Signals of Physiological Status Modulate

Food- and Food-Cue-Driven Activity of Dopamine Neurons

BY

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THESIS

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TABLE OF CONTENTS

CHAPTER **PAGE**

CHAPTER I: INTRODUCTION 1

A. Preface 1

B. Neural control of eating behaviors 1

C. Dopamine control of motivated behavior 6

D. The neuroanatomical organization of the mesolimbic dopamine system 9

E. Eating behavior is multifaceted and is encoded in the mesolimbic dopamine system 13

F. Hunger modulates aspects of eating behavior related to dopamine signaling 15

G. The hindbrain is a gatekeeper in gut-brain communication 16

H. Glucose utilization is a harbinger of hunger 19

I. Glucagon-like peptide 1 signaling regulates satiety and the suppression of motivated eating behavior 21

J. Implications for current studies 23

CHAPTER II: CYTOGLUCOPENIA POTENTIATES DOPAMINE NEURON ACTIVITY EVOKED BY FOOD REWARD AND FOOD PREDICTIVE CUES 25

A. Introduction 25

B. Materials and methods 27

B. 1. Subjects 27

B. 2. Surgeries 27

B. 3. Intraoral infusion and photometry sessions 28

B. 4. Drug injections 29

B. 5. In Vivo Fiber Photometry 30

B. 6. Signal normalization and processing 31

B. 7. Immunohistochemistry and verification of recording sites 31

B. 8. Statistical analyses 32

C. Results 33

C. 1. Food restriction enhances phasic dopamine signaling in the ventral tegmental area to food 33

TABLE OF CONTENTS (continued)

C. 2. 5TG induced cytoglucopenia potentiates food evoked phasic dopamine signaling 34

C. 3. Insulin induced cytoglucopenia potentiates food evoked phasic dopamine signaling 35

C. 4. 5TG fails to potentiate phasic dopamine signaling to intraoral water 37

C. 5. Central glucagon-like peptide 1 receptor (GLP-1R) stimulation suppresses phasic dopamine signaling to food 37

C. 6. Food-cues evoke robust phasic dopamine signaling in the ventral tegmental area 38

C. 7. 5TG potentiates food-cue evoked and sucrose evoked dopamine signaling in a site-specific manner 39

D. Discussion 39

CHAPTER III: PHASIC DOPAMINE RESPONSES TO A FOOD-PREDICTIVE CUE ARE SUPPRESSED BY THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST EXENDIN-4 51

A. Introduction 51

B. Materials and methods 53

B. 1. Subjects 53

B. 2. Behavior 54

B. 3. Surgery 54

B. 4. Fiber photometry 55

B. 5. Signal normalization 56

B. 6. Pharmacology 56

B. 7. Immunohistochemistry and verification of recording sites 57

B. 8. Statistical analyses 57

C. Results 58

C. 1. Selective expression of calcium-dependent fluorescent construct captures dynamic fluctuations in dopamine signaling 58

C. 2. GLP-1R activation suppresses sucrose directed behavior 60

C. 3. Central GLP-1R activation selectively suppresses cue evoked dopamine activity in the VTA 61

C. 4. Magnitude of cue-evoked dopamine is correlated with indices of behavior 62

D. Discussion 62

CHAPTER IV: GENERAL DISCUSSION 75

A. Physiological state modulates the neural representation of taste 76

TABLE OF CONTENTS (continued)

B. Dopamine responses to food predictive cues are modulated by physiological state 78

C. Cytoglucopenia potentiates dopamine signaling to food and food-cues 80

D. Glucagon-like peptide 1 suppresses dopamine signaling to food and food-cues..............84

E. The implications of a multiplexed dopamine signaling for obesity....................................87

CITATIONS 90

COPYRIGHT STATEMENT 139

CURRICULUM VITAE 140

TABLE OF FIGURES

CHAPTER II

Figure II.1: Intraoral sucrose evokes dopamine neuron activity in the VTA. 45

Figure II.2: 5TG induced cytoglucopenia modulates sucrose evoked dopamine neuron activity in a site- and time-dependent manner. 46

Figure II.3: Peripheral insulin induced cytoglucopenia potentiates sucrose evoked dopamine. . 47

Figure II.4: 5TG fails to potentiate water evoked dopamine neuron activity. 48

Figure II.5: Satiety inducing glucagon-like 1 receptor (GLP-1R) stimulation suppresses sucrose evoked dopamine neuron activity. 49

Figure II.6: 5TG potentiates CS+ and sucrose evoked dopamine signaling in a site-specific manner. 50

CHAPTER III

Figure III.1: Selective expression of calcium-dependent fluorescent construct in dopamine neurons captures dynamic fluctuations in dopamine signaling. 70

Figure III.2: Ex4 dose-dependently suppresses indices of sucrose-directed behavior (n=5 male and n=5 female rats). 71

Figure III.3: Spontaneous VTA dopamine transients are not modulated by central Ex4. 72

Figure III.4: Central Ex4 suppresses cue evoked dopamine activity. 73

Figure III.5: Magnitude of cue evoked dopamine activity is correlated with indices of sucrose-directed behavior. 74

LIST OF ABBREVIATIONS

2DG	2-deoxy-glucose
4V	fourth ventricle
5TG	5-thio-D-glucose
aCSF	artificial cerebrospinal fluid
ADP	adenosine monophosphate
AMPK	5' adenosine monophosphate activated protein kinase
AngII	angiotensin II
ANOVA	analysis of variance
AP	anterior-Posterior
ARC	arcuate nucleus
ATP	adenosine triphosphate
CA	catecholamine
Ca2+	calcium
cAMP	cyclic adenosine monophosphate
CCK	cholecystokinin
CGRP	calcitonin gene-related peptide
CNS	central nervous system
Cre	cre recombinase
CS-	negative conditioning stimulus
CS+	positive conditioning stimulus
DV	dorsal-ventral
Ex4	exendin-4
Ga	guage
GABA	gamma-aminobutyric acid
GC/mL	genome copies per mL
GFP	green fluorescent protein
GLP-1	glucagon-like peptide 1
GLP-1R	glucagon-like peptide 1 receptor
GPCR	g protein-coupled receptor
h	hours
HLM	hierarchical linear model
Hz	hertz
i.c.v.	intracerebroventricular
i.p.	intraperotineal
ICSS	intracranial self stimulation
kg	kilogram
KPBS	potassium phosphate buffered saline
LDTg	lateral dorsal tegmental nucleus
LH	lateral Hypothalamus
LHA	lateral Hypothalamus
lPBN	lateral parabrachial nucleus
LV	lateral ventricle

mg	milligram
min	minutes
ML	medial-lateral
mL	milliliter
mm	millimeter
n	sample size
NAc	nucleus accumbens
NaCl	sodium chloride
ng	nanograms
nm	nanometer
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
PBS	phosphate buffered saline
PKA	protein kinase A
PR	progressive ratio
PVN	paraventricular nucleus of the hypothalamus
RPE	reward prediction error
s	seconds
s.c.	subcutaneous
sEPSC	spontaneous excitatory postsynaptic currents
TH	tyrosine hydroxylase
U/kg	units per kilogram
VTA	ventral tegmental area
ΔF/F	ratiometric change in fluorescence
µL	microliter
µm	micrometer

Summary

An animal's survival depends on a stable internal physiological status. Homeostasis must be maintained to adaptively navigate the world. Various physiological mechanisms are controlled within narrow ranges and deviations from homeostasis are detrimental to the organism's survival. Return to homeostasis is critical and largely governed by the organism's adaptive motivated behaviors. One neural substrate that initiates and maintains motivated behaviors is the mesolimbic dopamine system. Food and food associated stimuli evoke phasic increases in dopamine neuronal activity and dopamine release that correlate with approach behavior. Moreover, physiological state changes how food and food-cues influence behaviors that are dependent on phasic dopamine signaling. However, it is unclear how physiological state modulates dopamine signaling to generate appropriate behaviors. Identifying modulators of dopamine response magnitude may serve as viable interventions for maladaptive motivated behavior. The present thesis aims to explore signals that convey physiological state information to the mesolimbic dopamine system in the service of motivated behaviors and ultimately, homeostasis. Within homeostasis, energy status is tightly controlled. Perturbations in available energy generate hunger or satiety, leading an organism to engage in or cease behaviors directed at calories, respectively. Manipulating physiological state and understanding its role in modulating dopamine signaling will help unravel the neural circuitry of motivated behaviors.

