work using *in vivo* electrophysiology has also reported a negative correlation between DS-evoked activity and reward-seeking latency (Richard et al., 2016, 2018a), as well as a positive relationship with other behavioral measures of cue-evoked motivation, including the speed of the upcoming approach movement and the animal's proximity to the movement target at cue onset (Lederman et al., 2021). Approximately 20-25% of neurons are reported to have significant negative correlations between post-cue firing and reward-seeking latency; our findings suggest that at least some of these neurons are GABAergic. The positive correlation between pre-cue (and early post-cue) VP GABA activity and trial-by-trial latency reported here was unexpected, as it has not been reported in prior work using electrophysiology, at least on average across VP single units. Whether this correlation is related to our cell-type specific approach, or to calcium activity specifically is an open question.

2.5.3 Elevation of calcium activity following port entry and during initial reward consumption

The encoding model revealed that VP GABA neuron calcium activity is also evoked by the operant behavior (port entry) required to obtain the sucrose reward, and by initial reward consumption (initial lick). This is consistent with prior work (Richard et al., 2016) showing that VP single units are excited at both the time of an operant response (lever press) and at entry into the reward port. In the Stage 5 version of the DS task, it is difficult to tell whether port entry-evoked calcium activity is related to the action itself, or to reward expectation or evaluation, as the timing of the action (port entry) and reward delivery are confounded. To address this, we added a delay between port entry and reward delivery as well as DS probe trials where no reward was delivered following port entry. When the timing of reward delivery after port entry is delayed, the timing of the calcium activity shifts to match. This suggests that it is not an action related response, but either reward expectation or evaluation. When we separated reward trials from probe trials, we observed very similar calcium responses in neurons no matter the trial type, suggesting that the calcium response is more related to expectation than reward evaluation or hedonic impact.

Finally, the encoding model showed that part of the calcium response was associated with the first lick after rewarded port entry (i.e. the beginning of sucrose consumption). This was expected, as previous work has found that VP GABA neurons respond to rewards (Stephenson-Jones et al., 2020) and have been implicated in consumption of palatable reward in sated animals (Farrell et

al., 2021). However, the same VP GABA activity was also observed on probe trials. This differs from previous work showing that a subset of VP neurons are sensitive to reward identity and encode reward value (Ottenheimer et al., 2020a; Stephenson-Jones et al., 2020).

2.5.4 Caveats and Future Directions

Here, we used population-level calcium imaging to examine encoding of behavioral events. While the time scale of neuronal activation is captured more accurately with electrophysiological recordings, cell-type specific recording is easier to achieve with calcium imaging approaches, as is measurement of activity from neurons with low basal firing rates. We found that VP GABA calcium activity increases in response to a reward-predictive cue and at the time of expected reward consumption. Unexpectedly, we found that the calcium response at the time of expected reward delivery was insensitive to reward omission. This is surprising due to the finding that VP neurons are sensitive to the relative reward value and can encode reward prediction errors (Ottenheimer et al., 2020a). This discrepancy could be due in part to the limitations of bulk calcium imaging. Recent work suggests that fiber photometry recordings of calcium activity may be more indicative of non-somatic activity, representing inputs to neurons as opposed to outputs of the neurons (Legaria et al., 2022). We may be capturing dendritic calcium changes, which may not reflect the same electrophysiological responses that encode relative reward value or reward

prediction error signals. Future work could examine soma-specific calcium activity in the DS task using soma-targeted viral expression and/or miniscope imaging (Aharoni and Hoogland, 2019; Chen et al., 2020; Shemesh et al., 2020). Our finding that calcium activity persists even when reward is omitted could also be due to heterogeneity in the response patterns of VP GABA neurons. Importantly, signals encoding relative reward value and reward-prediction error-like signals have just been observed in subsets of VP neurons (Ottenheimer et al., 2018, 2020a, 2020b). VP GABA neurons may differ in their encoding and functional contributions based on more specific cell-type subdivisions or output pathways. For instance, some work points to parvalbumin neurons being a subset of VP GABA neurons involved in cue-evoked behaviors (Prasad et al., 2020a), whereas PAS 1-positive VP GABA neurons may contribute to stress and anxiety-related behaviors (Morais-Silva et al., 2022). Additionally, VP GABA neurons have many projection targets that have varied roles in appetitive behavior (Covelo et al., 2014; Mahler et al., 2014; Leung and Balleine, 2015; Root et al., 2015; Prasad and McNally, 2016; Farrell et al., 2021; Vachez et al., 2021; Morais-Silva et al., 2022). Probing a more specific subset of VP GABA cells (cell-type or projection-specific) may reveal greater, or lack of, reward-predictive cue encoding and latency correlations.

2.6 Conclusions

Ventral pallidal (VP) circuitry is a major driver of cue-evoked behaviors. Previous work has found that VP neurons have heterogenous responses and

contributions to reward-seeking behavior. This functional heterogeneity is due to differences of neurochemical subtypes and projections of VP neurons. Understanding the heterogenous responses amongst, and within, VP neuronal cell types is a necessary step in further understanding how cue-evoked behavior becomes maladaptive. Our work explores the canonical GABAergic VP neuron, and how the calcium activity of these cells encodes components of cue-evoked reward-seeking, including the vigor and persistence of reward-seeking. Here, we used an intersectional viral approach with fiber photometry to record bulk calcium activity in VP GABAergic (VP GABA) neurons in male and female rats as they learned and performed the DS task. We found that VP GABA neurons are excited by reward-predictive cues but not neutral cues, and that this response develops over time. We also found that this cue-evoked response predicts reward-seeking behavior. Additionally, we found increased VP GABA calcium activity at the time of expected reward delivery, which occurred even on trials when reward was omitted. Together, these findings suggest that VP GABA neurons encode reward expectation and calcium activity in these neurons is predictive of the vigor of cue-elicited reward-seeking.

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Chapter 3: The Contribution of Ventral pallidal GABAergic neurons to consumption of chow and sucrose

(In Preparation)

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3.1 Chapter Summary

Cues are powerful modulators of motivated behaviors, including food intake. Eating behavior is impacted by environmental cues that invigorate reward-seeking. Neural circuits integrate these cues with peripheral homeostatic signals to initiate consumption. However, excessive cue reactivity can lead to eating beyond the caloric need for energy homeostasis. Determining the neural circuitry responsible for non-homeostatic eating and how this circuitry responds to environmental reward signals is an important area of focus in obesity and motivation research. The ventral pallidum (VP) is a brain region implicated in controlling both cue-elicited reward seeking and consumption of rewards. The predominate VP cell type is gamma-Aminobutyric acidergic (GABA) neurons. These neurons are excited by rewards and reward-related cues and inhibition of

VP GABA neurons can decrease consumption of chow or highly palatable reward in hunger rats. However, VP GABA neuronal contribution to consumption of chow or sucrose in sated rats remains unknown. Here, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to activate or inhibit VP GABA neurons in sated male and female rats during chow and sucrose consumption tasks. We found that activation of VP GABA neurons increases consumption of chow and sucrose in sex specific manner. We also found that inhibition of VP GABA neurons trends toward decreasing sucrose consumption in rats. Together, these findings suggest that activation of VP GABA neurons can stimulate consumption of routine or highly palatable rewards and that sex impacts this effect.

3.2 Introduction

Food intake involves multiple converging signals: the primary attributes of food (i.e. taste and smell), environmental cues that attract individuals to the food, and hormonal signals that provide information about energy homeostasis. Neural circuits integrate these cues and signals to initiate food-seeking and consumption (Morton et al., 2014). However, excessive cue reactivity can lead to eating beyond the caloric need for energy homeostasis. Environmental cues paired with food (i.e. packaging and food imagery) elicit strong food cravings and encourage individuals to overeat (Jansen et al., 2003). Food cue reactivity can predict both food intake and weight gain (Boswell and Kober, 2016). Excessive weight gain is

a major risk factor for metabolic diseases as well as obesity. Identifying the neural circuitry responsible for overeating and how this circuitry integrates environmental and internal signals is an important area of focus in motivation and obesity research.

The ventral pallidum (VP) is a brain region implicated in controlling both consumption of highly palatable rewards, routine consumption, and cue-elicited reward seeking. Disinhibition of the VP increases food intake in rats (Stratford and Kelley, 1999; Stratford and Wirtshafter, 2013), especially of food high in fat (Covelo et al., 2014). The VP is also a major region responsible for the hedonic impact of rewards; extensive cell death in VP reduces hedonic “liking” reactions in rodents (Ho & Berridge, 2014). Additionally, innate differences in VP neuronal activity may be a predictor of weight gain (Gendelis et al., 2020). The VP is also an essential node in the neural circuitry that underlies motivation to food/reward-seek. VP neurons are known to respond to cues predictive of reward and these neuronal responses encode the motivational value of said cues (Richard et al., 2016, 2018). Recent research has begun to probe which VP neuronal cell-types contribute to consumption and reward seeking. The VP contains 3 major neuronal subtypes: cholinergic, glutamatergic, and gamma-Aminobutyric acidergic (GABA). VP glutamatergic neurons are excited by aversive stimuli and constrain reward seeking (Tooley et al., 2018). In contrast, many VP GABA neurons are excited by rewards and Pavlovian reward-paired cues (Stephenson-Jones et al., 2019). Additionally, as described in Chapter 2, VP GABA neurons also encode reward-predictive cues, actions, and initial consumption of reward.

Some recent work has shown mixed effects VP GABA manipulations on food-seeking and consumption. Chemogenetic inhibition of VP GABA neurons decreases food-seeking (i.e. operant responding) of chow and palatable food (banana pellets) in food-restricted rats (Farrell et al., 2021), whereas inhibition of VP GABA neuronsin sated rats reduces palatable food-seeking but not chow-seeking behavior. In contrast, inhibition of VP GABA neurons failed to significantly reduce non-operant consumption of M&Ms (Farrell et al., 2021). Prior work also found that activation of VP GABA neurons does not increase consumption of chow in food-restricted mice (Li et al., 2020). Together, these findings might suggest that VP GABA neurons control food-seeking but not consumption, which would be surprising considering the prior work discussed above (Cromwell and Berridge, 1993; Stratford and Wirtshafter, 2013; Covelo et al., 2014). Yet, the internal state of animals in known to impact VP GABA responses to reward and reward-seeking (Stephenson-Jones et al., 2020; Farrell et al., 2021, 2022). Additionally, VP manipulations have been shown to have differential influence over different macronutrients (Covelo et al., 2014). Overall, it is still unclear how manipulation of VP GABA neurons in sated animals affects consumption of home chow or rewards of single macronutrient origin. Additionally, the prior work has not disaggregated testing subjects by sex.

Here we used chemogenetic methods, with a highly specific ligand (Bonaventura et al., 2019), to test whether VP GABA activation or inhibition effects consumption of chow or 10% liquid sucrose in sated rats. We found that inhibition of VP GABA neurons does not significantly change either home chow or sucrose

intake in sated rats, however rats did tend to consume less sucrose when VP GABA neurons were inhibited. We also found that VP GABA activation increases both chow and sucrose intake in a sex specific manner. These results suggest that VP GABA neurons modulate consumption of home chow and sucrose in sated rats. Our work also indicates that sex, internal state, and macronutrient make-up of rewards are important variables to consider when assessing VP GABA neuronal contribution to consumption.

3.3 Materials and Methods

3.3.1 Subjects

Male and female Long Evans rats (n = 62, 32 male, 30 female; Envigo), weighing 250–275 grams at arrival, were individually housed in a temperature- and humidity-controlled colony room on a 12 hr light/dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Minnesota and were carried out in accordance with the guidelines on animal care and use of the National Institutes of Health of the United States.

3.3.2 Surgeries

During surgery, rats were anesthetized with isoflurane (5%) and placed in a stereotaxic apparatus, after which surgical anesthesia was maintained with isoflurane (0.5–2.0%). Rats received preoperative injections of carprofen (5

mg/kg) for analgesia and cefazolin (75 mg/kg) to prevent infection. Syringes for viral delivery were aimed at the ventral pallidum (VP) using the following coordinates in comparison to bregma: +0.3 mm AP, +/- 2.3 mm ML, - 8.3 mm DV. To achieve cell-type specific expression of designer receptors exclusively by designer drugs (DREADDs) 0.60-0.80 uL of a 1:1 mixture of AAV8-GAD1-cre (8.29x10^13; University of Minnesota Viral Vector Core) and DREADD virus was injected bilaterally into VP. Gq DREADD virus (n=23, 12M, 11F: AAV8-hSyn-DIO-hM3Dq-mCherry, ≥ 4×10^{12} vg/mL, Addgene; Watertown, MA, USA) was mixed with GAD1-cre to express excitatory designer receptors in VP GABA neurons. Gi DREADD virus (n=25,13M, 12F: AAV8-hSyn-DIO-hM4Di-mCherry ,≥ 1×10^{13} vg/mL, Addgene; Watertown, MA, USA) was mixed with GAD1-cre to express inhibitory designer receptors in VP GABA neurons. For external controls (n=14, 7M, 7F) 0.6-0.8 uL of a 1:1 mixture of GAD1-cre and mCherry virus (AAV_8-hSyn-DIO-mCherry, ≥ 1×10^{13} vg/mL; Addgene, Watertown, MA, USA) was injected bilaterally into VP. A subset of rats (n=6, 3M, 3F) also received an infusion of AAV9-Syn-Flex-GCaMP6f and fiber implant (.48 NA, 400 micron,9mm; Doric) unilaterally into VP, along with bilateral Gi (n=2, 1M, 1F), Gq (n=2, 1M, 1F) or mCherry (n=2, 1M, 1F) viruses. Implants were secured to the skull with bone screws and dental acrylic. The DREADD viruses did not express on the side where the calcium indicator and fiber were placed (n=5). Therefore, these animals were excluded from analysis for lack of bilateral DREADD expression. Virus was delivered to VP through 28-gauge injectors at a rate of 0.1 µl per min. Injectors were left in place for 10 min following the infusion to allow virus to

diffuse away from the infusion site. Following viral injections, rats used for behavioral testing received fiber implants in VP for optical measurement of calcium activity. Rats recovered for at least one week before beginning handling. Rats recovered for at least 4 weeks before training to allow time for sufficient viral expression.

3.3.3 DREADD Ligand and Administration

Injections of DREADD ligand were administered on test days through intraperitoneal (IP) injection. Novel DREADD ligand, JHU37160 (JHU: HB6261, Hello Bio, Princeton, NJ, USA; Bonaventura et al., 2019) was dissolved in saline and diluted to a concentration of 0.05 mg/kg and 0.50 mg/kg. Ligands were injected at 1 mL/kg of volume to body weight. Saline vehicle injections were drawn directly from the solution used to dissolve the ligand and were also delivered IP.

3.3.4 Behavioral Testing

3.3.4.1 Chow Consumption Task

Rats were habituated to the testing environment 3 days before DREADD ligand or saline injection test days. The testing environment consisted of individually housed cages with corn cob pellet bedding and water. On test days, chow was pre-measured at 20 grams for each subject. After IP injection, rats were

immediately placed in test cages. One group (n=28) had the chow placed into the cage at the time of injection, meaning these animals had an hour and a half for consumption. Another group (n=34) received chow 30 minutes post-injection, meaning that these animals had an hour for consumption. The final amount of chow was recorded, and the amount consumed was calculated. Observation days followed each test day to ensure that DREADD ligand did not have any lingering consequences on behavior. Injection order was counterbalanced across animals. All testing was recorded for later video behavior analysis.

3.3.4.2 Sucrose Consumption Task

Sucrose Consumption Testing

10% sucrose was initially provided to subjects *ad libitum* overnight prior to the start of the experiment to ensure that any changes in consumption were not due to the novelty of the sucrose. Rats were then habituated to the testing environment 3 days before DREADD ligand or saline injection test days. The testing environment consisted of individually housed cages with corn cob pellet bedding and a two-bottle top with water and sucrose bottles. Bottle placement was balanced across subjects. Additionally, Arduino lickometers using a capacitance sensor (MPR121, Adafruit Industries, NY) were attached to the top of the cage and recorded the number of licks for sucrose and water bottles on the corresponding cage. On test days, water and sucrose bottles were weighed before the task. After IP injection was given, rats were immediately placed in the

testing chambers. One group (n=15) received water and sucrose availability at the time of injection, meaning that the animals had access to water and sucrose for an hour and a half. Another group (n=22) received access to water and sucrose 30 minutes post-injection, meaning the animals had access to water and sucrose for an hour. The amount of water and sucrose was recorded after 90 minutes, and the amount consumed was calculated. Observation days followed each test day to ensure that the DREADD ligand did not have any lasting impacts on behavior. Injection order was counterbalanced across animals. All testing was recorded for later video behavior analysis. Sucrose preference was calculated and the difference between the amount of sucrose consumed and the amount of water consumed, divided by the total amount of liquid consumed.

3.3.4.3 Discriminative Stimulus Task

Four to six weeks after surgery, a subset of rats (n=28) were trained in a discriminative stimulus (DS) task (Ottenheimer et al., 2018; Richard et al., 2018; Gómez-A et al., 2022). The DS task is an instrumental task where rats learn to discriminate between reward-predictive (DS) and neutral cues (NS). Auditory cues consisted of a siren or white noise, with cue assignments counterbalanced across subjects. Rats received reward if they entered the port during the DS cue but not the NS cue. Over time rats learn to discriminate between the cues, responding almost exclusively to the DS.

3.3.5 Histology

3.3.5.1 Region specific DREADD expression validation

To confirm that DREADD virus was expressed in VP, 40um sections were imaged on a wide field fluorescent microscope (Olympus MVX10). We verified the location of DREADD expression in all animals (n=62) based on anatomical markers of VP (anterior commissure, lateral ventricle size). 7 animals were excluded for lack of DREADD expression. To quantify the regional selectivity of DREADD virus expression we further examined tissue from the VP as well as the regions anterior to (Bregma 2.28-0.72)the VP and posterior to the VP,(Bregma -0.72 to -1.72 of representative rats (n=8; n=4, 2M, 2F of 0.60uL viral injections; n=4, 2M, 2F of 0.80uL viral injections). For each rat was assessed 3 representative sections throughout each brain region to assess the viral spread throughout the brain. All images on all sections were acquired with the same acquisition settings. The QUINT workflow (EBRAINS) was then used to assess the 3D spread through the brain regions. Briefly, the 2D images of each section, from each animal were matched to atlas (Waxholm Space Atlas v4) segmentations in the QuickNII software. Then, masks of mCherry expression, of each section/animal, were made in the iLatsik software, with labels differentiating between background and expression. Finally, quantification of regional expression was calculated with atlas segmentations and masks of sections in the Nutil software. The final output contained all atlas brain regions in our sections and the amount of mCherry expression (pixel quantification) in each region.

3.3.5.2 DREADD Functional Validation

Representative rats from each DREADD group (for Gi, Gq, mCherry, M and F) were administered either 0.05mg/kg JHU, 0.50mg/kg JHU, or saline solution 1 hour prior to perfusion. For perfusion, rats were deeply anesthetized with pentobarbital and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed, post-fixed in 4% paraformaldehyde for 4–24 hr, cryoprotected in 30% sucrose for >48 hr, and sectioned at 40 um on a microtome. 40um brain sections were run through a cfos immunohistochemistry protocol. Briefly, sections were washed in PBS, blocked in Normal Donkey Serum, and incubated in the cfos primary antibody (1:2500 rabbit anti-cFos, Cell Signaling Technology,5348S) overnight at room temperature. After primary antibody incubation, sections were washed in PBS, and then incubated in the fluorescent conjugated secondary antibody (1:250 Alexa 488 Donkey anti-rabbit (Invitrogen # A-21206)) for 2 hours at room temperature. After secondary antibody incubation, sections were washed in PBS, wet-mounted on coated glass slides in PBS, air-dried, coverslipped with Vectashield mounting medium with DAPI. Slides were stored at 4 degrees Celsius until imaged. Imaging of the tissue was done on a confocal microscope (Nikon upright c2, University Imaging Centers at UMN) at 20x with 2x digital zoom, and settings were optimized with the help of the imaging core. The exact same acquisition settings were used on all tissue samples. HALO software (Indica Labs) quantified the amount of DREADD expressing cells (mCherry positive cells) that colocalized with the early activation gene, cfos. Briefly, total cell number and location within an image was

determined by nuclear staining (DAPI). mCherry and cfos were assigned their respective fluorophores within the software and the software compared the location of each fluorophore to the location of the DAPI labeled cells to confirm fluorophore expression is associated with a self-defined nuclei and cell size. HALO analysis software and software settings were optimized for the tissue from all animals and then settings were used to batch analyze cell counts of mCherry and cfos positive cells.

3.3.6 Statistical Analysis

Behavioral Analysis

Statistical significance was measured by fitting a linear mixed model to examine the effects of the designer drugs within DREADD groups (i.e., Gq, Gi, or mCherry), with fixed effects for sex and injection type and random effect of rat. Follow-up comparisons with Sidak corrections were done when LME effects were found to be significant.

Region-specific DREADD expression validation

Statistical significance was measured by fitting a linear mixed model to examine the effects of injection volume on viral spread and expression of DREADD virus. LME's were fit for fixed effects for sex, volume and brain region, and random effect of rat. Follow-up comparisons with Sidak corrections were done when LME effects were found to be significant.

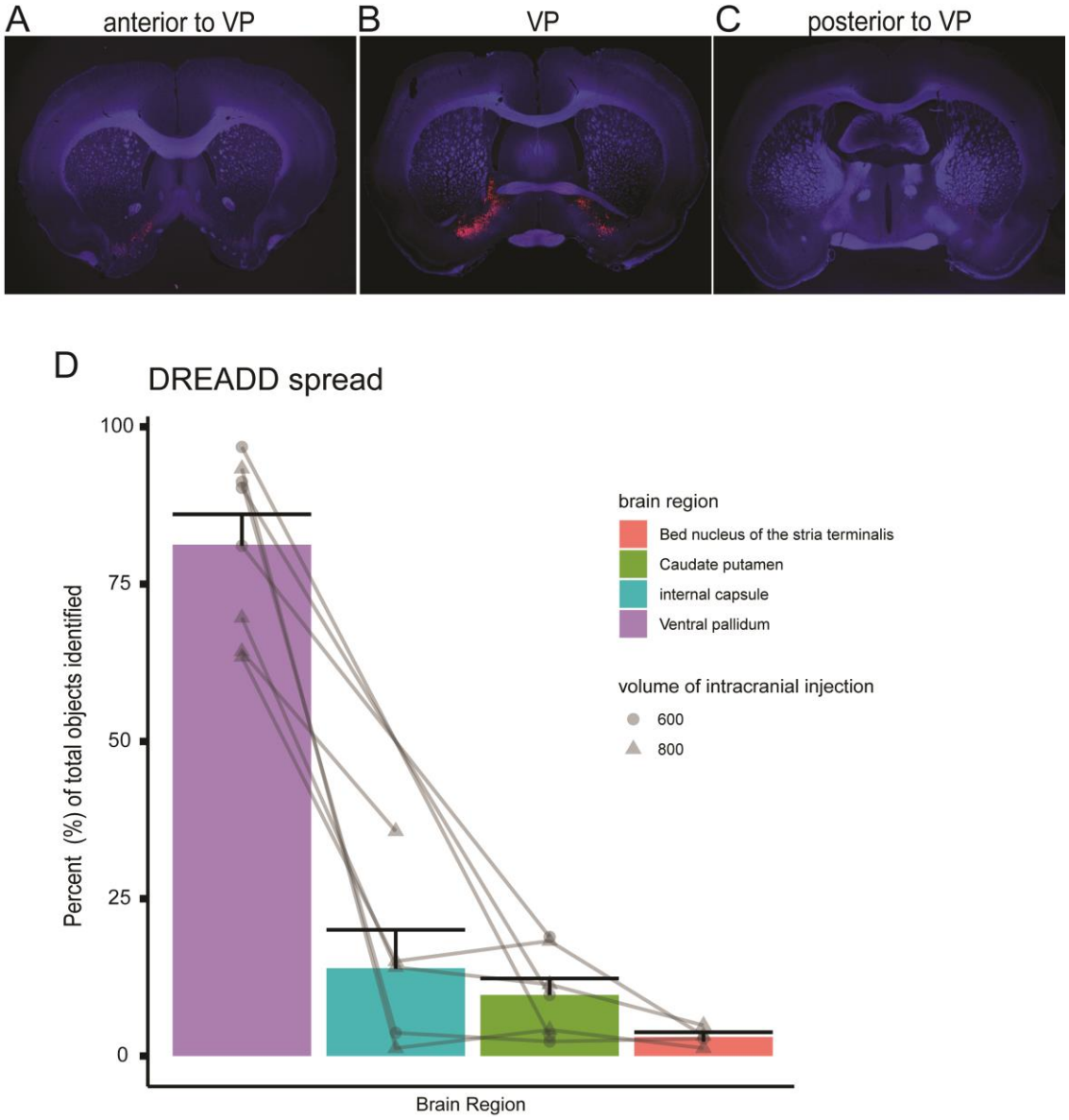

Figure 1. *DREADD virus is primarily expressed in VP.* (A) Representative section from striatum, (B) ventral pallidum (VP), (C) septum, used for analysis in QUINT workflow. (D) Output results of QUINT workflow, showing all brain regions where mCherry (DREADD virus reporter) was detected. The VP atlas region was where 72.72% +/- 9.65 of objects (proxy for cell count) were detected by analysis software. LME showed and significant effect of brain region $F(10,7)=6.34$, $p=.01$, on spread of virus, which follow-up comparisons show that VP was significantly greater in expression compared to other brain regions that were found to have expression in multiple animals (corticofugal tract and corona radiata 17.15% +/- 9.93, caudate putamen 7.66% +/- 5.25, bed nucleus of the stria terminalis 2.20% +/- 0.97), *$p<0.005$.

3.4 Results

3.4.1 Inactivation of VP GABA neurons has no significant effect on chow or sucrose consumption

Chow Consumption

Gi rats (n=22: 13M, 9F) were habituated to chow and sucrose testing chambers before each respective consumption tasks to control for any effect on movement or consumption due to novelty of the testing chamber. After 3 days of habituation in the same testing environment, test days with ligand or vehicle IP injections were done. Novel DREADD ligand, JHU37160 dihydrochloride, was given at 0.50 mg/kg (high) and 0.05 mg/kg (low) doses to examine if there was a dose dependent effect on consumption. Days of the high dose, low dose and saline (vehicle) injections were counterbalanced across rats to control for any lingering effects of ligand. Additionally, rats were given at least 48 hours after each test day to allow ligand to leave the bloodstream completely and for recovery of any stress the injection/ restraint may have caused on the first day of testing. For chow experiments, 20 grams of home chow (Teklad Global 18% Protein Rodent Diet, pellets, 2818, Envigo) was pre-weighted before the start of any injections. Injections were pulled into syringes and labeled before any injections were given to allow for the least amount of time between each animal injection. Before placing each animal in the testing chambers, animals were given ligand or vehicle injections IP. Animals were placed directly into the testing chamber after injection and given chow 0-30 mins after being placed in the chamber. All animals spent 90 minutes in the testing chamber, to allow for an hour of testing

when the ligand had reached the central nervous system (Bonaventura et al. 2019). Once all rats were removed from testing chambers, chow was collected and reweighed. The amount of chow consumed was determined by the difference between 20 grams and the amount of grams of chow left. On average, Gi rats consumed 2.55+/- 0.44 grams of chow on saline test days, 2.13 +/- 0.41 grams on low ligand test days, and 2.59 +/- 0.46 on high ligand test days. No significant effect of injection ($F(2,32.75) = 0.64$, $p = 0.53$) or sex ($F(1,19.07) = 0.004$, $p = 0.95$),or sex*injection interaction ($F(2,32.75) = 0.57$, $p = 0.57$) was observed in a linear mixed effect model.

Sucrose Consumption

The same protocol was followed for ligand preparation and injections in sucrose consumption experiments. Rats were placed in the testing chamber immediately after injections and water and 10% sucrose bottles were flipped over 0-30 mins after injection to allow access to both solutions. Sucrose and water bottles were filled and weighed before placing the bottles on the cages. Once rats were out of testing chambers, bottles were reweighed, and the amount of sucrose and water consumed was determined by the difference between pre and post-test bottle weight. Preference for sucrose solution was also calculated based on the difference in amount of sucrose and water consumed. On average, Gi sucrose rats (n=11, 7M, 4F) consumed 14.05 +/- 1.62 grams of sucrose on saline test days, 11.44 +/- 1.12 grams on low ligand test days, and 12.04 +/- 1.51 on high ligand test days. No significant effect of injection ($F(2,18) = 2.60$, $p = 0.10$) or sex

(F(1,9) = 0.08, p = 0.78) or sex*injection interaction (F(2,18) = 1.39, p = 0.27) was observed in a linear mixed effect model. Similar to chow consumption, sucrose consumption was not significantly affected by VP GABA inhibition. However, there is a trend of decreased consumption on ligand injection days, which is consistent with a decrease in palatable food consumption in sated rats reported previously (Farrell et al. 2021).

Preference Consumption

Sucrose preference consumption was measured as the percentage of total liquid solution consumed (water and sucrose solutions) that was sucrose, during the sucrose consumption task. All rats were noted to have a baseline sucrose preference on habituation days. Gi sucrose rats (n=11, 7M, 4F) did not differ in their preference for sucrose across saline (89.86%+/- 2.23), low ligand (88.36% +/- 2.31), and high ligand (86.79% +/- 2.85) test days. No significant effect of injection (F (2,18) = 1.38, p = 0.28) or sex (F(1,9)=1.70, p = 0.23) or interaction of injection by sex (F(2,18) = 1.69, p = 0.21) was observed in a linear mixed effect model. Therefore, chemogenetic inhibition of VP GABA neurons showed no significant effect on sucrose preference.