The central nervous system to conveys energy status through many signals, including glucose and glucagon-like peptide 1 (GLP-1). Glucose is a ubiquitous energy substrate monitored by the brain. Importantly, low glucose utilization (cytoglucopenia) is detected by the brain to promote robust motivated behavior directed at food - and is refered as a 'hunger' signal. Conversely, GLP-1 acts to suppress food intake and is known as a 'satiety' signal. Here, I manipulated these signals while measuring mesolimbic dopamine neural activity to characterize a node in a circuit responsive to appetitive and consummatory food reward.

My research elucidates the neural mechanisms, originating in the mesolimbic dopamine system, that process food and food-cues in the face of caloric deficit and surfeit. In the first study I recorded real-time dopamine neuron activity in the ventral tegmental area (VTA) using a calcium sensor, GCaMP, while subjects were given intraoral sucrose. Central cytoglucopenia increased the magnitude of phasic VTA dopamine signaling evoked by intraoral sucrose and by sucrose associated cues. Interestingly, cytoglucopenia failed to augment water evoked phasic dopamine signaling, supporting that this circuit is tuned toward caloric stimuli. Furthermore, forebrain cytoglucopenia but not hindbrain cytoglucopenia potentiated sucrose-cue evoked phasic VTA dopamine signaling, suggesting a forebrain control of learned associations. In contrast, only hindbrain cytoglucopenia potentiated sucrose evoked VTA dopamine signaling, implying that hindbrain circuits may provide the VTA information regarding the sucrose reward. In the second study, I recorded activity in dopamine neurons with GCaMP while subjects were allowed to approach and ingest sucrose from a sipper which was preceded by an audio cue. I showed that GLP-1 suppressed phasic dopamine signaling to sucrose and sucrose-associated cues, while also suppressing food-directed behaviors. Together, this work supports that cytoglucopenia and GLP-1R signaling modulate eating behavior via central dopamine signaling.

Chapter I: Introduction

A. <u>Preface</u>

Many physiological parameters (e.g., pH, temperature, energy balance) of an organism are tightly controlled and are essential for survival. Any perturbations from the homeostatic set points result in compensatory behaviors to restore balance in that system. For example, food seeking and eating behaviors are compensatory responses to caloric deficiency and are a significant part of our daily life. However, we don't engage in eating behaviors passively to replenish our energy deficit. Rather, hunger and satiety inform how we view food and food-associated cues, which in turn have powerful influence on our decisions on whether to seek and consume food. The signals and neural pathways that integrate physiological state and the drive directed toward food are only recently being elucidated.

Clinical and preclinical studies have characterized and implicated specific regions of the central nervous system in the expression of food-related behaviors during caloric deficiency. However, in today's obesogenic society, where the "sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals," (Swinburn et al., 1999) environmental factors undoubtedly influence the problem of obesity. To help ameliorate the global obesity crisis, we must understand the mechanisms through which signals that convey physiological state modulate neural representations of environmental cues and food stimuli.

In this manuscript, I will first provide a general overview of the neural control of eating behaviors. Then, I will describe the mesolimbic dopamine system's role in driving motivated behaviors, specifically in the context of eating. I will continue by discussing the hindbrain as a key region in the communication between the periphery and the brain via neural mechanisms and hormonal mechanisms. Then, I will discuss the role of glucose-sensing in hunger and the role of GLP-1 in satiety. This introduction section will culminate with the main implications and

questions answered by my work— those questions regarding how physiological state modulates mesolimbic dopamine signaling to appetitive and consummatory food reward. Collectively, these novel studies show that cytoglucopenia (signal of caloric deficit) and GLP-1 (signal of caloric surfeit) are both integrated in dopamine representations of food and food-cues that guide motivated behaviors toward food.

B. Neural control of eating behaviors

Eating is a motivated behavior directed toward food and is aroused by interoceptive and exteroceptive stimuli (Watts, 2001). Eating consists of four phases: initiation, appetitive (e.g. foraging), consummatory (e.g. biting, chewing, swallowing, etc.), and termination (Craig, 1917). Decades of research have been dedicated to understanding the neural substrates that govern each phase of eating behavior, with one of the largest contributions made by Eliot Stellar in 1954. He established a “dual-center” hypothesis that posited that the hypothalamus contained two population of neurons, one excitatory and one inhibitory that orchestrated the drive for and the cessation of motivated behaviors (Stellar, 1954). In Stellar’s scheme, exteroceptive sensory stimuli and interoceptive physiological signals are integrated at the hypothalamus. The importance of the hypothalamus in appetitive behaviors was highlighted by pioneering work by Harvey Grill and Ralph Norgren. Rats were decerebrated by transecting the brain at the level of the superior colliculus, leaving the hypothalamus disconnected from the rest of the central nervous system. Decerebrate rats failed to approach food and only eat when food was delivered directly into their oral cavities (H. Grill & Norgren, 1978). Because decerebrate rats do not spontaneously eat, changes in food intake are measured by the amount of intraoral sucrose consumed until the rats reject the sucrose solution by passively letting it drip out of their mouths. Therefore, the hindbrain is sufficient to enact consummatory behaviors (the consummatory phase—stereotypical action that acquires the goal and terminates the motivated behavior (Freeman & Sherrington, 2006)). However, the motivation to eat (the appetitive phase—

component of motivated behavior that increases the likelihood in acquiring the goal (Freeman & Sherrington, 2006)) seems to be controlled somewhere in the forebrain. While not necessary for consummatory behavior, communication between the hypothalamus and the hindbrain is critical for appetitive drive.

Although decerebration provides much needed insight into motivated behaviors, it essentially removes the whole forebrain, making it difficult to isolate the importance of the hypothalamus. More focal lesions of the hypothalamus were performed in seminal work by Bal Anand and John Brobeck, who Stellar featured in his appraisal of the hypothalamus in his hypothesis. Within the Stellar dual-center hypothesis, an excitatory center in the hypothalamus provides the drive for motivated behavior and the excitatory center is held in check by an upstream inhibitory hypothalamic center. In Anand and Brobeck's experiments, large bilateral electrolytic lesions in the lateral hypothalamus (LH) abolished food intake, whereas bilateral lesions of the ventromedial hypothalamus (VMH) resulted in voracious eating (Anand & Brobeck, 1951). Interestingly, the hypophagia of LH-lesioned animals could not be rescued with lesions in the VMH. Such lesions supported Stellar's idea that the LH served as an excitatory circuit held in check by VMH inhibition. Strong hypophagia resulting from LH destruction came to be known as "LH syndrome." The syndrome was defined with four clear-cut stages in the recovery from lateral hypothalamic lesions: aphagia and adipsia; anorexia and adipsia; adipsia with a secondary dehydration-aphagia; and recovery (Teitelbaum & Epstein, 1962). Due to LH ablation effects on eating behaviors, the LH was named the "eating center" of the brain and the VMH was named the "satiety center."