Water Consumption

Water consumption was calculated as the difference in weight of the water bottle before and after each test day. On average Gi sucrose rats (n=11, 7M, 4F) did not change their water intake across saline (1.40 grams +/- 0.30), low dose (1.40 grams +/- 0.27), and high dose(1.55 grams +/- 0.31) test days. No significant effect of injection ($F(2,18) = 0.38$, $p = 0.69$), sex ($F(1,9) = 1.78$, $p = 0.21$), or injection by sex interaction ($F(2,18) = 0.28$, $p = 0.76$) was found in a linear mixed effect model. VP GABA inhibition had no significant effect on water consumption.

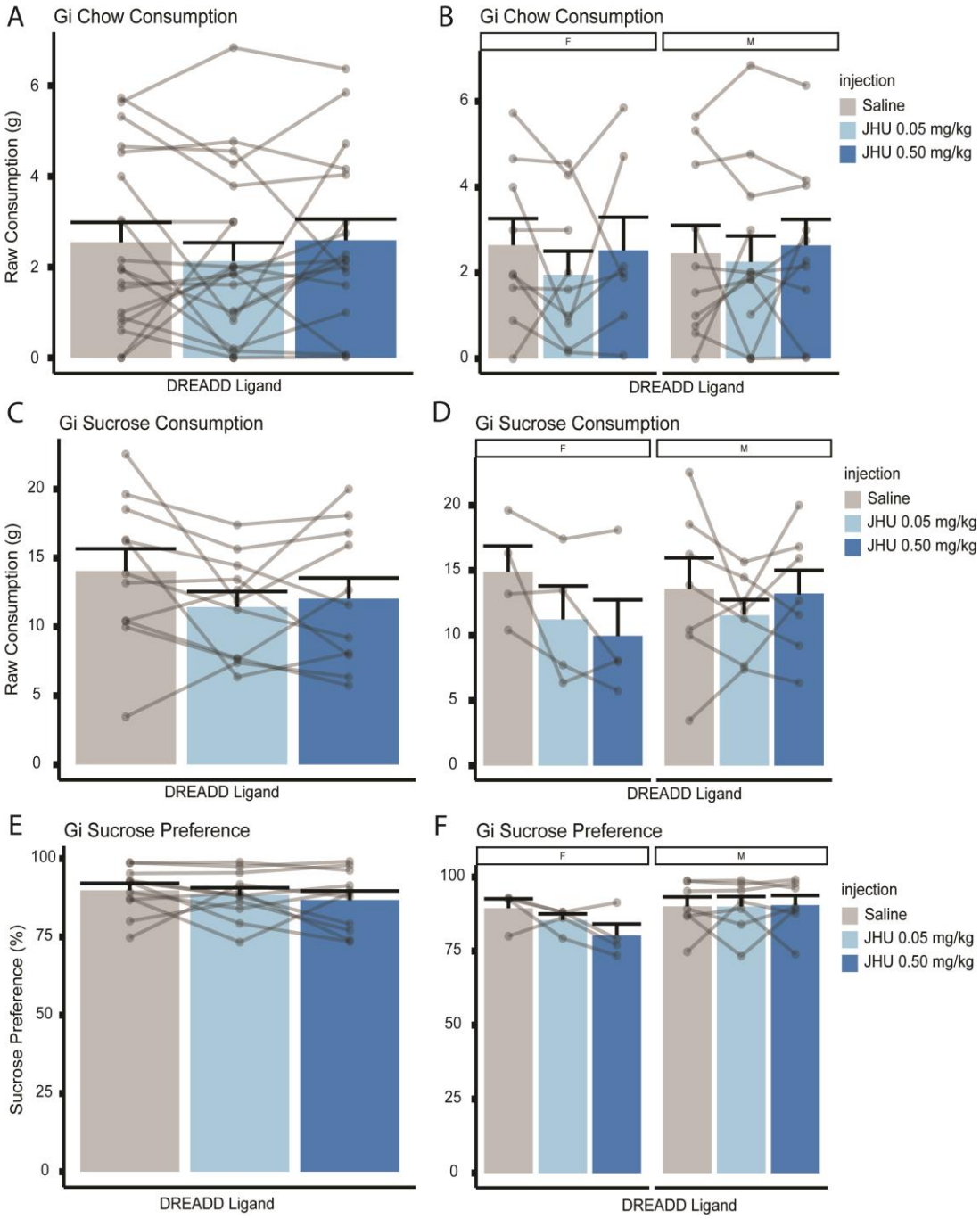

Figure 2*. VP GABA inhibition has no significant effects on consumption of chow, sucrose, and sucrose preference*. (A) Gi rat chow consumption when delivered control saline (gray; 2.55+/- 0.44 grams), low dose of DREADD ligand (light blue; 2.13 +/- 0.41 grams) and high dose of DREADD ligand (dark blue; and 2.59 +/- 0.46), via IP injections (B) Gi chow consumption split by sex: saline(M:2.46 +/- 0.46,F:2.65 +/- 0.62), low dose(M:2.26 +/- 0.60,F:1.96+/-0.55), high dose(M:2.64+/-0.61,F:2.53+/-0.77), LME shows no significant effect of injection (F

(2,32.75)=0.64, p=0.53) or sex (F(1,19.07)=0.004, p=0.95),or sex*injection interaction (F(2,32.75)=0.57, p=0.57). (C) Gi sucrose consumption: saline(14.05 +/- 1.62), low dose(11.44 +/- 1.12), high dose(12.04 +/- 1.51) (D)Gi sucrose consumption split by sex: saline(M:13.58+/-2.28,F:14.88+/- 1.99), low dose(M:11.56+/-1.19,F:11.23+/-2.57), high dose(M:13.22+/-1.78,F:9.97+/-2.76), LME shows no significant effect of injection (F (2,18)=2.60, p=0.10) or sex (F(1,9)=0.08, p=0.78) or sex*injection interaction (F(2,18)=1.39, p=0.27) (E) Gi sucrose preference: saline(89.86%+/-2.23), low dose(88.36%+/-2.32), high dose(86.79+/-2.85) (F) Gi sucrose preference split by sex: saline(M:90.08%+/-3.19,F:89.48%+/-3.15), low dose(M:90.02%+/-3.39,F:85.46%+/-2.10), high dose(M:90.53%+/-3.26,F:80.26%+/-3.88), LME shows no significant effect of injection (F (2,18)=1.38, p=0.28) or sex (F(1,9)=1.70, p=0.23) or interaction of injection by sex (F(2,18)=1.69, p=0.21). Data are represented as mean +/- SEM. Dots and lines represent individual rats.

3.4.2 Activation of VP GABA neurons causes a significant increase in chow and sucrose consumption/preference in a sex specific manner

Chow Consumption

Gq rats (n=21, 11M, 10F) tested on the chow consumption task were habituated and tested as described above. On average, Gq rats consumed 2.57 +/- 0.39 grams of chow on saline test days, 1.68 +/-0.36 grams on low ligand test days, and 3.69 +/- 0.63 on high ligand test days. A significant effect of injection ($F(2,34.75) = 7.76$, $p = 0.002$), sex ($F(1,18.65) = 0.08$, $p = 0.002$) and injection by sex interaction ($F(2,34.74) = 6.90$, $p = 0.003$) was observed in a linear mixed effect model. Follow-up comparisons showed that there was a significant difference between male saline injections and low ($p = 0.02$) and high dose ($p = .002$) of the ligand, as well as a significant difference between male ligand groups ($p < 0.0001$). However, female saline and ligand injections showed no significant differences. The saline ($p = 0.03$) and high dose ($p < 0.0001$) ligand groups

showed a significant sex difference in chow consumption. These results indicate that activation of VP GABA neurons with the novel DREADD ligand may decrease chow consumption in male rats at lower concentrations and increase chow consumption in male rats at higher concentrations, compared to vehicle controls. Additionally, there seems to be a sex difference where at the higher dose of ligand male chow consumption is increased compared to control and female chow high ligand consumption. Female rat chow consumption remains consistent across vehicle and ligand groups - further confirming there is a sex specific effect of ligand.

Sucrose Consumption

Gq rats (n=13, 7M, 6F) tested on the sucrose consumption task were habituated and tested as described above. On average, Gq rats consumed 13.88 +/-1.24 grams of chow on saline test days, 14.99 +/-1.21 grams on low ligand test days, and 17.21 +/-1.33 on high ligand test days. Similar to the results for chow consumption, significant effects of injection ($F(2,22) = 4.27$, $p = 0.027$) and sex ($F(1,11) = 9.60$, $p = 0.010$), and an injection by sex interaction ($F(2,22) = 4.40$, $p = 0.025$) were observed in a linear mixed effect model. Follow-up comparisons showed that there was a significant increase in sucrose between male saline injections and low ($p = 0.015$) and high dose ($p = 0.001$) of the ligand, as well as a significant increase in sucrose consumption between male ligand groups ($p < 0.0001$). However, female saline and ligand injections showed no significant differences. The saline ($p = 0.0013$) and high dose ($p = 0.006$) ligand conditions

had a significant sex difference between male and female rat's sucrose consumption. These results indicate that activation of VP GABA neurons with the novel DREADD ligand increases sucrose consumption in male rats at low and high concentrations, compared to vehicle controls. Additionally, there is a sex difference at each dose of ligand; male sucrose consumption is increased compared to female sucrose consumption. Female rat sucrose consumption remains consistent across vehicle and ligand groups- further confirming there is a sex specific effect of ligand. VP GABA activation elicits increased consumption of both chow and sucrose, suggesting that VP GABA neurons impact general consumption, not dependent on reward type, although this does not rule out differential effects of VP GABA activation of different rewards.

Preference Consumption

Sucrose preference consumption was measured as described above. All rats were noted to have a baseline sucrose preference on habituation days. Gq sucrose rats (n=13, 7M, 6F) did not differ in their preference for sucrose across saline (87.70% +/- 1.36), low ligand (87.95% +/- 1.19), and high ligand (88.95% +/- 1.20) test days. No significant effect of injection ($F(2,22) = 0.38$, $p=0.69$) or sex ($F(1,11) = 1.16$, $p = 0.31$) was observed in a linear mixed effect model. However, an injection by sex interaction ($F(2,22) = 5.88$, $p = 0.009$) was observed in the model. Follow up comparisons revealed low doses of ligand significantly ($p = 0.03$) decreased sucrose preference in female rats compared the vehicle controls, whereas both low and high doses of ligand significantly ($p =$

0.03, p = 0.03) increased sucrose preference in male rats. This is aligned with sucrose consumption findings where males consumed more following both doses of ligand and females did not significantly change their sucrose consumption but tended to decrease their consumption compared to vehicle control. Additionally, low dose ligand consumption was significantly different between male and female rats (p = 0.02). This is another sex specific effect where activation of VP GABA neurons increases sucrose preference in male rats to levels that are significantly different from female rats.

Water Consumption

Water consumption was calculated as the difference in weight of the water bottle before and after each test day. On average, Gq sucrose rats (n=13, 7M, 6F) did not change their water intake across saline (1.89 grams +/- 0.24), low dose (1.94 grams +/- 0.16), and high dose (2.05 grams +/- 0.21) test days. No significant effects of injection ($F(2,22) = 0.21$, $p = 0.81$), or sex ($F(1,11) = 0.71$, $p = 0.42$), or injection by sex interaction ($F(2,22)=0.62$, $p=0.54$) were found in a linear mixed effects model. Overall, VP GABA activation had no significant effect on water consumption.

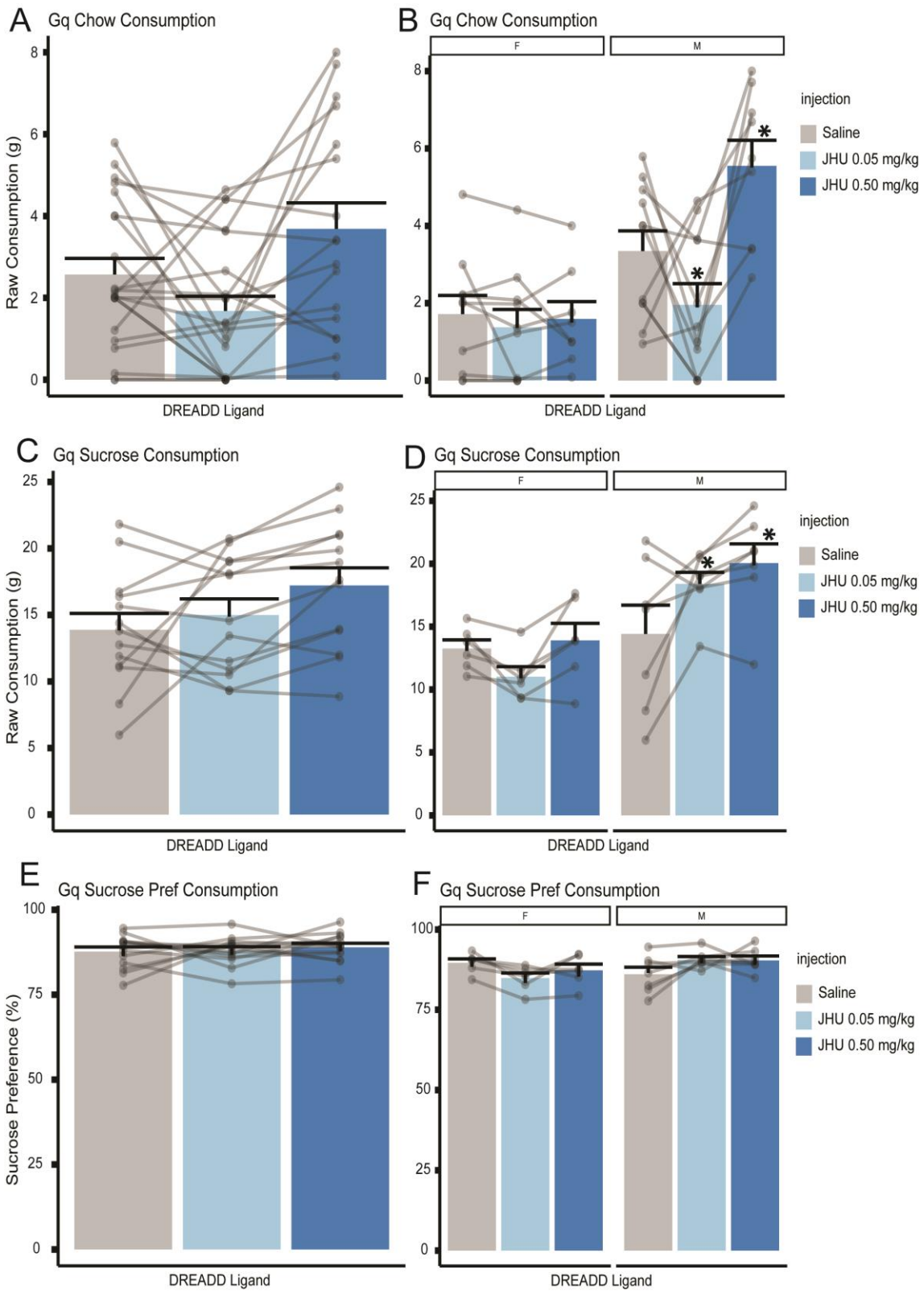

***Figure 3**. VP GABA activation increases consumption of chow and sucrose at high ligand dose and decreases chow consumption at low ligand doses, specifically in male rats*.

(A) Gq rat chow consumption when delivered control saline (gray; 2.57+/- 0.39 grams), low dose of DREADD ligand (light blue; 1.68 +/- 0.36 grams) and high dose of DREADD ligand (dark blue; and 3.69 +/- 0.63), via IP injections (B) Gq chow consumption split by sex: saline(M:3.35 +/- 0.53,F:1.72 +/- 0.48), low dose(M:1.96 +/- 0.55,F:1.38+/-0.46), high dose(M:5.55+/-0.66,F:1.59+/- 0.45), LME shows a significant effect of injection ($F(2,34.75)=7.76$, $p=0.002$), sex ($F(1,18.65)=0.08$, $p=0.002$) and injection*sex interaction ($F(2,34.74)=6.90$, $p=0.003$) was observed in a linear mixed effect model. Follow-up comparisons show significant decrease in chow consumption with low ligand injection ($p=0.02$) and high dose ($p=.002$) of the ligand, compared to controls. The saline ($p=0.03$) and high dose ($p=<0.0001$) ligand groups showed a significant sex difference in chow consumption.
(C) Gq sucrose consumption: saline(13.88 +/- 1.24), low dose(14.99 +/- 1.21), high dose (17.21 +/- 1.33) (D)Gq sucrose consumption split by sex: saline(M:86.05+/-2.20,F:89.62+/- 1.25), low dose(M:90.60+/-1.01,F:84.88+/-1.56), high dose(M:90.38+/-1.36,F:87.37+/-1.95), LME shows significant effect of injection ($F (2,22)=4.27$, $p=0.03$) or sex ($F(1,11)=9.60$, $p=0.01$) or sex*injection interaction ($F(2,22)=4.40$, $p=0.02$)
(E) Gq sucrose preference: saline(87.70%+/-1.36), low dose(87.95%+/-1.19), high dose(88.95+/- 1.20) (F) Gq sucrose preference split by sex: saline(M:90.08%+/-3.19,F:89.48%+/-3.15), low dose(M:90.02%+/-3.39,F:85.46%+/-2.10), high dose(M:90.53%+/-3.26,F:80.26%+/-3.88), LME shows no significant effect of injection ($F (2,22)=0.38$, $p=0.69$) or sex ($F(1,11)=1.16$, $p=0.31$) but a significant interaction of injection by sex ($F(2,22)=5.88$, $p=0.01$). Data are represented as mean +/- SEM. Dots and lines represent individual rats.

3.4.3 Injection of ligand has no significant effect on chow or sucrose consumption in non-DREADD expressing animals, but may have off target effects on water consumption

Chow Consumption

mCherry control vector rats (n=12, 6M, 6F) were habituated and tested on the chow task as described above. On average, mCherry rats consumed 3.14 +/- 0.52 grams of chow on saline test days, 3.34 +/-0.59 grams on low ligand test days, and 3.83 +/- 0.42 on high ligand test days. No significant effects of injection ($F(2,20) = 1.95$, $p = 0.17$), or sex ($F(1,10) = 0.92$, $p = 0.36$), or injection by sex interaction ($F(2,20) = 0.36$, $p = 0.70$) were observed in a linear mixed effects

model. Chow consumption seems to be consistent in control animals that do not express DREADDs across all test days, therefore there is no obvious off-target effect of ligand or injections that affects chow consumption in either sex.

Sucrose Consumption

mCherry control vector rats (n=10, 5M, 5F) were habituated and tested on the sucrose task as described above. On average, mCherry rats consumed 15.23 +/- 1.45 grams of sucrose on saline test days, 14.74 +/-1.36 grams on low ligand test days, and 15.81 +/-1.44 on high ligand test days. No significant effects of injection ($F(2,16) = 1.67$, $p = 0.22$), or sex ($F(1,8) = 1.34$, $p = 0.28$) or injection by sex interaction ($F(2,16) = 1.60$, $p = 0.23$) was observed in a linear mixed effect model. Sucrose consumption remains consistent in control animals that do not express DREADDs across all test days, therefore there is no obvious off-target effect of ligand or injections that affects sucrose consumption in either sex.

Preference of Sucrose

Sucrose preference consumption was measured as described above. All rats were noted to have a baseline sucrose preference on habituation days. mCherry control rats (n=10, 5M, 5F) preference for sucrose across saline (94.34%+/- 1.22), low ligand (94.00% +/- 1.40), and high ligand (93.19% +/- 1.52) test days. No significant effect of injection ($F(2,16) = 1.98$, $p = 0.17$) or sex ($F(1,8) = 0.58$, $p = 0.47$) was observed in a linear mixed effect model. However, an injection*sex interaction ($F(2,16) = 5.01$, $p = 0.021$) was observed in the model. Follow up

comparisons revealed that high doses of ligand significantly (p = 0.02) decreased sucrose preference (91.21% +/- 2.60) in female rats compared the vehicle controls (93.33% +/- 2.15) and low doses (93.92% +/- 2.46; p=0.006) of ligand. No effect of ligand was seen on sucrose preference in male rats. This indicates there may be off target consumption effects of the high dose of ligand injection in females that is not related to sucrose consumption.

Water Consumption

When just water consumption was observed in control animals, a linear mixed effect model showed a significant effect of injection ($F(2,16) = 4.30$, $p = 0.032$) and a significant sex*injection interaction ($F(2,16) = 4.35$, $p = 0.031$) on water consumption. Follow up comparisons revealed a significant increase in water consumption in female rats on high dose ligand test days (1.10 +/- 0.23) compared to vehicle (0.78 +/- 0.16, $p = 0.01$) and low dose (0.66 +/- 0.23, $p = 0.001$) test days. This increase in female water intake explains the decrease in sucrose preference females show on high ligand test days. It seems there is an off-target effect of high ligand concentration on water consumption of females, even though water consumption remains low relative to sucrose consumption.

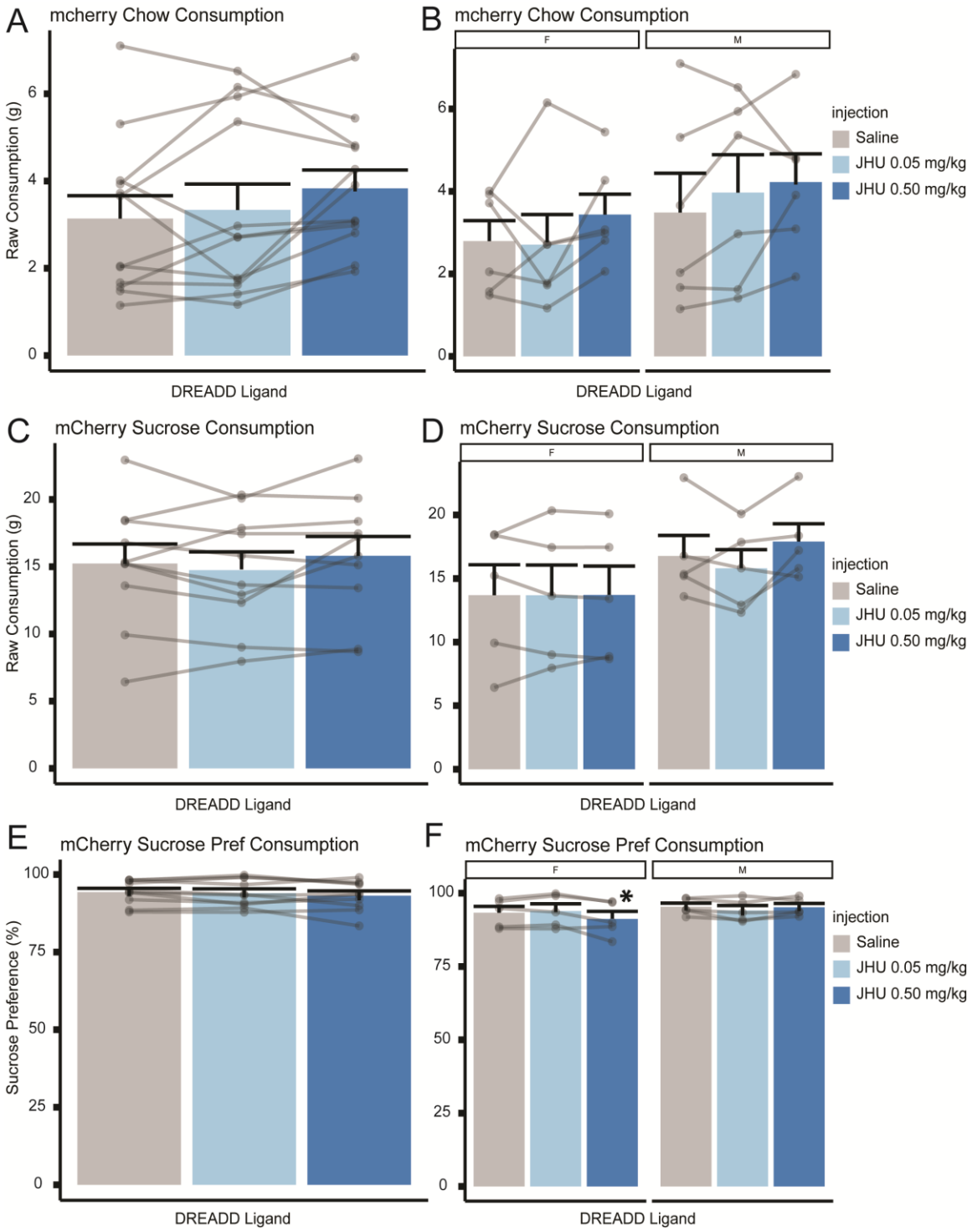

Figure 4*. DREADD ligand has no significant effects on consumption of chow or sucrose, however does impact sucrose preference*. (A) mCherry rats chow consumption when delivered control saline (gray; 3.14+/- 0.52 grams), low dose of DREADD ligand (light blue; 3.39 +/- 0.59 grams) and high dose of DREADD ligand (dark blue; and 3.83 +/- 0.42), via IP injections (B) mCherry chow consumption split by sex: saline (M:3.49 +/- 0.95,F:2.79 +/- 0.50), low dose (M:3.97 +/-

0.92,F:2.71+/-0.73), high dose (M:4.23+/-0.69,F:3.44+/-0.49), LME shows no significant effect of injection (F (2,20)=1.95, p=0.17) or sex (F(1,10)=0.92, p=0.36), or sex*injection interaction (F(2,20)=0.36, p=0.70).

(C) mCherry sucrose consumption: saline (15.23 +/- 1.45), low dose (14.74 +/- 1.36), high dose (15.81 +/- 1.44) (D) mCherry sucrose consumption split by sex: saline (M:16.77+/-1.62,F:13.69+/-2.39), low dose (M:15.80+/-1.47,F:13.68+/-2.38), high dose (M:17.91+/-1.40,F:13.70+/-2.28), LME shows no significant effect of injection (F (2,16)=1.67, p=0.22) or sex (F(1,8)=1.34, p=0.28) or sex*injection interaction (F(2,16)=1.60, p=0.23)

(E) mCherry sucrose preference: saline (94.34%+/-1.22), low dose (94.01%+/-1.40), high dose(93.19+/-1.52) (F) Gi sucrose preference split by sex: saline (M:95.36%+/-1.24,F:93.33%+/-2.15), low dose (M:94.10%+/-1.68,F:93.92%+/-2.46), high dose (M:95.17%+/-1.32, F:91.21%+/-2.60), LME shows no significant effect of injection (F (2,16)=1.98, p=0.17) or sex (F(1,8)=0.58, p=0.47) but did find a significant interaction of injection by sex (F(2,16)=5.01, p=0.02). Data are represented as mean +/- SEM. Dots and lines represent individual rats.

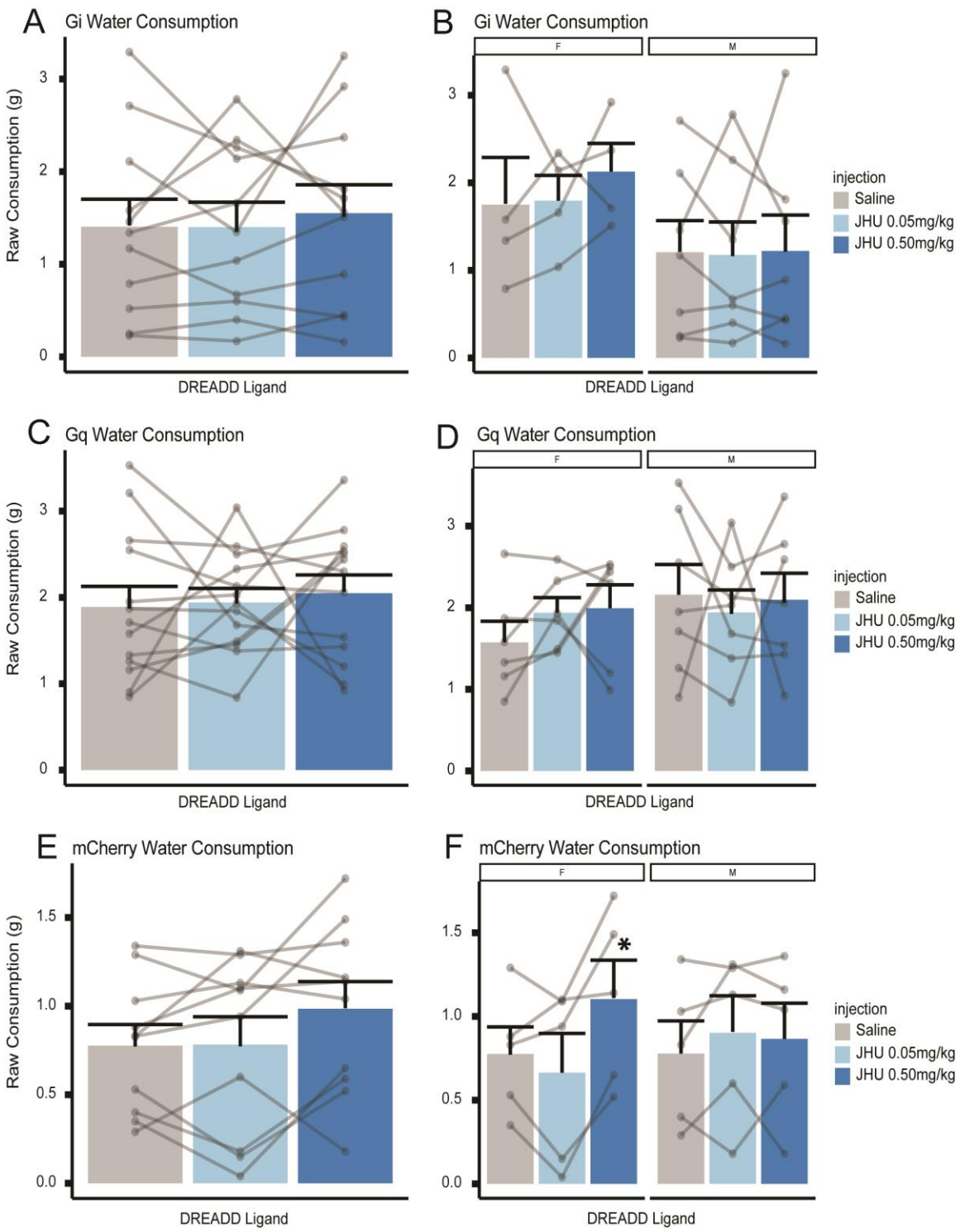

Figure 5*. DREADD ligand has off target effects on water consumption*. (A) Gi rat water consumption when delivered control saline (gray; 1.40+/- 0.30 grams), low dose of DREADD ligand (light blue; 1.40 +/- 0.27 grams) and high dose of DREADD ligand (dark blue; and 1.55 +/- 0.31), via IP injections (B) Gi water consumption split by sex: saline(M:1.21 +/- 0.36,F:1.75 +/-

0.54), low dose(M:1.18 +/- 0.38,F:1.80+/-0.29), high dose(M:1.22+/-0.41,F:2.13+/-0.32), LME shows no significant effect of injection (F (2,18)=0.38, p=0.69) or sex (F(1,9)=1.78, p=0.21),or sex*injection interaction (F(2,18)=0.28, p=0.76).
(C) Gq water consumption: saline(1.89 +/- 0.24), low dose(1.94 +/- 0.16), high dose(2.05 +/- 0.21) (D)Gq water consumption split by sex: saline(M:2.16+/-0.37,F:1.58+/- 0.26), low dose(M:1.94+/-0.27,F:1.94+/-0.19), high dose(M:2.10+/-0.33,F:1.99+/-0.29), LME shows no significant effect of injection (F (2,22)=0.21, p=0.81) or sex (F(1,11)=0.71, p=0.42) or sex*injection interaction (F(2,22)=0.62, p=0.54)
(E) mCherry water consumption: saline(0.78+/-0.12), low dose(0.78+/-0.16), high dose(0.99+/-0.15) (F) mCherry water consumption split by sex: saline(M:0.78+/-0.20,F:0.78+/-0.16), low dose(M:0.90+/-0.22,F:0.66+/-0.23), high dose(M:0.87+/-0.21,F:1.10+/-0.23), LME shows significant effect of injection (F (2,16)=4.30, p=0.03), no significant effect of sex (F(1,8)<0.001, p=1.00) and significant interaction of injection by sex (F(2,16)=4.35, p=0.03). Follow-up comparisons revealed high dose of ligand showed a significant difference compared to saline (p=0.001) in female rats. Data are represented as mean +/- SEM. Dots and lines represent individual rats.