While the hypothalamic lesions linked the hypothalamus to motivated behaviors, gross lesions failed to account for any cell-type specificity. Recent literature has characterized cell-specific contributions to eating behavior. Firstly, studies that specifically destroyed neurons and spared any fibers of passage show that the LH contains neurons that are necessary for eating

(Grossman et al., 1978; Grossman & Grossman, 1982; Marshall et al., 1976). Glutamate receptor activation in the LH increases food intake (Stanley et al., 1993), while activating γ-Aminobutyric acid (GABA) receptors suppresses food intake (Kelly et al., 1979). The LH contains different cell types that express a variety of combinations of receptors and neuropeptides (Allen & Cechetto, 1995; Burdakov & Alexopoulos, 2005; Goforth et al., 2014; Griffond & Risold, 2009; Knight et al., 2012; Laque et al., 2013; Leinninger et al., 2009; Rosin et al., 2003). Majority of these cell types are either GABAergic or glutamatergic and some co-express neuropeptides. Some GABAergic neurons co-express melanin-concentrating hormone (MCH) and some glutamatergic neurons co-express orexin. MCH and orexin are unique to the LH and are never expressed in the same neurons (Broberger et al., 1998). Indeed, the stimulation of either of these populations stimulates eating behavior (Barson et al., 2013). Although orexin (Peyron et al., 1998) and MCH (Bittencourt, 2011) neurons project throughout the brain, MCH is also released into the cerebrospinal fluid (Noble et al., 2018) as a complementary means of stimulating MCH receptors throughout the brain. The LH has a diverse population of neuronal cell types and is still under intense investigation.

In addition to sending efferents that control eating behavior, the LH neurons receive input from various other nuclei that are involved in food intake, including the arcuate nucleus (ARC). The ARC contains cell bodies that are necessary for eating and is located at the base of the brain near the median eminence, a fenestrated region with highly permeable blood vessels (Ciofi, 2011). The ARC has privileged hormonal access and can regulate various eating behaviors based on physiological signals (R. Cone et al., 2001). Indeed, ARC neurons express receptors for various circulating factors that regulate eating behaviors, such as leptin, ghrelin, glucose, and insulin (Könner et al., 2009; Q. Wang et al., 2014; R. Wang et al., 2004). Key ARC neurons involved in hunger can be distinguished by their production of neuropeptide Y (NPY) and agouti-related peptide (AgRP). The NPY/AgRP neurons stimulate eating behaviors by

releasing NPY, especially after a fasting period (Kalra et al., 1991). Selective stimulation of NPY/AgRP neurons increases eating (Aponte et al., 2011; Krashes et al., 2011) and selective ablation decreases eating and body weight (Gropp et al., 2005; Luquet et al., 2005). However, decreasing the expression of either NPY or AgRP alone, or both together, does not decrease eating in the same fashion (Palmiter et al., 1998; Qian et al., 2002), suggesting that there are other necessary mechanisms that add to the control of eating. Conversely, ARC neurons that produce proopiomelanocortin (POMC) generally have the opposite effect on feeding-behavior relative to ARC NPY/AgRP neurons. POMC neurons co-express and release α-melanocyte-stimulating-hormone (α-MSH) and stimulate melanocortin receptor 4 (MC4R) of paraventricular nucleus of the hypothalamus (PVN) neurons to suppress eating (Fenselau et al., 2017). Interestingly, AgRP acts as an inverse agonist on the MC4R (Nijenhuis et al., 2001) and inhibits α-MSH action (Ollmann et al., 1997). NPY/AgRP neurons also co-express GABA and inhibit POMC neurons through GABA-A receptor activation (Tong et al., 2008). Furthermore, AgRP neurons directly innervate the PVN to increase eating (Atasoy et al., 2012) and AgRP overexpression leads to obesity (Yen et al., 1994). In general, AgRP neurons drive eating via direct action on MC4R on PVN neurons and indirect inhibition of POMC neurons. Meanwhile POMC neurons counter the AgRP drive to eat by α-MSH action on MC4R of PVN neurons to suppress eating. Collectively, the ARC acts on the PVN with inputs that can either drive or suppress eating behaviors.

The PVN sits next to the third ventricle and contains a dense population of heterogeneous neurons (Sawchenko & Swanson, 1983; Swanson & Sawchenko, 1983). PVN administration of almost any orexigenic (i.e., to generate eating) signal results in increased food intake (Dube et al., 1999; Kalra et al., 1991; Kelly et al., 1979; Kelly & Grossman, 1979; Morley, 1987; Stanley & Leibowitz, 1984), suggesting that the PVN is a critical site of action of signals that convey physiological state. Experiments that label cFos, an immediate early gene

expressed in recently active cells, show that there is increased activity in PVN neurons that receive input from orexigenic (B.-H. Li et al., 1994; Xu et al., 1995) and anorxigenic signals (Elmquist et al., 1997; Schwartz et al., 1996; Yokosuka et al., 1998). Lesions in the PVN cause hyperphagia and obesity, further highlighting the importance of this region in the regulation of food intake (Leibowitz et al., 1981; Weingarten, 1985). Therefore, the ARC, LH, and PVN all act together to regulate eating behaviors. LH neuronal activity is correlated with either appetitive or with consummatory behaviors (Jennings et al., 2015). However, the influences of the hypothalamus alone are insufficient to explain how activity in these regions influence motivating drive. The following section will describe how this missing link between orexigenic factors and motivation is provided by the brain's dopamine circuitry.

C. <u>Dopamine control of motivated behavior</u>

Lesions in the LH destroy eating behaviors (Anand & Brobeck, 1951). Although LH cell bodies are critical for producing behaviors toward food, electrolytic lesions not only destroy cell bodies but also sever fibers of passage. Fibers of passage in the LH comprise the medial forebrain bundle, a majority of which are dopamine axons that course from the midbrain to the striatum (Andén et al., 1966). Therefore, it is possible that the loss of dopamine signaling may contribute to the loss of eating behaviors. Urban Ungerstedt addressed the ambiguity of whether eating behavior was controlled solely by the LH cell bodies or also by dopamine fibers of passage. Bilateral LH injections of a dopamine-specific neurotoxic compound, 6-OHDA, produced rats that did not approach or consume food, recapitulating the hypophagic nature of "LH syndrome" (Ungerstedt, 1970). This strategy of only destroying the dopamine fibers while leaving LH cell bodies intact was a first step in suggesting a role for dopamine signaling in eating behaviors. Later work by Richard Palmiter and colleagues further supported dopamine involvement by showing that mice could be made hypoactive, adipsic, and aphagic by a selective knockout of tyrosine hydroxylase, the rate limiting enzyme for the cellular production of

dopamine (Zhou & Palmiter, 1995). Eating behavior in dopamine deficient mice could then be rescued by restoring dopamine production via administering L-DOPA, a dopamine precursor (Szczypka et al., 2001). Collectively, data support that dopamine signaling is necessary in the expression of the approach behavior (appetitive phase) toward food.