3.4.4 Injection of ligand has no significant effect on animal movement in DREADD expressing or control animals

Finally, we wanted to assess if there were any effects of ligand injections and VP GABA manipulation on movement, to rule out this as a potential variable driving changes in consumption. We have previously tested a subset of DREADD animals (n=28; 16 M, 12 F) in the discriminative stimulus (DS) task before consumption tasks. Rats were trained on an operant discriminative stimulus task where reward availability was signaled by a reward-predictive cue (DS). Rats received a 10% sucrose reward if they entered the operant chamber port (port entry) during DS presentations, but not if they entered during the control cue (NS) or non-cue periods (Fig. 2A, Chapter 1). DREADD manipulation of VP GABA neurons showed no significant impact on measures of cue-elicited reward-

seeking (DS port entry probability and latency) in Gq, Gi and mCherry groups. During the task, we also recorded the total port entries each animal made as a measure of general movement across test day conditions.

Gi rats (n=9, 5M, 4F) maintained total PE frequency across saline test days (137.78 +/- 15.26), low ligand test days (154.22 +/- 26.31), and high ligand test days (139.29 +/- 25.26). There was no significant effect of injection ($F(2,12.31)=0.47$, $p=0.64$) or interaction with sex ($F(2,12.31)=0.50$, $p=0.87$) on total PEs. Therefore inhibition of VP GABA neurons had no off-target effect on animal movement that could impact food-seeking. Gq rats (n=13, 8M, 5F) maintained total PE frequency across saline test days (140.08 +/- 19.06), low ligand test days (170.54 +/- 20.81), and high ligand test days (162.36 +/- 26.41). There was no significant effect of injection ($F(2,21.05)=0.62$, $p=0.55$) or interaction with sex ($F(2,21.05)=0.17$, $p=0.84$) on total PEs. Therefore activation of VP GABA neurons had no off-target effect on animal movement that could impact consumption. mCherry rats (n=6, 3M, 3F) maintained total PE frequency across saline test days (174.43 +/- 18.83), low ligand test days (151.00 +/- 11.43), and high ligand test days (129.83 +/- 8.59). Injection type had no significant effect ($F(2,12)=2.43$, $p=0.13$) or interaction with sex ($F(2,12)=1.63$, $p=0.24$) on total PEs. Therefore injections of vehicle and ligand had no off-target effect on animal movement that could impact consumption. Overall, no significant effect of injections were seen in any of the DREADD or control groups,

suggesting that injections and DREADD ligand have no off target or unexpected effects on locomotion.

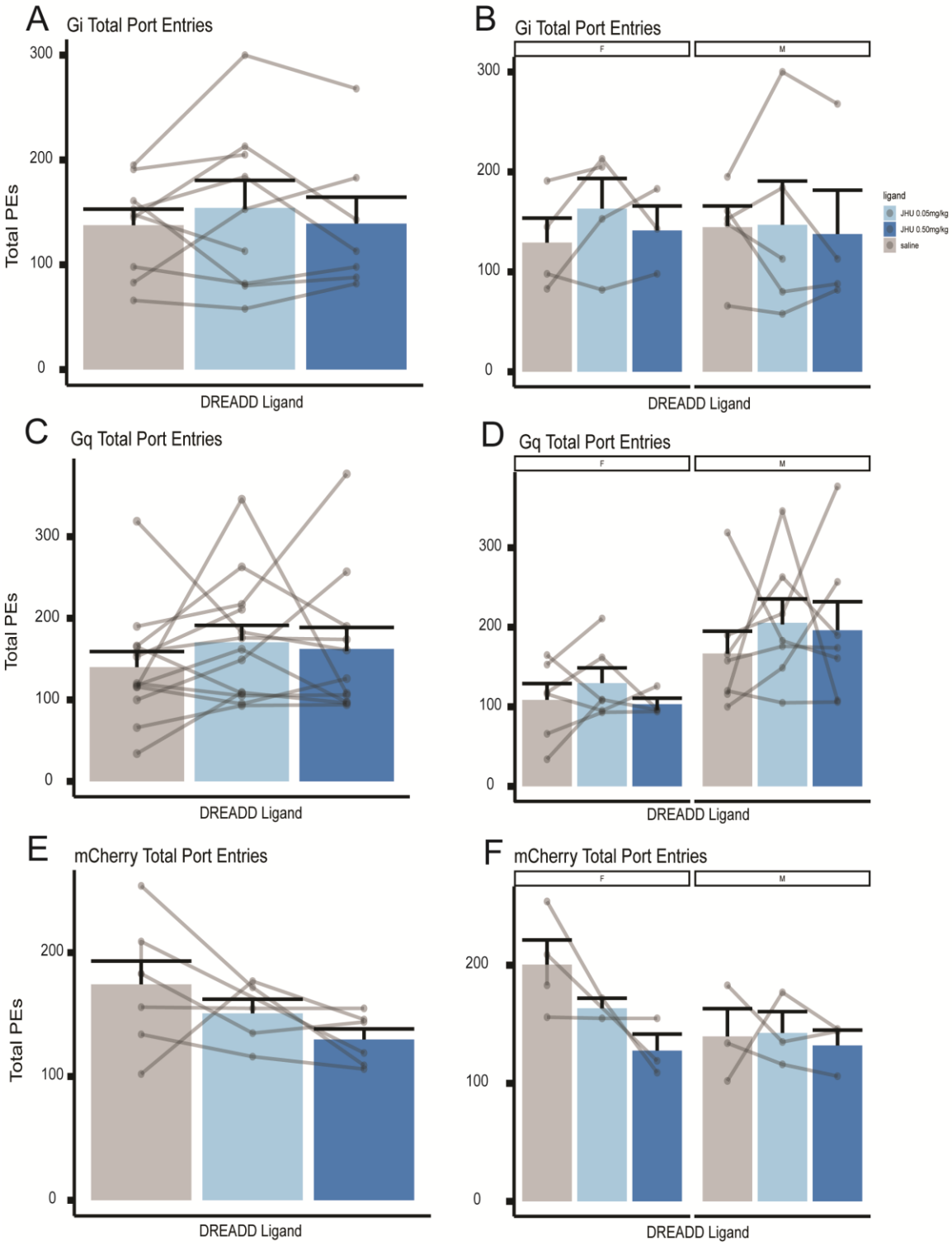

Figure 6*. VP GABA inhibition or activation as well as DREADD ligand injections, have no significant effects on locomotion for reward-seeking*. (A) Gi port entries when delivered control saline (gray; 137.78+/- 15.26 grams), low dose of DREADD ligand (light blue; 154.22 +/- 26.31 grams) and high dose of DREADD ligand (dark blue; and 139.29 +/- 25.26), via IP injections (B) Gi port entries split by sex: saline(M:144.60 +/- 21.29,F:129.25 +/- 24.46), low dose(M:147.00 +/- 43.79,F:163.25+/-30.18), high dose(M:137.75+/-43.93,F:141.33+/-24.55), LME shows no significant effect of injection ($F(2,12.31)=0.47$, $p=0.64$) or sex ($F(1,7.12)=0.87$, $p=0.87$),or sex*injection interaction ($F(2,12.31)=0.50$, $p=0.62$).
(C) Gq port entries: saline(140.08 +/- 19.06), low dose(170.54 +/- 20.81), high dose(162.04 +/- 26.41) (D)Gq port entries split by sex: saline(M:167.00+/-28.04,F:108.67+/- 20.56), low dose(M:205.57+/-30.02,F:129.67+/-19.24), high dose(M:196.14+/-35.88,F:103.25+/-7.60), LME shows no significant effect of injection ($F(2,21.05)=0.62$, $p=0.55$) a significant effect of sex ($F(1,9.85)=9.85$, $p=0.009$) but no significant sex*injection interaction ($F(2,21.05)=0.17$, $p=0.84$) (E) mCherry port entries: saline(174.43+/-18.83), low dose(151.00%+/-11.43), high dose(129.83+/-8.59) (F) mCherry port entries split by sex: saline(M:139.67+/-23.56,F:200.50+/-20.86), low dose(M:142.67+/-18.02,F:163.50+/-8.50), high dose(M:132.00+/-13.01,F:127.67 +/-13.97), LME shows no significant effect of injection ($F(2,12)=2.43$, $p=0.13$) or sex ($F(1,12)=2.69$, $p=0.13$) or interaction of injection by sex ($F(2,12)=1.63$, $p=0.24$). Data are represented as mean +/- SEM. Dots and lines represent individual rats.

3.5 Discussion

Summary of Results

Here we assessed how functional manipulation of the activity of VP GABA neurons impacted rats' consumption of regular chow and 10% sucrose solution. When VP GABA neurons were activated with a novel DREADD ligand male rats consumed more sucrose compared to saline delivery test days, in a dose dependent manner. Additionally, male rats consumed more chow when delivered a high dose of the ligand, and less chow when tested with a lower concentration of ligand, compared to saline controls. None of these VP GABA activation effects were seen in female rats. Inactivation of VP GABA neurons did not significantly

affect consumption of either chow, sucrose or water, though we observed a trend toward a decrease in sucrose consumption. Non-DREADD expressing rats had no change in sucrose or chow consumption across ligands or saline test days. However, ligand injections did significantly alter water consumption in non-DREADD expressing female rats, resulting in a significant decrease in their sucrose preference. No group significantly changed their locomotor behavior in response to ligand injections. Overall, activation of VP GABA neurons increases consumption of reward specifically in male rats.

3.5.1 Inactivation of VP GABA neurons has no significant effect on chow or sucrose consumption in non-food restricted animals

When we inactivated VP GABA neurons in ad-libitum fed rats before consumption tasks, we saw no significant effect on chow, sucrose or water consumption. This is a similar effect that recent work from Farrell et al. 2022 showed, where sated rats did not significantly change chow-seeking or free access consumption of palatable food (M&Ms) when VP GABA neurons were inactivated. However, sated rats did decrease their palatable food-seeking (banana pellets) when VP GABA neurons were inactivated (Farrell et al., 2021). When we inactivated VP GABA neurons, we found that our non-food restricted rats trended toward a decrease in 10% sucrose consumption, especially visible in female rats (Fig. 2D). This may also decrease sucrose preference compared to water (Fig. 2F). Our experiments may yield similar results to Farrell et at. 2022 if VP GABA inactivation was paired with other contextual cues (operant task) or

food-restriction. Additionally, palatable banana pellets were used as a reward in Farrell et al. 2022 sated rats palatable food-seeking task. These pellets are approximately 95% carbohydrates (Bioserve, #F0024) but also contain fat, banana flavoring, and have a different texture than our liquid solution. Disinhibition of VP causes a strong preferential increase in fat consumption of rats (Covelo et al., 2014). Food-seeking for these pellets compared to sugar water may elicit a higher motivation (Smith and Berridge, 2005; Smith et al., 2011) for seeking of these pellets, allowing for a more discernible effect. Macronutrient differences in consumption when VP GABA neurons are inhibited should be explored further.

It is likely that there are multiple VP GABA populations (cell type specific and projection specific) that affect consumption. It could be possible that inhibition of all these populations leads to variable results in consumption tasks. It has been shown that VP GABA projections can have a distinct impact on reward seeking behavior (Prasad et al., 2020a). Disconnection of VP GABA neurons projecting to the lateral hypothalamus does not significantly impact reacquisition of alcohol seeking in rats, whereas the exact same procedure targeted at VTA projecting VP GABA neurons significantly decreased reacquisition of alcohol seeking (Prasad et al., 2020a). The canonical feeding center is the lateral hypothalamus (LH) (Stuber and Wise, 2016a; Rossi and Stuber, 2018). VP GABA neurons project to LH, and to other areas that also project to LH neurons (nucleus accumbens, lateral habenula, VTA) (Stratford and Wirtshafter, 2013; Root et al., 2015; Stuber and Wise, 2016; Sharpe et al., 2017). Therefore, VP

GABA inhibition could be impacting the LH in multiple opposing ways. It is important to follow up on inhibiting specific projection subtypes of VP GABA neurons during consumption tasks and compare these results to those of global VP GABA inhibition.

VP GABA neurons also have a number of cell type specific populations (Zhu et al., 2017; Prasad et al., 2020; Morais-Silva et al., 2022) such as npas1 neurons, somatostatin and parvalbumin GABA neurons. Different VP GABAergic cell type populations can project to distinct areas of the brain involved in reward seeking and consumption behavior (Prasad and McNally, 2016; Zhu et al., 2017; Prasad et al., 2020). Research indicates there may be different effects of basal forebrain GABA neuronal subtypes on consumption of highly palatable rewards (Zhu et al., 2017). Additionally, VP GABA neurons have a heterogeneous response to reward paired cues and reward (Stephenson-Jones et al., 2020). Finally, VP GABA parvalbumin neurons have a distinct role acquisition of alcohol seeking behavior (Prasad et al., 2020). Therefore, probing the VP GABA cell types that are involved in consumption tasks may lead to a greater understanding of VP GABA contribution to the brain circuitry involved in consumption.

3.5.2 Activation of VP neurons impacts chow consumption in a sex specific manner

When we activated VP GABA neurons in ad-libitum fed rats before consumption tasks, we saw a significant decrease in chow consumption with low dose of ligand and an increase on chow consumption with a high dose of ligand. This

was unexpected compared to our hypothesis that activation of VP GABA neurons with ligand doses would cause an increase in chow consumption in a dose dependent manner. One explanation for this is that VP GABA neurons project to the lateral hypothalamus as well as a number of brain regions that also project to the lateral hypothalamus and to the VP (Castro et al., 2015; Prasad and McNally, 2016; Gendelis et al., 2020; Prasad et al., 2020). Activation of VP GABA neurons could have conflicting effects on indirect and direct connections to brain areas that contribute to initiation of consumption. VP GABA neurons are shown to make monosynaptic connections with LH GABA neurons (Prasad et al., 2020), and LH GABA neurons moderate consummatory behavior (Navarro et al., 2016; Sharpe et al., 2017). However, nucleus accumbens neurons are also shown to make connections with LH GABA neurons and, and the NAc also has reciprocal connections with the LH and VP (Stratford and Kelley, 1999; Castro et al., 2015; Root et al., 2015). VP neurons respond to reward predictive cues and relative reward value more quickly than NAc neurons (Ottenheimer et al., 2018), therefore, VP GABA activation may inhibit input from NAc into LH GABA neurons that also may be important in initiating consumption behavior(Stratford and Kelley, 1999; Stratford and Wirtshafter, 2013). This is also in contrast to what was found in Li et al. 2020 where activation of VP GABA neurons had no significant effect on food intake in food-restricted (hungry) mice (n=7) (Li et al., 2020). This difference could be explained by our use of a higher affinity DREADD ligand (Bonaventura et al., 2019). Also, we did not test how different hunger

states affect consumption in our paradigm. Future experiments should test the impact of internal state on VP GABA activation effects we observed (Figure 3).

The decrease and increase in chow consumption at low and high dose of ligand respectively, was seen specifically in male rats. This sex specific effect could be due to ovarian hormones' effect on consumption behavior (Alonso-Caraballo and Ferrario, 2019; Ma et al., 2020). Food intake of average chow was measured across the estrous cycle in female obesity prone and resistant rats and in both of the groups home-chow intake significantly decreased in estrus phase (Alonso-Caraballo and Ferrario, 2019). Estrus phase is characterized by low levels of progesterone and estrogen in the rodent (Smith et al., 1975). These levels of hormones that differ between our intact male and female rats may account for some of the sex differences we found in our dataset.

When we activated VP GABA neurons in rats and measured sucrose consumption, we also saw a significant increase of sucrose consumption in male rats but not in female rats. Additionally, sucrose preference increased significantly in the male but not the female rats (Fig. 3F). However, female rats trended toward a decrease in sucrose consumption when VP GABA neurons were activated with ligand. This sex specific effect could be due to circulating sex specific hormones (Butera, 2010; Xu et al., 2011; Alonso-Caraballo and Ferrario, 2019; Ma et al., 2020). Additionally, the internal hunger state of each animal may differ and this could be due to sex hormones affecting metabolism and consumption (Butera, 2010; Xu et al., 2011). Estrogen delivery in ovariectomized female rats, causes a significant decrease in food intake when coupled with

ghrelin delivery, compared to vehicle and saline controls (Butera, 2010) Estrogen-receptor deletion in most brain regions regulated measures of energy homeostasis, mainly increasing body weight in male and female rats, increasing visceral fat mass and daily food intake in female rats, and decreasing heat production, ambulatory movements and rearing counts in female rats (Xu et al., 2011). These differences provide evidence for follow-up experiments controlling for effects of circulating sex hormones on metabolism and internal state of the animal.

3.5.3 Non-selective effects of DREADD ligand on water consumption

When we injected mCherry vector control rats with saline or ligand doses we saw no significant effect on chow or sucrose consumption in both male and female rats. However, ligand injections increased water consumption specifically in female rats. This change in water consumption also decreased measures of sucrose preference, seemingly independent from sucrose consumption, in female rats. This change in water consumption is most likely not due to any off-target effects on movement, as our measurements of movement during reward seeking tasks were not significant (Fig. 5) and previous research also confirms this (Bonaventura et al., 2019). Additionally, other studies that have measured off target effect on water consumption and showed no significant off target effect of CNO (Zhang et al., 2019; Stanojlovic et al., 2021). Alternatively, this change in water consumption could be due to the effect IP injections have on stress responses or anxiety behavior (Martinez et al., 2019). Also, DREADD ligand

JHU37160 has a higher affinity to muscarinic receptors (Bonaventura et al., 2019; Goutaudier et al., 2019), that could impact its effect on endogenous muscarinic receptors leading to more parasympathetic nervous system effects that should be explored further.

3.5.4 Caveats and Future Directions

Here, we used DREADD to bidirectionally manipulate VP GABA neurons during consumption tasks to examine how inactivation or activation of VP GABA neurons impact on feeding behavior. We found that VP GABA neuronal inactivation does not significantly affect chow or sucrose consumption. This effect on chow consumption was expected from previous work (Farrell et al., 2021). When VP GABA neurons were activated, we found a significant effect on chow and sucrose consumption. VP activation increased sucrose consumption in a dose dependent manner. Unexpectedly, we found that chow consumption was differentially changed with different doses of ligand, specifically in male rats. Activation of VP GABA neurons with a low dose of ligand caused a significant decrease in chow consumption, whereas activation with a high dose of ligand significantly increased chow consumption, compared to internal controls. This decrease in chow consumption at low ligand doses was surprising due to the finding that VP GABA neurons are involved in appetitive behaviors, including reward consumption (Stephenson-Jones et al., 2020). This discrepancy could be due to confounding variables of VP GABA cell type (projection or cellular markers), and how these cell types differentially impact consumption circuitry

(Prasad and McNally, 2016; Zhu et al., 2017; Gendelis et al., 2020; Prasad et al., 2020; Kupchik and Prasad, 2021; Morais-Silva et al., 2022).

The sex specific effect of VP GABA activation on chow and sucrose consumption was also unexpected. Although we accounted for sex in our mixed effect models we did not expect to see such a drastic sex specific effect in chow and sucrose consumption. This sex difference could be accounted for by circulating sex hormones (Fukushima et al., 2015; Alonso-Caraballo and Ferrario, 2019; Buczek et al., 2020; Ma et al., 2020). Future experiments should appropriately power experiments in females to assess how the 4 phases of estrus phase impact activation of VP GABA on consumption of chow and sucrose, or alternatively ovariectomize and castrate all rats to account for the impact of circulating sex specific hormones. Additionally, it will be important to examine how functional manipulation of VP GABA subtypes, both projection and cell-type, impacts consumption behavior across sexes to see if there are any structural differences in connectivity.

It was also surprising to see that VP GABA inactivation showed no significant effect on sucrose consumption because past work (Farrell et al., 2021) has shown a decrease in palatable food-seeking following VP GABA inactivation. This could be due to a different training context, a difference in macronutrient make-up of palatable rewards. Future work should examine the effect of VP GABA inactivation on different palatable macronutrient profiles, how contextual cues can impact consumption during VP GABA neurons are inactivated and how internal state directly effects both of these paradigms.

3.6 Conclusions

Understanding the neural circuitry for both these behaviors and how the neural circuitry integrates homeostatic signals about internal state to initiate consumption is necessary in understanding how food-seeking becomes maladaptive. Ventral pallidal (VP) circuitry is a major driver of cue-evoked behaviors including routine consumption and consumption of palatable rewards. Previous work has found that inhibition of the canonical, GABAergic, VP neurons can decrease consumption of chow or palatable reward in hungry animals. Additionally, activation does not affect intake of chow in hungry animals. However, how manipulation of VP GABA neurons impacts sated rats consumption of chow or a single macronutrient palatable reward has yet to be explored. Our work explores how activation or inhibition of VP GABA neurons affects consumption of chow or sucrose in sated. Here, we used an intersectional viral approach to manipulate activity of VP GABAergic (VP GABA) neurons in male and female rats as they had access to chow or sucrose reward. We found that activation of VP GABA neurons increases both chow and sucrose intake in sated male rats. We also found inhibition of VP GABA neurons does not significantly impact chow or sucrose intake in sated rats, but a trend of decrease of sucrose consumption was seen. Together, these findings suggest that VP GABA neurons are an important neuronal component that contribute to consumption behavior. Additionally, sex and internal state are important variables to control for when examining other neural components of chow or sucrose

consumption.

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Chapter 4: Conclusions

Cue-elicited reward seeking is an important learned behavior, necessary for animals and humans to understand and navigate their environment. However, modern advancements have led to more maladaptive cue-elicited reward seeking or overconsumption of rewards that have established a need for understanding the origin of such maladaptive behaviors. For this reason, it is important to understand the neural components contributing to cue-elicited reward seeking and how they contribute to reward seeking and reward consumption. The ventral pallidum is a brain region long associated with reward impact and consumption. However, there is heterogeneity amongst VP cell types in their neural responses and contribution to rewarding or aversive stimuli. There is a need to disentangle the encoding and contribution of VP cell types to cue-elicited reward seeking and reward consumption behavior. This dissertation aimed to address how the VP GABA cell type encodes and contributes to cue elicited reward seeking and consumption. This dissertation characterizes the encoding of reward-predictive cues in VP GABA neurons and how these neural signals are predictive of reward-seeking actions (Chapter 2). In addition, we assess of VP GABA bidirectional modulation effects consumption of different reward types in male and female rats (Chapter 3). These studies advance our understanding of the neural sources contributing to both routine and maladaptive cue-elicited reward seeking and reward consumption.

4.1 Summary of Findings

4.1.1 VP GABA neurons encode reward predictive cues and this neural response in predictive of the vigor of reward-seeking behavior

In chapter 2, we used fiber photometry and an intersectional viral approach to record calcium activity in VP GABA neurons while rats learned a discriminative stimulus instrumental reward-seeking task. Our recordings show the VP GABA population calcium activity developed in response to reward predictive cues much more so than neutral cues never paired with rewards. A regression-based encoding model revealed that VP GABA population calcium activity encodes reward predictive auditory cues, the operant action of entering the port, and the initial consumption of the reward, once rats learn the discriminative stimulus task. Additionally, the calcium activity in response to learned reward predictive cues is predictive of how quickly animals enter the port to initiate reward delivery, post auditory cue presentation. These findings show that VP GABA neurons are significantly involved in cue-eliciting reward seeking and vigor of reward-seeking.

4.1.2 VP GABA neuronal response to reward predictive cues is modulated by absence of reward, but persists even in the absence of reward

In chapter 2, we also examined how VP GABA population calcium response changes when the reward-predictive cue changes value, where it is no longer predictive of reward delivery, and is followed by no reward delivery. Rat behavior is nearly abolished throughout extinction days, and VP GABA activity decreases

across extinction days. However, VP GABA activity is still persistent post reward predictive cue, even when rats stop responding to reward predictive cues. This show that VP GABA neurons persist their responding, even in the absence of reward, suggesting this response may be a neural correlate of persistent or maladaptive cue-elicited reward seeking behavior.

4.1.3 VP GABA neurons contribute to consumption of chow and sucrose reward in sated male rats

In chapter 3, we used chemogenetic methods to examine how VP GABA neurons contribute to passive reward consumption of both a home chow and palatable sucrose. We used designer receptors exclusively activated by designer drugs (DREADDs) and a novel, high specific, DREADD ligand to either activate of inhibit VP GABA neurons during consumption tasks or either chow or sucrose. Male and female rats were tested in each task and sex was accounted for in statistical analysis. VP GABA activation proved to have a significant effect on chow and sucrose consumption specifically male rats. In chow consumption tasks, low dose of ligand decreased chow consumption compared to internal controls whereas high dose of ligand increased chow consumption compared to internal controls. In sucrose consumption tasks, sucrose consumption was increased in a dose dependent manner. These results show VP GABA neurons contribute to consumption of both routine and palatable rewards and show a need for further examination on direct effects of internal state and sex on VP GABA contributions to reward consumption behavior.

4.2 Future Directions

4.2.1 VP GABA neuronal subtypes in cue-elicited reward-seeking and consumption

Importantly, previous studies have also shown some VP GABA neurons are inhibited by rewards and Pavlovian reward-paired cues (Stephenson-Jones et al., 2020), suggesting there is even heterogeneity within the VP GABA subtype. It was surprising to find that VP GABA response to reward predictive cues was persistent in the absence of reward due to the finding that VP neurons are sensitive to the relative reward value and can encode reward prediction errors (Ottenheimer et al., 2020a). This finding could also be due to heterogeneity in the response patterns of VP GABA neurons. Importantly, signals encoding relative reward value and reward-prediction error-like signals have just been observed in subsets of VP neurons (Ottenheimer et al., 2018, 2020a, 2020b). VP GABA neurons may differ in their encoding and functional contributions based on more specific cell-type subdivisions or output pathways. Additionally, recent research found that chemogenetic activation of VP GABA neurons does not impact intake of standard chow (Li et al., 2020), suggesting that the VP GABA induced feeding we see may be initiated by a subset of VP GABA neurons.

VP GABA neuronal marker specific subtypes

Some work points to parvalbumin neurons being a subset of VP GABA neurons involved in cue-evoked behaviors (Prasad et al., 2020). PV neurons

make up about 26% of all VP neurons and overlap with about 70% of cells that express VP GABA markers (McKenna et al., 2013; Prasad et al., 2020; Kupchik and Prasad, 2021). VTA GABAergic neurons exclusively receive input from GABAergic VP PV neurons (Knowland et al., 2017). Another subtype, PAS 1-positive VP GABA, were identified and shown to contribute to stress and anxiety-related behaviors (Morais-Silva et al., 2022). A subpopulation of GABAergic neurons also co-express enkephalin (VP PENK). VP PENK neurons may receive preferential innervation from D1-expressing accumbens neurons. Chemogenetic stimulation of VP GABA and VP PENK neurons also potentiates cue induced reinstatement of cocaine seeking in mice (Heinsbroek et al., 2020). These studies make PV and PENK subtypes of VP GABA neurons of particular interest in following up on which neurons contribute to cue-elicited reward seeking behaviors, and parsing out which neurons are sensitive to reward value and which neurons are not. Using intersectional viral approaches with fiber photometry and optogenetics to selectively record or modulate VP GABA subtypes during the discriminative task will help reveal the distinct role of each VP GABA subtype in cue-elicited reward seeking.

VP GABA projection specific subtypes

VP GABA neurons have many projection targets that have varied roles in appetitive behavior (Covelo et al., 2014; Mahler et al., 2014; Leung and Balleine, 2015; Root et al., 2015; Prasad and McNally, 2016; Farrell et al., 2021; Vachez et al., 2021; Morais-Silva et al., 2022). Recording or manipulating more specific

subset of projection-specific VP GABA cells (cell-type or projection-specific) may reveal greater, or lack of, reward-predictive cue encoding, latency correlations, or contributions to consumption.