Dopamine plays an essential role in producing and maintaining motivated behaviors, including those directed at rewarding stimuli such as food, sex, or drugs of abuse. For example, all drugs abused by humans (e.g., opiates, ethanol, nicotine, amphetamine, and cocaine) increase extracellular dopamine concentrations in the nucleus accumbens and in the caudate nucleus (Chiara & Imperato, 1988). Dopamine lesions eliminate reward-seeking toward stimulants or opiates (Bozarth & Wise, 1986). Furthermore, blocking dopamine receptors with pimozide suppresses motivated behaviors toward food (Wise et al., 1978) and toward sex (Pfaus et al., 1995; Pfaus & Phillips, 1989; Wenkstern et al., 1993). To understand whether the behavioral effects of blocking dopamine were related to dopamine release, Fibiger and colleagues measured dopamine concentrations in the terminal regions using microdialysis. A food-associated cue elicits higher dopamine concentrations in the nucleus accumbens (a site of dopamine release) relative to cues not associated with food (Blackburn et al., 1989). In general, dopamine release is critical for motivated behavior for various rewarding stimuli, including food.

Interestingly, an extrinsic reward isn't required to elicit motivated behavior. Behaviors can be reinforced by inducing neuronal activity via intracranial self-stimulation (ICSS) in specific brain regions (Margules & Olds, 1962; Olds & Milner, 1954). Behaviors are said to be positive reinforced when a desired stimulus (e.g. food) increases the probability of that behavior to occur again in the future (Thorndike, 1898). Seminal work of James Olds and Peter Milner shows that rats engage in reinforced behavior to work for electrical stimulation (i.e., ICSS), which serves in itself as a reward (Olds & Milner, 1954). ICSS-reinforced behaviors suggest that the regions being stimulated contribute to reward-seeking behavior. ICSS-sensitive regions are proximal to

catecholamine (i.e., dopamine and norepinephrine) cell bodies and their processes (Crow, 1972; Fibiger, 1978; Stein, 1964). Detailed mapping studies show that the dopamine neuron density at an ICSS stimulator's tip is proportional to the magnitude of behavioral responding (Corbett & Wise, 1980), suggesting that dopamine neurons stimulate motivated behaviors. However, ICSS is a nonspecific approach that recruits more than just dopamine circuits. Recent studies show that dopamine recruitment alone is sufficient to recapitulate the data observed by Olds and Milner. Specific optogenetic stimulation dopamine neurons is sufficient to reinforce behavior (Steinberg et al., 2014). Stimulation of dopamine neurons is dependent on frequency and ICSS reinforces behavior only at stimulation frequencies of greater than 20Hz—frequencies that elicit phasic bursts of activity (Carlezon & Chartoff, 2007; Liebman, 1983). Phasic dopamine activity is defined as the brief bursts of dopamine neuron action potentials and the resulting dopamine release in the terminal regions. Only phasic stimulation of dopamine cell bodies or terminals, not slow tonic stimulation, reinforces behavior (Berke, 2018; Palmiter, 2008; Tsai et al., 2009). The causal link between phasic dopamine signaling and reinforcement is corroborated by the fact that dopamine neurons burst fire upon the receipt of reward and cues that predict reward (Romo & Schultz, 1990; Schultz & Romo, 1990). Seminal work from Wolfram Schultz and colleagues shows that dopamine neurons fire to food reward (Apicella et al., 1991; Schultz, 1986; Waelti et al., 2001). If cues that predict reward are presented, then over training, dopamine neurons develop a response to the earliest cue that predicts reward (Schultz, 2007; 2013). Recent work shows that brief increases in dopamine neuronal firing and dopamine release is correlated with approach behavior toward reward (J. J. Day et al., 2007; Hoffmann & Nicola, 2014; Roitman et al., 2004). Phasic dopamine signaling is also involved in the reinforcing aspects of reward (K. M. Kim et al., 2012; Steinberg et al., 2014; Wise et al., 1978) and reward value (Hamid et al., 2015; Howe et al., 2013; Roitman et al., 2008). Clearly, dopamine systems are essential for the motivating drive toward reward, including food rewards.

To understand how motivation translates to behavior, it is necessary to understand how dopamine modulates activity in dopamine receptor expressing neurons—discussed below.

D. The neuroanatomical organization of the mesolimbic dopamine system

Dopamine cell bodies of the mesolimbic dopamine system originate in the ventral tegmental area (VTA) and their axons project to cortical and limbic structures (Domesick, 1988). One such limbic structure is the nucleus accumbens (NAc), also referred to as the ventral striatum. Dopamine cell bodies are identified histologically by their expression of the rate limiting enzyme in the production of dopamine, tyrosine hydroxylase (TH) (Kaufman & Milstein, 2013). Dopamine neurons are heterogeneous in that they co-release either GABA or glutamate (Morales & Margolis, 2017) and send topographical projections to the NAc (Ikemoto, 2007). The lateral VTA projects mainly to the NAc shell subregion, whereas more medial portions of the VTA project to the NAc core subregion. Dopamine projections mainly innervate GABAergic medium spiny neurons (MSNs) in the striatum (Gerfen et al., 1990). MSNs are the main output neurons of the basal ganglia, a circuit mainly responsible for motor control of behaviors (Lanciego et al., 2012). Although the anatomical delineation suggests that VTA subcompartments might be functionally divided, a single dopamine neuron can innervate a vast region of the striatum (Aransay et al., 2015; Matsuda et al., 2009) while also diffusely releasing dopamine through volume transmission (Dreyer et al., 2010; Garris et al., 1994; Rice & Cragg, 2008). Conversely, a single dopamine neuron may receive input from a variety of regions, including the laterodorsal tegmental nucleus (LDTg), superior colliculus, the nucleus of the solitary tract (NTS), and the hypothalamus (Alhadeff et al., 2012; Watabe-Uchida et al., 2012). Collectively, it's clear that the diverse inputs into VTA dopamine neurons affords them the ability to integrate a variety of information and project to output regions (i.e. NAc) to influence behavior.

Dopamine is a ligand to multiple g-protein coupled receptors, of which there are two categories: D1-like and D2-like. The D1-like category consists of D1 (D1R) and D5 receptors, whereas the D2-like category is comprised of D2 (D2R), D3, and D4 receptors. In the striatum, dopamine acts mainly on D1Rs and D2Rs (Beckstead et al., 1988; Berendse & Richfield, 1993), which are largely located extrasynaptically on distinct MSNs (Gerfen, 1992; Gerfen et al., 1990; Levey et al., 1993; Weiner et al., 1991; Yung et al., 1995). D1Rs activate the $G\alpha_s$ and the $G\alpha_{olf}$ g-proteins, activating adenylate cyclase, increasing intracellular cyclic adenosine monophosphate (cAMP), stimulating protein kinase A (PKA) activity, and ultimately regulating a variety of ion channels and gene products (Neve et al., 2004). In general, D1Rs in the striatum increase sodium and L-type calcium currents, while attenuating potassium currents (Dong et al., 2004; Gorelova & Yang, 2000; Paupardin-Tritsch et al., 1985; Yang et al., 1999). On the other hand, D2Rs are coupled to $G_{i/o}$ g-proteins and have the opposite effect on ion channels than the D1Rs via an inhibition of cAMP (Neve et al., 2004; Stoof & Kebabian, 1981). Effectively, D1Rs increase excitability and D2Rs decrease excitability of MSNs. Additionally, D2R affinity for dopamine is approximately 100-fold higher than D1R (Martel & McArthur, 2020). The imbalance in D1R and D2R affinity results in mostly D2R receptor activation at low extracellular dopamine concentrations and both D1R and D2R activation at high extracellular dopamine concentrations (Caravaggio et al., 2019; Dreyer et al., 2010; Nair et al., 2014). Therefore, dopamine concentrations may trigger functionally different pathways—the so-called movement-related “direct” and “indirect” pathways of the basal ganglia (Calabresi et al., 2014). These functionally distinct pathways will be further discussed later in this section. A notable exception in the D1R and D2R cellular segregation lies in the NAc (ventral striatum), where some neurons express both receptors (Meredith et al., 2008). In this case, D1R and D2R may work cooperatively, as only co-activation of both D1Rs and D2Rs elicits an increase in PKA activity (Hopf et al., 2003). This cooperative activation is also evident in behavioral studies where animals will work to self-