Connectivity between VP and LH has been implicated in both cue-driven reward-seeking and consummatory behavior. For example, VP GABA projections to LH are recruited during renewal of alcohol seeking by a context cue and this renewal is absent when VP-LH connections are disrupted using contralateral disconnection methods (Prasad et al., 2020). Additionally, increases in food intake that occur after disinhibition of VP are reduced in rats with lesions of the ipsilateral LH (Stratford and Wirtshafter, 2013). These findings provide indirect evidence for a role of VP projections to LH in cue-driven behavior and consumption. Future work should use intersectional viral approaches to record from and manipulate VP GABA projections to LH in cue-elicited reward seeking and consumption tasks.

Disconnection studies also indicate that VP Gad1 neurons promote reacquisition of cue elicited reward seeking however through their connection to LH however the same was not true for VP PV neurons. VP PV neurons seem to promote acquisition of cue-elicited reward seeking via their connections to the VTA (Prasad et al., 2020). This is further evidence that cellular subtype within VP neurons and the heterogeneity in where these neurons project to are important variables to consider in future research assessing VP GABA contributions to cue-elicited reward seeking and consumption.

4.2.2 Internal state effect on VP GABA encoding and contribution to cue-elicited reward seeking and consumption

Internal state (i.e., hunger or thirst) is known to modulate VP GABA response and impact on cue-elicited reward seeking behavior and reward consumption. When sated animals hear a cue-predictive of water delivery and receive water VP GABA neuronal firing rate shows no significant changes to the cue or water delivery. However, when animals are thirsty a large subset of VP GABA neurons increase their firing rate in response to the auditory cue and water delivery (Stephenson-Jones et al., 2020). It was also shown that inhibition of VP GABA neurons decreases intake of chow in hungry animals but not in sated animals (Farrell et al., 2021). Therefore, internal state of animal effects the impact VP GABA neurons can have on cue-elicited reward seeking and consumption. However, studies comparing the effect of internal sate, sex and VP GABA modulation has not been done. Our studies indicated a need for this to clarify the bidirectional impact VP GABA modulation can have on consumption. Additionally, recording from VP GABA neurons during operant reward seeking tasks in different internal states has yet to be done. It would be of interest to understand of internal state impacts the acquisition and encoding of cues predictive of sucrose reward in VP GABA neurons. Follow-up work should determine whether internal sate impacts how predictive VP GABA responses are of reward seeking vigor.

4.2.3 VP GABA contribution to cue-elicited reward seeking with substances of abuse

Finally, VP GABA neuronal encoding of cues predictive of substances of abuse are of particular interest in addition neuroscience research. VP GABA neurons are found to respond and contribute to predictive cues/contexts and reward-seeking behavior for multiple substances of abuse. Tonic VP GABA neuronal calcium activity increased during cue-induced reinstatement for cocaine seeking. Phasic activity of VP PENK neurons increased during reinstatement (Heinsbroek et al., 2020). Chemogenetic activation of VP GABA or of VP PENK neurons increases cocaine seeking in extinguished mice in the absence of drug reward paired cues. However, chemogenetic activation of VP PENK but not VP GABA neurons potentiates cue-induced reinstatement. These results suggest that VP PENK neurons may be involved in cue-evoked cocaine relapse (Heinsbroek et al., 2020). Chemogenetic inhibition of the VP reduces alcohol seeking and the reacquisition of alcohol seeking (Prasad and McNally, 2016). Projection specific VP cell types may contribute to cue elicited alcohol seeking behavior. Chemogenetic inhibition of VP GABA neurons that project to the LH significantly decreases contextual cue induced alcohol-seeking behavior. However, inhibition of VTA projecting VP GABA neurons does not have any significant effect on contextual cue induced alcohol-seeking (Prasad et al., 2020). Inhibition of VP PV neurons, a large proportion of VP GABA neurons, that project to the LH does not impact contextual cue-induced alcohol seeking. However, inhibition of VTA projecting VP PV neurons significantly decreases contextual cue-inducted

alcohol seeking (Prasad et al., 2020). Chemogenetic stimulation of VP GABA neurons increases relapse to cue-elicited renewal of reward- seeking of the opioid drug remifentanil, even in a punishing context (Farrell et al., 2022). All these studies indicate a need for further exploration of how VP GABA neural cell-type, projection-type and their intersections differentially impact cue-elicited reward seeking in drugs of abuse. Use of intersectional viral approaches and optogenetics more work can be done to define the circuitry involved in relapse and how these might act as potential neuromodulation targets in substance use disorders.

I also would like to finish this thesis by recognizing substance abuse disorder is not just biologically based. Substance use and abuse is also a product of institutional failures-leading to poor living conditions, poor health outcomes and higher levels of substance abuse (Saah, 2005; Ewald et al., 2019) Lower socioeconomic status is a major social determinate of poor health outcomes (Administration (US) and General (US), 2016; Taylor et al., 2016; Ewald et al., 2019). Chronic stress associated with lower socioeconomic status directly impacts brain function and activity (Freudenberg, 2001; Ewald et al., 2019). Lower socioeconomic status disproportionately effects oppressed and marginalized communities including Black, Indigenous, transgender, disabled and neurodivergent folx (Freudenberg, 2001; Gibson et al., 2020). These realities should guide future allocation of funds aimed at reducing rates of addition toward social programs that address these social determinates and guide a more

preventive approach (i.e., universal basic income, housing for substance users, etc.) for reduction of substance use disorders.

References

Administration (US) SA and MHS, General (US) O of the S (2016) VISION FOR THE FUTURE: A PUBLIC HEALTH APPROACH. US Department of Health and Human Services. Available at: https://www.ncbi.nlm.nih.gov/books/NBK424861/ [Accessed March 4, 2023].

Aharoni D, Hoogland TM (2019) Circuit Investigations With Open-Source Miniaturized Microscopes: Past, Present and Future. Front Cell Neurosci 13 Available at: https://www.frontiersin.org/articles/10.3389/fncel.2019.00141 [Accessed December 18, 2022].

Ahrens AM, Meyer PJ, Ferguson LM, Robinson TE, Aldridge JW (2016) Neural Activity in the Ventral Pallidum Encodes Variation in the Incentive Value of a Reward Cue. J Neurosci 36:7957–7970.

Alonso-Caraballo Y, Ferrario CR (2019) Effects of the estrous cycle and ovarian hormones on cue-triggered motivation and intrinsic excitability of medium spiny neurons in the Nucleus Accumbens core of female rats. Horm Behav 116:104583.

Ambroggi F, Ghazizadeh A, Nicola SM, Fields HL (2011) Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. J Neurosci Off J Soc Neurosci 31:6820–6830.

Anand BK, Brobeck JR (1951) Hypothalamic control of food intake in rats and cats. Yale J Biol Med 24:123–140.

Asensio N, Brockelman WY, Malaivijitnond S, Reichard UH (2011) Gibbon travel paths are goal oriented. Anim Cogn 14:395–405.

Baker PM, Jhou T, Li B, Matsumoto M, Mizumori SJY, Stephenson-Jones M, Vicentic A (2016) The Lateral Habenula Circuitry: Reward Processing and Cognitive Control. J Neurosci Off J Soc Neurosci 36:11482–11488.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191:391–431.

Bonaventura J et al. (2019) High-potency ligands for DREADD imaging and activation in rodents and monkeys. Nat Commun 10:4627.

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013) The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl) 229:453–476.

Boswell RG, Kober H (2016) Food cue reactivity and craving predict eating and weight gain: a meta-analytic review. Obes Rev Off J Int Assoc Study Obes 17:159–177.

Buczek L, Migliaccio J, Petrovich GD (2020) Hedonic Eating: Sex Differences and Characterization of Orexin Activation and Signaling. Neuroscience 436:34–45.

Butera PC (2010) Estradiol and the control of food intake. Physiol Behav 99:175–180.

Cannon CM, Palmiter RD (2003) Reward without dopamine. J Neurosci Off J Soc Neurosci 23:10827–10831.

Castro DC, Cole SL, Berridge KC (2015) Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. Front Syst Neurosci 9 Available at: https://www.frontiersin.org/articles/10.3389/fnsys.2015.00090/full [Accessed July 10, 2019].

Chen T-W, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499:295–300.

Chen Y, Jang H, Spratt PWE, Kosar S, Taylor DE, Essner RA, Bai L, Leib DE, Kuo T-W, Lin Y-C, Patel M, Subkhangulova A, Kato S, Feinberg EH, Bender KJ, Knight ZA, Garrison JL (2020) Soma-Targeted Imaging of Neural Circuits by Ribosome Tethering. Neuron 107:454-469.e6.

Covelo IR, Patel ZI, Luviano JA, Stratford TR, Wirtshafter D (2014) Manipulation of GABA in the ventral pallidum, but not the nucleus accumbens, induces intense, preferential, fat consumption in rats. Behav Brain Res 270:316–325.

Cromwell HC, Berridge KC (1993) Where does damage lead to enhanced food aversion: the ventral pallidum/substantia innominata or lateral hypothalamus? Brain Res 624:1–10.

Dallimore JE, Mickiewicz AL, Napier TC (2006) Intra-ventral pallidal glutamate antagonists block expression of morphine-induced place preference. Behav Neurosci 120:1103–1114.

Dana H, Novak O, Guardado-Montesino M, Fransen JW, Hu A, Borghuis BG, Guo C, Kim DS, Svoboda K (2018) Thy1 transgenic mice expressing the red fluorescent calcium indicator jRGECO1a for neuronal population imaging in vivo. PLOS ONE 13:e0205444.

Ewald DR, Strack RW, Orsini MM (2019) Rethinking Addiction. Glob Pediatr Health 6:2333794X18821943.

Faget L, Zell V, Souter E, McPherson A, Ressler R, Gutierrez-Reed N, Yoo JH, Dulcis D, Hnasko TS (2018) Opponent control of behavioral reinforcement by inhibitory and excitatory projections from the ventral pallidum. Nat Commun 9:1–14.

Farrar AM, Font L, Pereira M, Mingote S, Bunce JG, Chrobak JJ, Salamone JD (2008) Forebrain circuitry involved in effort-related choice: Injections of the GABAA agonist muscimol into ventral pallidum alter response allocation in food-seeking behavior. Neuroscience 152:321–330.

Farrell MR, Esteban JSD, Faget L, Floresco SB, Hnasko TS, Mahler SV (2021) Ventral Pallidum GABA Neurons Mediate Motivation Underlying Risky Choice. J Neurosci 41:4500.

Farrell MR, Ye Q, Xie Y, Esteban JSD, Mahler SV (2022) Ventral pallidum GABA neurons bidirectionally control opioid relapse across rat behavioral models. Addict Neurosci 3:100026.

Freudenberg N (2001) Jails, prisons, and the health of urban populations: A review of the impact of the correctional system on community health. J Urban Health Bull N Y Acad Med 78:214–235.

Fukushima A, Hagiwara H, Fujioka H, Kimura F, Akema T, Funabashi T (2015) Sex differences in feeding behavior in rats: the relationship with neuronal activation in the hypothalamus. Front Neurosci 9 Available at: https://www.frontiersin.org/articles/10.3389/fnins.2015.00088/full [Accessed February 23, 2020].

Geisler S, Marinelli M, DeGarmo B, Becker ML, Freiman AJ, Beales M, Meredith GE, Zahm DS (2008) Prominent activation of brainstem and pallidal afferents of the ventral tegmental area by cocaine. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 33:2688–2700.

Geisler S, Zahm DS (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. J Comp Neurol 490:270–294.

Gendelis S, Inbar D, Inbar K, Mesner S, Kupchik YM (2020) Metaplasticity in the ventral pallidum as a potential marker for the propensity to gain weight in chronic high-calorie diet. J Neurosci Available at: http://www.jneurosci.org/content/early/2020/11/10/JNEUROSCI.1809-20.2020 [Accessed December 1, 2020].

Gibson M, Hearty W, Craig P (2020) The public health effects of interventions similar to basic income: a scoping review. Lancet Public Health 5:e165–e176.

Gómez-A A, Shnitko TA, Caref KL, Nicola SM, Robinson DL (2022) Stimuli predicting high-calorie reward increase dopamine release and drive approach to food in the absence of homeostatic need. Nutr Neurosci 25:593–602.

Goutaudier R, Coizet V, Carcenac C, Carnicella S (2019) DREADDs: The Power of the Lock, the Weakness of the Key. Favoring the Pursuit of Specific Conditions Rather than Specific Ligands. eNeuro 6:ENEURO.0171-19.2019.

Heimer L, Switzer RD, Van Hoesen GW (1982) Ventral striatum and ventral pallidum: Components of the motor system? Trends Neurosci 5:83–87.

Heinsbroek JA, Bobadilla A-C, Dereschewitz E, Assali A, Chalhoub RM, Cowan CW, Kalivas PW (2020) Opposing Regulation of Cocaine Seeking by Glutamate and GABA Neurons in the Ventral Pallidum. Cell Rep 30:2018-2027.e3.

Heinsbroek JA, Neuhofer DN, Griffin WC, Siegel GS, Bobadilla A-C, Kupchik YM, Kalivas PW (2017) Loss of Plasticity in the D2-Accumbens Pallidal Pathway Promotes Cocaine Seeking. J Neurosci 37:757–767.

Hiroi N, White NM (1993) The ventral pallidum area is involved in the acquisition but not expression of the amphetamine conditioned place preference. Neurosci Lett 156:9–12.

Hur EE, Zaborszky L (2005) Vglut2 afferents to the medial prefrontal and primary somatosensory cortices: a combined retrograde tracing in situ hybridization study [corrected]. J Comp Neurol 483:351–373.

Inutsuka A, Yamanaka A (2013) The physiological role of orexin/hypocretin neurons in the regulation of sleep/wakefulness and neuroendocrine functions. Front Endocrinol 4:18.

Jansen A, Theunissen N, Slechten K, Nederkoorn C, Boon B, Mulkens S, Roefs A (2003) Overweight children overeat after exposure to food cues. Eat Behav 4:197–209.

Johansson O, Hökfelt T, Elde RP (1984) Immunohistochemical distribution of somatostatin-like immunoreactivity in the central nervous system of the adult rat. Neuroscience 13:265–339.

Knowland D, Lilascharoen V, Pacia CP, Shin S, Wang EH-J, Lim BK (2017) Distinct Ventral Pallidal Neural Populations Mediate Separate Symptoms of Depression. Cell 170:284-297.e18.

Kupchik YM, Prasad AA (2021) Ventral pallidum cellular and pathway specificity in drug seeking. Neurosci Biobehav Rev 131:373–386.

Lederman J, Lardeux S, Nicola SM (2021) Vigor Encoding in the Ventral Pallidum. eNeuro 8:ENEURO.0064-21.2021.

Legaria AA, Matikainen-Ankney BA, Yang B, Ahanonu B, Licholai JA, Parker JG, Kravitz AV (2022) Fiber photometry in striatum reflects primarily nonsomatic changes in calcium. Nat Neurosci 25:1124–1128.

Leung BK, Balleine BW (2015) Ventral pallidal projections to mediodorsal thalamus and ventral tegmental area play distinct roles in outcome-specific Pavlovian-instrumental transfer. J Neurosci Off J Soc Neurosci 35:4953–4964.