administer D1R and D2R agonists together but will not work to receive either agonist alone (Ikemoto et al., 1997). VTA dopamine neuron activity likely engages both D1Rs and D2Rs to facilitate motivated behaviors.

Dopamine concentration in the terminals is regulated by (1) burst firing of dopamine neurons, (2) D2R inhibition, (3) dopamine transporters, and (4) cholinergic interneurons. In addition to slow tonic firing (~4Hz), dopamine neurons fire brief (phasic) bursts of action potentials (~20Hz), which are dependent on LDTg input (Lodge & Grace, 2006). It is likely that it is phasic activity (and not the periodic slow firing rate) in dopamine neurons that is responsible for effective dopamine modulation of MSNs (Berke, 2018). Due to D1R and D2R's differential affinity for dopamine, it is theorized that phasic release of dopamine engages D1R neurons as dopamine reaches high enough concentrations (Dreyer et al., 2010; Nair et al., 2014). Furthermore, phasic dopamine signaling also drives synaptic plasticity (Wieland et al., 2015), suggesting that although phasic signaling occurs at short time scales, its effects last longer than the signaling itself. Secondly, presynaptic D2Rs on dopamine neurons are activated by volumetric transmission of dopamine out of the synaptic cleft, resulting in negative feedback and suppression of dopamine release (Kennedy et al., 1992). On longer time scales D2Rs also decrease terminal dopamine production by downregulating tyrosine hydroxylase phosphorylation (Lindgren et al., 2001). Although a few regions of the brain use dopamine breakdown (Bigl et al., 1974; Weller et al., 1987), dopamine transporters (DAT) are the main mechanism through which dopamine is recycled (Ciliax et al., 1995). Since dopamine receptors and DATs are mainly expressed on axons rather than in synapses (Ciliax et al., 1995), it is thought that DAT-facilitated dopamine reuptake allows for dopamine to act on receptors over longer distances (past the synapse) than other neurotransmitters, such as glutamate. Finally, recent reports show that dopamine release may not only be activity-dependent but also is regulated by local cholinergic innervation (Cachope et al., 2012; Threlfell et al., 2012). Nicotinic

acetylcholine receptors on dopamine terminals can elicit *de novo* activity-independent dopamine release (Cachope et al., 2012). Cholinergic interneurons also enhance activity-dependent dopamine release (Threlfell et al., 2012). Taken together, dopamine concentration in the striatum is tightly orchestrated by both intracellular and extracellular architecture to modulate NAc MSN output to the basal ganglia.

In addition to dopamine innervation, the NAc receives input from cortical and limbic structures, such as the amygdala (Kelley et al., 1982), hippocampus (Brog et al., 1993), and thalamus (Berendse & Groenewegen, 1990), while sending projections to structures related to the production of motor responses, such as the ventral pallidum (Groenewegen et al., 1999). Therefore, the NAc is named the "limbic-motor" interface (Mogenson et al., 1980) to emphasize that the NAc is a central hub of integrating memory, emotion, and complex cognition into activity that results in action. Indeed, the NAc is thought to be the interface between the production of movement and the inhibition of movement via the direct and indirect pathways of the basal ganglia, respectively (Calabresi et al., 2014). It is largely accepted that D1R activation in the striatum activates movement through the direct pathway, while D2R activation inhibits movement via the indirect pathway (Gerfen & Surmeier, 2011; Yawata et al., 2012). The NAc acts as a conduit for encoding reinforcement to rewarding stimuli (Cardinal et al., 2002; Kelley & Berridge, 2002; Robbins & Everitt, 1996) and linking motivation to the action necessary for consummation (Mogenson et al., 1980). Pharmacologically interrupting this NAc conduit indeed alters eating behaviors (Bakshi & Kelley, 1993; Stratford & Kelley, 1997). Because NAc dopamine input from the VTA integrates a host of signals that convey physiological state (Alhadeff et al., 2012; J. J. Cone et al., 2014; Hsu et al., 2020; X. F. Wang et al., 2015), Mogenson's view of the NAc as a converter of motivation to action is largely supported. Therefore, the mesolimbic dopamine system that arises from the VTA to innervate the NAc is a critical component in producing appetitive and consummatory behaviors toward food.

E. <u>Eating behavior is multifaceted and is encoded in the mesolimbic dopamine system</u>

Dopamine is necessary for eating behaviors and movement (Szczypka et al., 2001; Ungerstedt, 1971; Zhou & Palmiter, 1995). Clinically, the loss of dopamine neurons in Parkinson's disease patients results in a severe movement disorder (Fahn, 2008; Sacks, 1973). However, it is important to note that movement is not completely abolished, as rats move to an alerting stimulus (Marshall et al., 1976) and patients can make fast and effective motor responses to salient events such as an earthquake (Bonanni et al., 2010; Distler et al., 2016), in a phenomenon called "paradoxical kinesia." Similarly, preservation of specific consummatory movements are observed in dopamine lesioned or decerebrated rats, where rats perform orofacial movements associated with the "liking" of reward identically to intact rats (Berridge et al., 1989; H. Grill & Norgren, 1978). Since dopamine lesioned rats can eat but don't expend effort to seek food, dopamine is less necessary for some consummatory behaviors but is necessary for appetitive behaviors.

Initiating eating behaviors (i.e., initiating a meal) requires that the previous behavior be terminated and then a sufficient "agitating" stimulus to eat be present (Craig, 1917). First, behavioral selection occurs, where specific motor sequences are selected to carry out the prioritized behavior (Klaus et al., 2016). Then an appropriate series of actions (e.g., approach, licking, biting, chewing, and swallowing) for that motor program are executed in a specific order (Aldridge & Berridge, 1998). This sequence of motor actions, or behavioral syntax, can be as complex as human behaviors or can be highly stereotyped species-specific sequences (Sachs & Richmond, 1980). To initiate behaviors, various interoceptive and exteroceptive cues provide agitating stimuli (Anderson, 2016), which are then integrated by multiple neural networks, which then feed pertinent information to circuits that execute the motor programs (Arber & Costa, 2018). One of these integrators is the mesolimbic dopamine system.

Regions such as the LH, paraventricular nucleus of the hypothalamus (PVN), and arcuate nucleus (ARC) funnel prioritizing information (likely through GABAergic disinhibition) into the dopamine system to initiate appropriate motor programs (Arber & Costa, 2018; Lammel et al., 2012; Nieh et al., 2016; Yamanaka et al., 2003). For example, the dopamine system is essential in predator hunting (M. Huang et al., 2021; Pribadi et al., 2022). However, due to the physical and practical limitations of experimental design, very few studies describe mechanisms that govern naturalistic food acquisition (e.g., foraging, hunting, planning, etc.). Thus, more focus has been targeted toward the phases of eating that are more proximal to consummatory behaviors. Appetitive behaviors such as an animal poking its nose into an opening to receive a sugar pellet or an animal pressing a lever for reward (termed operant behavior) are much easier to identify and quantify (Atalayer & Rowland, 2007; D. E. Day & Bartness, 2003; McMurray et al., 2014) than naturalistic behaviors such as an animal's attempts to procure food in its natural environment. An example of the relationship of dopamine neurons to rewards and operant behaviors was demonstrated by seminal work from Wolfram Schultz and colleagues. Animals can associate food with cues, which then elicit phasic firing in dopamine neurons (Ljungberg et al., 1992; Romo & Schultz, 1990; Schultz, 1998; 2015; Schultz & Romo, 1990; Waelti et al., 2001). Additionally, dopamine neurons fire immediately before and during actions toward food rewards (J. J. Cone et al., 2014; Hsu et al., 2020; Wilson et al., 1995), suggesting that dopamine neurons are necessary to initiate and maintain motivated behaviors. Furthermore, the magnitude of dopamine signaling scales with the physiological state and with the vigor with which animals engage in food seeking behaviors (J. J. Cone et al., 2014; Hoffmann & Nicola, 2014; Hsu et al., 2020; Palmiter, 2008; Salamone et al., 1997; Salamone & Correa, 2012; Wilson et al., 1995). Taken together, data support that dopamine signaling is critical in producing appetitive behaviors while integrating information regarding physiological state from various sources.

Physiological state and the value of food stimuli inform the learning of post-ingestive consequences, terminating eating behaviors, and updating future behaviors. The value of food may vary based on the caloric density, the flavor preference, post-ingestive history, emotional status, and especially physiological state (Berridge, 1991; Hajnal et al., 2004; Hamid et al., 2015; Roitman et al., 2005; Smith, 2004; Spector et al., 1993; Willner et al., 1991). Phasic dopamine signaling dynamically encodes hedonic (subjectively positive) value of food reward (Schultz et al., 2015). Dopamine representations of reward value change at fast time scales based on current sensory properties as well as prior experience (Berke, 2018). Termination of eating behavior is determined by dynamically updating value of actions via signals that convey physiological state and other competing motivated behaviors (e.g., sex, grooming, etc.). Various satiety signals such as cholecystokinin (CCK) (Brodie & Dunwiddie, 1987; Damonte et al., 2022), oxytocin (C. M. Liu et al., 2020), amylin (Mietlicki-Baase et al., 2015), and GLP-1 (Alhadeff et al., 2012) all act on VTA dopamine neurons directly or indirectly. Signals that convey satiety information suppress dopamine signaling, decreasing the invigoration with which behaviors are executed, increasing the likelihood of the termination of a meal. Because of the cyclical nature of eating behaviors, i.e. initiation-appetition-consummation-termination (Craig, 1917), dopamine responses to reward stimuli that flexibly encode value profoundly impact both present and future behaviors.

F. <u>Hunger modulates aspects of eating behavior related to dopamine signaling</u>

Eating food is more rewarding while hungry than while sated. Food restriction profoundly influences motivation to seek food (Stellar, 1954; Wilson et al., 1995), enhances dopamine neuronal activity (Branch et al., 2013), and dopamine release in the NAc (Carr et al., 2003; J. J. Cone et al., 2014; Wilson et al., 1995). Specifically, food depriving rats increases dopamine release when food is made available and during its consumption (Wilson et al., 1995). A caveat to this study falls in its microdialysis-approach, which is unable to capture phasic dopamine

dynamics due to low temporal resolution. In naturalistic conditions appetitive and consummatory behaviors occur at a subsecond time scale and it is difficult to attribute dopamine release to any specific component of motivated behavior using a low temporal resolution technique such as microdialysis. Dopamine measurements at subsecond resolution through fast scan cyclic voltammetry show that food restriction augments dopamine concentration during food retrieval (J. J. Cone et al., 2014) and during cues that predict food (Aitken et al., 2016). The VTA dopamine neurons integrate physiological state (e.g. hunger) information and sensory information to produce behavior. For example, a hunger-associated hormone, ghrelin, potentiates food evoked dopamine and drives eating behavior (J. J. Cone et al., 2014; 2016). Intra-LH ghrelin, but not intra-VTA ghrelin, increases dopamine signaling and motivated behavior (J. J. Cone et al., 2014). Interestingly, intra-VTA orexin blockade reduced LH ghrelin-induced eating, suggesting a multisynaptic circuit via ghrelin action on LH orexin-A neurons that project to the VTA (J. J. Cone et al., 2014). LH to VTA input is important for homeostatic modulation of mesolimbic dopamine control of eating behavior. Recent reports that exploit cell specific chemogenetic and optogenetic approaches further support that physiological state is encoded in LH networks (Fenselau et al., 2017; Jennings et al., 2013; 2015). The throughline in all these studies is that physiological state integration with circuits governing motivated behavior in the central nervous system, by way of the LH or otherwise, profoundly modulates phasic dopamine activity.

G. <u>The hindbrain is a gatekeeper in gut-brain communication</u>

Motivated behavior is both aroused and directed. Internal signals that arouse or suppress eating (e.g, hunger or satiety) can arise from the gut. Gut-brain communication is essential in controlling relevant behavior to restore energy balance. A region of the hindbrain, nucleus of the solitary tract (NTS), acts as a hub to collect and disseminate peripheral information to the rest of the brain. The facial, glossopharyngeal, and vagus nerves that carry

gustatory and visceral information make their first synapse onto neurons in the NTS (Aström, 1953; F. Kerr, 1962; Torvik, 1956). NTS neurons then project to structures throughout the brain, such as the medial and lateral hypothalamus (Ricardo & Koh, 1978; Rinaman, 1999), VTA (Alhadeff et al., 2012), and many others (Alhadeff et al., 2014; Norgren, 1978; Ricardo & Koh, 1978; Rinaman, 2010). Therefore, the NTS is poised to integrate both neural and hormonal signals from the periphery and convey such critical information to the rest of the brain to enact restorative behaviors.