Levi LA, Inbar K, Nachshon N, Bernat N, Gatterer A, Inbar D, Kupchik YM (2020) Projection-Specific Potentiation of Ventral Pallidal Glutamatergic Outputs after Abstinence from Cocaine. J Neurosci 40:1276–1285.

Li Y-D, Luo Y-J, Xu W, Ge J, Cherasse Y, Wang Y-Q, Lazarus M, Qu W-M, Huang Z-L (2020) Ventral pallidal GABAergic neurons control wakefulness associated with motivation through the ventral tegmental pathway. Mol Psychiatry:1–17.

Liu Y-J, Ehrengruber MU, Negwer M, Shao H-J, Cetin AH, Lyon DC (2013) Tracing Inputs to Inhibitory or Excitatory Neurons of Mouse and Cat Visual Cortex with a Targeted Rabies Virus. Curr Biol 23:1746–1755.

Ma R, Mikhail ME, Culbert KM, Johnson AW, Sisk CL, Klump KL (2020) Ovarian Hormones and Reward Processes in Palatable Food Intake and Binge Eating. Physiology 35:69–78.

Mahler SV, Vazey EM, Beckley JT, Keistler CR, McGlinchey EM, Kaufling J, Wilson SP, Deisseroth K, Woodward JJ, Aston-Jones G (2014) Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. Nat Neurosci 17:577–585.

Martinez VK, Saldana-Morales F, Sun JJ, Zhu PJ, Costa-Mattioli M, Ray RS (2019) Off-Target Effects of Clozapine-N-Oxide on the Chemosensory Reflex Are Masked by High Stress Levels. Front Physiol 10 Available at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00521 [Accessed March 4, 2023].

McFarland K, Kalivas PW (2001) The Circuitry Mediating Cocaine-Induced Reinstatement of Drug-Seeking Behavior. J Neurosci 21:8655–8663.

McHugh RK, Hearon BA, Otto MW (2010) Cognitive-Behavioral Therapy for Substance Use Disorders. Psychiatr Clin North Am 33:511–525.

McKenna JT, Yang C, Franciosi S, Winston S, Abarr KK, Rigby MS, Yanagawa Y, McCarley RW, Brown RE (2013) Distribution and Intrinsic Membrane Properties of Basal Forebrain GABAergic and Parvalbumin Neurons in the Mouse. J Comp Neurol 521:1225–1250.

Mehrkam LR (2020) The Cognitive Abilities of Wild Animals. In: Zoo Animal Learning and Training, pp 15–34. John Wiley & Sons, Ltd. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118968543.ch2 [Accessed March 2, 2023].

Meng C, Zhou J, Papaneri A, Peddada T, Xu K, Cui G (2018) Spectrally Resolved Fiber Photometry for Multi-component Analysis of Brain Circuits. Neuron 98:707-717.e4.

Morais-Silva G, Campbell RR, Nam H, Basu M, Pagliusi M, Fox ME, Chan S, Iñiguez SD, Ament S, Cramer N, Marin MT, Lobo MK (2022) Molecular, circuit, and stress response characterization of Ventral Pallidum Npas1-neurons. J Neurosci Off J Soc Neurosci:JN-RM-0971-22.

Morgane PJ (1961) Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. Am J Physiol-Leg Content 201:420–428.

Morton GJ, Meek TH, Schwartz MW (2014) Neurobiology of food intake in health and disease. Nat Rev Neurosci 15:367–378.

Navarro M, Olney JJ, Burnham NW, Mazzone CM, Lowery-Gionta EG, Pleil KE, Kash TL, Thiele TE (2016) Lateral Hypothalamus GABAergic Neurons Modulate Consummatory Behaviors Regardless of the Caloric Content or Biological Relevance of the Consumed Stimuli. Neuropsychopharmacology 41:1505–1512.

Negrete JC, Emil S (1992) Cue-evoked arousal in cocaine users: a study of variance and predictive value. Drug Alcohol Depend 30:187–192.

Nicola SM, Yun IA, Wakabayashi KT, Fields HL (2004a) Firing of Nucleus Accumbens Neurons During the Consummatory Phase of a Discriminative Stimulus Task Depends on Previous Reward Predictive Cues. J Neurophysiol 91:1866–1882.

Nicola SM, Yun IA, Wakabayashi KT, Fields HL (2004b) Cue-Evoked Firing of Nucleus Accumbens Neurons Encodes Motivational Significance During a Discriminative Stimulus Task. J Neurophysiol 91:1840–1865.

Ottenheimer D, Richard JM, Janak PH (2018) Ventral pallidum encodes relative reward value earlier and more robustly than nucleus accumbens. Nat Commun 9:4350.

Ottenheimer DJ, Bari BA, Sutlief E, Fraser KM, Kim TH, Richard JM, Cohen JY, Janak PH (2020a) A quantitative reward prediction error signal in the ventral pallidum. Nat Neurosci 23:1267–1276.

Ottenheimer DJ, Wang K, Tong X, Fraser KM, Richard JM, Janak PH (2020b) Reward activity in ventral pallidum tracks satiety-sensitive preference and drives choice behavior. Sci Adv 6:eabc9321.

Parker NF, Cameron CM, Taliaferro JP, Lee J, Choi JY, Davidson TJ, Daw ND, Witten IB (2016) Reward and choice encoding in terminals of midbrain dopamine neurons depends on striatal target. Nat Neurosci 19:845–854.

Patel JM, Swanson J, Ung K, Herman A, Hanson E, Ortiz-Guzman J, Selever J, Tong Q, Arenkiel BR (2019) Sensory perception drives food avoidance through excitatory basal forebrain circuits. eLife 8.

Pérez SE, Wynick D, Steiner RA, Mufson EJ (2001) Distribution of galaninergic immunoreactivity in the brain of the mouse. J Comp Neurol 434:158–185.

Perry CJ, Zbukvic I, Kim JH, Lawrence AJ (2014) Role of cues and contexts on drug-seeking behaviour. Br J Pharmacol 171:4636–4672.

Pitchers KK, Sarter M, Robinson TE (2018) The hot ‘n’ cold of cue-induced drug relapse. Learn Mem 25:474–480.

Prasad AA, McNally GP (2016) Ventral Pallidum Output Pathways in Context-Induced Reinstatement of Alcohol Seeking. J Neurosci 36:11716–11726.

Prasad AA, Xie C, Chaichim C, Nguyen JH, McClusky HE, Killcross S, Power JM, McNally GP (2020a) Complementary Roles for Ventral Pallidum Cell Types and Their Projections in Relapse. J Neurosci 40:880–893.

Prasad AA, Xie C, Chaichim C, Nguyen JH, McClusky HE, Killcross S, Power JM, McNally GP (2020b) Complementary Roles for Ventral Pallidum Cell Types and Their Projections in Relapse. J Neurosci 40:880–893.

Rehman I, Mahabadi N, Sanvictores T, Rehman CI (2022) Classical Conditioning. In: StatPearls. Treasure Island (FL): StatPearls Publishing. Available at: http://www.ncbi.nlm.nih.gov/books/NBK470326/ [Accessed March 2, 2023].

Richard JM, Ambroggi F, Janak PH, Fields HL (2016) Ventral Pallidum Neurons Encode Incentive Value and Promote Cue-Elicited Instrumental Actions. Neuron 90:1165–1173.

Richard JM, Stout N, Acs D, Janak PH (2018a) Ventral pallidal encoding of reward-seeking behavior depends on the underlying associative structure. eLife 7:e33107.

Richard JM, Stout N, Acs D, Janak PH (2018b) Ventral pallidal encoding of reward-seeking behavior depends on the underlying associative structure Gold JI, ed. eLife 7:e33107.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247–291.

Root DH, Melendez RI, Zaborszky L, Napier TC (2015) The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors. Prog Neurobiol 130:29–70.

Rossi MA, Stuber GD (2018) Overlapping Brain Circuits for Homeostatic and Hedonic Feeding. Cell Metab 27:42–56.

Saah T (2005) The evolutionary origins and significance of drug addiction. Harm Reduct J 2:8.

Saunders BT, Richard JM, Margolis EB, Janak PH (2018) Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. Nat Neurosci 21:1072–1083.

Sharpe MJ, Marchant NJ, Whitaker LR, Richie CT, Zhang YJ, Campbell EJ, Koivula PP, Necarsulmer JC, Mejias-Aponte C, Morales M, Pickel J, Smith JC, Niv Y, Shaham Y, Harvey BK, Schoenbaum G (2017) Lateral Hypothalamic GABAergic Neurons Encode Reward Predictions that Are Relayed to the Ventral Tegmental Area to Regulate Learning. Curr Biol CB 27:2089-2100.e5.

Shemesh OA et al. (2020) Precision Calcium Imaging of Dense Neural Populations via a Cell-Body-Targeted Calcium Indicator. Neuron 107:470-486.e11.

Shields AK, Suarez M, Wakabayashi KT, Bass CE (2021) Activation of VTA GABA neurons disrupts reward seeking by altering temporal processing. Behav Brain Res 410:113292.

Shiffman S, Dunbar M, Kirchner T, Li X, Tindle H, Anderson S, Scholl S (2013) Smoker reactivity to cues: Effects on craving and on smoking behavior. J Abnorm Psychol 122:264–280.

Shimura T, Imaoka H, Yamamoto T (2006) Neurochemical modulation of ingestive behavior in the ventral pallidum. Eur J Neurosci 23:1596–1604.

Smith KS, Berridge KC (2005) The ventral pallidum and hedonic reward: neurochemical maps of sucrose “liking” and food intake. J Neurosci Off J Soc Neurosci 25:8637–8649.

Smith KS, Berridge KC, Aldridge JW (2011) Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. Proc Natl Acad Sci U S A 108:E255-264.

Smith KS, Tindell AJ, Aldridge JW, Berridge KC (2009) Ventral pallidum roles in reward and motivation. Behav Brain Res 196:155–167.

Smith MS, Freeman ME, Neill JD (1975) The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96:219–226.

Soares-Cunha C, Heinsbroek JA (2023) Ventral pallidal regulation of motivated behaviors and reinforcement. Front Neural Circuits 17:1086053.

Stanojlovic M, Pallais JP, Kotz CM (2021) Inhibition of Orexin/Hypocretin Neurons Ameliorates Elevated Physical Activity and Energy Expenditure in the A53T Mouse Model of Parkinson’s Disease. Int J Mol Sci 22:795.

Stephenson-Jones M, Bravo-Rivera C, Ahrens S, Furlan A, Fernandes-Henriques C, Li B (2019) Opposing contributions of GABAergic and glutamatergic ventral pallidal neurons to motivational behaviours. bioRxiv:594887.

Stephenson-Jones M, Bravo-Rivera C, Ahrens S, Furlan A, Xiao X, Fernandes-Henriques C, Li B (2020) Opposing Contributions of GABAergic and Glutamatergic Ventral Pallidal Neurons to Motivational Behaviors. Neuron Available at: http://www.sciencedirect.com/science/article/pii/S0896627319310487 [Accessed January 21, 2020].

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

Stratford TR, Wirtshafter D (2013) Lateral hypothalamic involvement in feeding elicited from the ventral pallidum. Eur J Neurosci 37:648–653.

Stuber GD, Wise RA (2016a) Lateral Hypothalamic Circuits for Feeding and Reward. Nat Neurosci 19:198–205.

Stuber GD, Wise RA (2016b) Lateral hypothalamic circuits for feeding and reward. Nat Neurosci 19:198–205.

Taylor LA, Tan AX, Coyle CE, Ndumele C, Rogan E, Canavan M, Curry LA, Bradley EH (2016) Leveraging the Social Determinants of Health: What Works? PLoS ONE 11:e0160217.

Teichroeb JA, Chapman CA (2014) Sensory information and associative cues used in food detection by wild vervet monkeys. Anim Cogn 17:517–528.

Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI, Jayaraman V, Svoboda K, Looger LL (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods 6:875–881.

Tindell AJ, Berridge KC, Aldridge JW (2004) Ventral pallidal representation of pavlovian cues and reward: population and rate codes. J Neurosci Off J Soc Neurosci 24:1058–1069.

Tindell AJ, Berridge KC, Zhang J, Peciña S, Aldridge JW (2005) Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22:2617–2634.

Tooley J, Marconi L, Alipio JB, Matikainen-Ankney B, Georgiou P, Kravitz AV, Creed MC (2018) Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula–Tegmental Circuitry and Constrain Reward Seeking. Biol Psychiatry 83:1012–1023.

Vachez YM, Tooley JR, Abiraman K, Matikainen-Ankney B, Casey E, Earnest T, Ramos LM, Silberberg H, Godynyuk E, Uddin O, Marconi L, Le Pichon CE, Creed MC (2021) Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption. Nat Neurosci 24:379–390.

Verbeke G (1997) Linear Mixed Models for Longitudinal Data. In: Linear Mixed Models in Practice: A SAS-Oriented Approach (Verbeke G, Molenberghs G, eds), pp 63–153 Lecture Notes in Statistics. New York, NY: Springer. Available at: https://doi.org/10.1007/978-1-4612-2294-1_3 [Accessed December 8, 2022].

Volkow ND, Fowler JS, Wang G-J, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry 9:557–569.

Wakabayashi KT, Feja M, Baindur AN, Bruno MJ, Bhimani RV, Park J, Hausknecht K, Shen R-Y, Haj-Dahmane S, Bass CE (2019) Chemogenetic activation of ventral tegmental area GABA neurons, but not mesoaccumbal GABA

terminals, disrupts responding to reward-predictive cues. Neuropsychopharmacology 44:372–380.

Wei Z, Lin B-J, Chen T-W, Daie K, Svoboda K, Druckmann S (2020) A comparison of neuronal population dynamics measured with calcium imaging and electrophysiology. PLOS Comput Biol 16:e1008198.

White NM (2011) Reward: What Is It? How Can It Be Inferred from Behavior? In: Neurobiology of Sensation and Reward (Gottfried JA, ed) Frontiers in Neuroscience. Boca Raton (FL): CRC Press/Taylor & Francis. Available at: http://www.ncbi.nlm.nih.gov/books/NBK92792/ [Accessed February 23, 2023].

Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, Zou F, Gent LM, Hahner LD, Khan SA, Elias CF, Elmquist JK, Clegg DJ (2011) Distinct Hypothalamic Neurons Mediate Estrogenic Effects on Energy Homeostasis and Reproduction. Cell Metab 14:453–465.

Young PT (1959) The role of affective processes in learning and motivation. Psychol Rev 66:104.

Zaborszky L, van den Pol A, Gyengesi E (2012) Chapter 28 - The Basal Forebrain Cholinergic Projection System in Mice. In: The Mouse Nervous System (Watson C, Paxinos G, Puelles L, eds), pp 684–718. San Diego: Academic Press. Available at: https://www.sciencedirect.com/science/article/pii/B9780123694973100287 [Accessed March 4, 2023].

Zhang L, Hernandez-Sanchez D, Herzog H (2019) Regulation of Feeding-Related Behaviors by Arcuate Neuropeptide Y Neurons. Endocrinology 160:1411–1420.

Zhu C, Yao Y, Xiong Y, Cheng M, Chen J, Zhao R, Liao F, Shi R, Song S (2017) Somatostatin Neurons in the Basal Forebrain Promote High-Calorie Food Intake. Cell Rep 20:112–123.