The sensation of being hungry is something to which anyone can relate. One may consciously feel their stomach during a hunger pang, but many alimentary signals are sensed by the brain unconsciously and drive us to eat. One of the earliest pieces of evidence of gut-brain communication was discovered in 1858 by a physician, Dr. W. Busch. Busch's patient was gored by a bull. During healing, a fistula developed in the small intestine such that ingested food exited the gut before being fully digested and absorbed. Busch reported that despite the patient's ravenous appetite and consumption of very large meals, she responded with no perception of satiation even though her stomach felt full. In both humans and rats, stomachs prevented from filling by use of a gastric fistula are met with continuous eating without satiation (Busch, 1858; Partosoedarso & Blackshaw, 2000). When the fistulas are closed, rats quickly cease eating, suggesting that a satiety signal is sent to the brain via the vagus nerve (Gonzalez & Deutsch, 1981) as soon as the stomach and the small intestine start to fill (Davis & Smith, 1990). It is not solely the act of eating that informs the brain but also the post-ingestive consequences (including gastric distension, absorption of nutrients, increase in blood glucose, etc.) that provide central feedback (Donovan & Watts, 2014; Hayes et al., 2009; Ly et al., 2017; Oesch et al., 2006; Pappas et al., 1989). Gastric distension induces satiation via a hindbrain neural pathway (Pappas et al., 1989), because the vagus nerve bidirectionally innervates a vast part of the stomach, allowing for direct central communication (Prechtl & Powley, 1990). Vagal

deafferentation experiments show that neural communication through the vagus nerve is essential in communicating satiation to the brain (Steinert et al., 2016). In this experiment, peripheral administration of an analog of the anorectic hormone glucagon-like peptide 1 (GLP-1) suppresses food intake but its effects are attenuated by vagal deafferentation (Steinert et al., 2016), suggesting that the peripheral neural component of GLP-1 action is an important part of satiation. The vagal afferent to the NTS is therefore critical in transducing peripheral status to the brain via activity-dependent means.

In addition to neural communication through the vagus nerve, gut-brain communication via endocrine pathways is also essential in the control of motivated behaviors toward food. Various hormones that cross the blood brain barrier have been shown to modulate eating behavior. For example, ghrelin is a stomach-derived hormone that can act throughout the neuraxis and increases food intake and motivated behavior to acquire food (Bron et al., 2013; J. J. Cone et al., 2014; Hsu et al., 2015; 2018; Olszewski et al., 2003; Skibicka et al., 2011). In human studies, ghrelin increases neural response to food pictures in the amygdala, orbitofrontal cortex, anterior insula, and the striatum (Malik et al., 2008). Receptors to similar peripherally derived peptides, such as amylin, insulin, leptin, and GLP-1 are found throughout the brain and can modulate eating behavior (Begg & Woods, 2012; 2013; J. Friedman, 2016; Mietlicki-Baase et al., 2015; Unger et al., 1991). Hormonal signaling can enter the brain via various circumventricular organs (Ganong, 2000; McKinley et al., 1998), especially via the area postrema in the hindbrain (H. J. Grill & Hayes, 2012; K.-P. Huang & Raybould, 2020) where the blood-brain barrier is the thinnest. The hormonal method of gut-brain communication has profound influence on modulating behavior. Vagus nerve signaling and ghrelin-induced food intake are examples of communication that highlight the importance of gut-brain communication as a complex dynamic system that depends on neuronal and hormonal signals from the periphery to produce appropriate eating and satiety behavior. Such physiological state

information filtered through the NTS is then disseminated to structures that are critical in producing motivated behaviors, one of which being the mesolimbic dopamine system.

H. Glucose utilization is a harbinger of hunger

One important signal that conveys hunger to the brain is glucose. In 1953, Jean Mayer first posited a "glucostatic hypothesis," where glucose was central in a mechanism through which hunger guides compensatory behaviors to bring the organism to homeostasis (Mayer, 1953). Mayer found blood glucose levels in oxygenated and deoxygenated blood, which reflect glucose utilization, were correlated with hunger (Mayer, 1953). Furthermore, small changes in blood glucose that precede spontaneous eating (Campfield et al., 1985) can be detected by neurons (Song et al., 2001; R. Wang et al., 2004). Consistent with the glucostatic hypothesis, pharmacologically decreasing blood glucose or brain glucose utilization (i.e. cytoglucopenia) initiates eating behaviors (Campfield et al., 1985; Dunn-Meynell et al., 2009; Louis-Sylvestre & Magnen, 1996; Melanson et al., 1999) and increases the subjective value of sucrose in humans (Thompson & Campbell, 1977). Blocking glucose utilization in the hindbrain, but not in the forebrain, is sufficient to elicit eating (R. C. Ritter et al., 1981). The hypothalamus and the hindbrain are glucose-sensitive due to a select few neurons that use extracellular glucose concentration to control their firing rates (Cancelliere & Ferguson, 2017; Izumi et al., 1994; Labouèbe et al., 2016; Medeiros et al., 2012; Mobbs et al., 2001; Nakano et al., 1986; Papp et al., 2007; Riediger et al., 2002; Yamanaka et al., 2003). Even some peripheral glucose-sensitive tissues (Donovan & Watts, 2014; Niijima, 1969) send projections to the brain, specifically to the dorsal medulla (Garcia-Luna et al., 2021). Numerous glucose-sensing structures in the brain and body send information to a central integrator. The convergence of various interoceptive cues ultimately controls food-motivated behavior in a complex circuit yet to be unraveled.

In general, organisms use fats, proteins, and carbohydrates to sustain life. Although some macronutrients are necessary in different proportions for different organisms for energy

balance (Remonti et al., 2016), one common form of energy that all cells need is adenosine triphosphate (ATP). The energy sensor 5' adenosine monophosphate activated protein kinase (AMPK) monitors ATP availability as the ratio of ATP to its metabolite adenosine diphosphate (ADP) (M. Friedman, 2008; Hardie, 2018; Krebs, 1964; Moore et al., 1991). Low ATP:ADP ratio in fasted animals results in elevated phosphorylated AMPK (active form) in the ARC, PVN, and in the NTS (Hayes et al., 2009; Minokoshi et al., 2004; Xue & Kahn, 2006). AMPK activity has behavioral consequences, in that inhibiting AMPK activity attenuates food intake (Hayes et al., 2009). AMPK has a foundational role in energy balance and in neural circuits that govern food motivated behaviors.

One of the many hunger-associated signals that modulate AMPK activity is glucose (Lin & Hardie, 2018). Not only is glucose the main energy fuel for the brain but its availability is a prime physiological signal that contributes to shape eating behaviors. Antiglycolytic agents, such as 5-thio-D-glucose (5TG) and 2-deoxy-D-glucose (2DG), induce cytoglucopenia and drive eating. Antiglycolytic agents competitively suppress glucose metabolism by blocking glucose uptake (Betz et al., 1975; 2013) and inhibiting hexokinase, a key enzyme in glycolysis (Bachelard et al., 1971; M. Chen & Whistler, 1975; Horton et al., 1973). Antiglycolytic agents are therefore important tools to determine the neural substrates that integrate glycemic information to yield appropriate behavior. Firstly, the hindbrain is essential for glucoprivic (in response to deprivation of glucose availability) eating. In decerebrate animals, antiglycolytic agents increase consumption of intraoral meals before the food is passively rejected (Darling & Ritter, 2009). Hindbrain, not forebrain, structures promote eating in response to decreased glucose availability (R. C. Ritter et al., 1981; S. Ritter et al., 2000). Although the hypothalamus contains glucose sensitive neurons, almost none of its nuclei elicit eating behavior in response to local glycolysis blockade (S. Ritter et al., 2000). Only the NTS and ventrolateral medulla increase eating in response to local administration of 5TG (S. Ritter et al., 2000). Sue Ritter found that

catecholamine (CA) neurons that project to the PVN are necessary for glucoprivic eating (S. Ritter et al., 2001). The identity of these CA neurons may be the NPY producing epinephrine neurons in the NTS that increase eating (J. Chen et al., 2020). Collectively, the hindbrain is critical for glucose-sensing and converts the decreases in glucose availability into ascending signals to stimulate eating behavior.

Although I have noted the importance and the necessity of hindbrain structures in glycemic regulation and glucoprivic eating, hypothalamic structures also have a marked role in preserving glycemic balance. Orexin neurons of the LH directly modulate the activity of VTA dopamine signaling based on glucose availability (Borgland et al., 2006; 2009; J. J. Cone et al., 2014; Rosin et al., 2003; Sheng et al., 2014). The LH to VTA circuit may, in part, contribute to reward seeking behaviors parallel to but distinct from those that originate from the hindbrain (Alhadeff et al., 2012; J. Chen et al., 2020; R. Wang et al., 2004). Decerebrate rats do retain consummatory behaviors and compensate for glucoprivation by consuming more intraoral sucrose before rejecting the solution (Flynn & Grill, 1983). However, decerebrate rates *do not* show appetitive responses to external stimuli (H. Grill & Norgren, 1978). Therefore, it is possible that the hypothalamic connections to the VTA might be transmitting information regarding the appetitive phase of eating behaviors.

I. Glucagon-like peptide 1 signaling regulates satiety and the suppression of motivated eating behavior

Satiation (i.e., meal termination) arises, in part, from the release of a variety of gut peptides. One such satiety signal is glucagon-like peptide 1 (GLP-1) and is secreted peripherally by the L cells in the small and large intestine. GLP-1 is released in response to the presence of mixed nutrients in the gastrointestinal tract (Herrmann et al., 1995), promoting the release of insulin and glucagon (D'Alessio et al., 1996). Although peripheral GLP-1R function is not necessary for long term energy balance (e.g. body weight, food intake, and heat production)

(Donahey et al., 1998), the knockdown of the GLP-1R in vagal afferents increases meal size and accelerates gastric emptying (Abbott et al., 2005), suggesting a role of the vagal afferents in providing crucial post-prandial feedback. The vagal pathway acts in series with a central pathway in the brain (Kanoski et al., 2011). Peripherally released GLP-1 acts in a paracrine fashion to stimulate vagal afferents, whose cell bodies are in the nodose ganglion (Krieger et al., 2016). This vagal pathway is an important input for the NTS pre-pro-glucagon neurons, a central source of GLP-1 signaling that impacts energy balance (Donahey et al., 1998; V. K. M. Han et al., 1986; Holst, 2007; Jin et al., 1988; W. E. Schmidt et al., 1985). The NTS serves in a feed-forward circuit, integrating information from vagal afferents and innervating a variety of neural substrates involved in motivated behavior, including the hypothalamus, hippocampus, and the mesolimbic dopamine system (Affleck et al., 2012; Alhadeff et al., 2012). It is technically possible that GLP-1 is acting through hormonal action because both central GLP-1 (Donahey et al., 1998) and peripherally administered longer lasting GLP-1 analogs decrease food intake (Kanoski et al., 2011; Turton et al., 1996). However, GLP-1 released in the periphery is rapidly degraded by vascular dipeptidyl peptidase-4 before crossing the blood brain barrier in meaningful concentrations to activate central GLP-1R (Holst, 2007). Furthermore, in animals that have vagal input removed, peripherally administered GLP-1 analogs still reduced food intake (Kanoski et al., 2011), suggesting a central site of action. Therefore, peripheral GLP-1 most likely acts in a paracrine fashion to stimulate NTS pre-pro-glucagon neurons resulting in central release of GLP-1.

After the discovery of GLP-1 and its hypophagic properties, GLP-1 analogs have been FDA approved as therapeutics for patients with type 2 diabetes (Lovshin & Drucker, 2009) and obesity (Fujioka, 2015). Exendin-4 (Ex4) and liraglutide are GLP-1 analogs which act centrally to reduce food intake and reduce motivated responding to seek food (Kanoski et al., 2011; Secher et al., 2014; Sisley et al., 2014). This therapy, however, is not without its significant

drawbacks. A portion of patients using liraglutide report nausea, which may be mediated through GLP-1R signaling (Kanoski et al., 2012). Although some may argue that GLP-1R induced hypophagia may be secondary to malaise, targeted intra-VTA and intra-NAc GLP-1R activations show reductions in food intake independent of nausea (Dickson et al., 2012). This suggests that mesolimbic circuitry may control satiety in a pathway parallel to and distinct from nausea.

Malaise aside, GLP-1R activation has been a promising therapeutic candidate. Intra-NTS Ex4 potently reduces various measures of food directed behaviors (Alhadeff & Grill, 2014; Richard et al., 2015). Specifically, GLP-1R activation in the NTS reduces progressive ratio (PR) operant responding, a dopamine sensitive behavior (Hamill et al., 1999; Richard et al., 2015). The NTS projects to the VTA and NAc (Alhadeff et al., 2012; Dossat et al., 2011; Rinaman, 2010)—prime nodes of the mesolimbic dopamine circuit, whose activity is essential in driving motivated behavior (Hoffmann & Nicola, 2014). Central GLP-1R activity (possibly in the VTA itself (Cork et al., 2015; Heppner et al., 2015; Merchenthaler et al., 1999)) modulates VTA tyrosine hydroxylase and dopamine D2 receptor expression (Anderberg et al., 2014; Mietlicki-Baase et al., 2013). However, it is unknown whether this change in VTA expression profile translates to a decrease in transient changes in motivated behavior. Clearly, central GLP-1R activation does decrease motivated responding for food (Dickson et al., 2012), as well as other rewarding stimuli, such as alcohol (Shirazi et al., 2013) and cocaine (Sørensen et al., 2015). Additionally, GLP-1R agonism suppresses cocaine evoked phasic dopamine signaling in the NAc (Fortin & Roitman, 2017). GLP-1R signaling seems to suppress reward related behaviors in a general fashion and may suppress dopamine signaling to food and food related stimuli.

J. Implications for current studies

Cues in our environment hold powerful sway over our decisions to find and consume food. The ubiquity of advertisements that provoke food purchases and consumption of various

obesogenic foods has likely contributed to the prevelance of obesity and type 2 diabetes (Burger & Stice, 2014; Halford et al., 2004; Mink et al., 2010). Obesity and type 2 diabetes are causing major impacts on public health and the economy (Abdelaal et al., 2017; Davidson et al., 2014; Murphy et al., 2020; Schelbert, 2009; Ward et al., 2021). In addition to signals that convey hunger and satiety, environmental cues as well as the subjective value of food can drive overconsumption (Harris et al., 2009; Johnson, 2013; Saelens et al., 2012; Zimmerman & Bell, 2010). Diseases that cause maladaptive overconsumption undermine the neurotypical function of dopamine signaling. Obesity causes significant changes in peripheral signaling of physiological state (Tschöp et al., 2001), dopamine receptor expression (Blum et al., 1996), dopamine release (Carlin et al., 2013), and reuptake (J. J. Cone et al., 2013). Understanding the mechanisms through which dopamine systems in the brain encode food stimuli and their predictive cues in an adaptive manner can shed light on how these systems may be highjacked in maladaptive diseases.

Hindbrain neurons in the NTS send projections to the forebrain that signal both cytoglucopenia and GLP-1 and can impact dopamine signaling. **The primary goal of this thesis is to investigate the impact of two signals of physiological state that convey either hunger (cytoglucopenia) or satiety (GLP-1) on VTA dopamine signaling to food and food-cues.** In a Pavlovian conditioning paradigm, I hypothesize that physiological signals will modulate dopamine signaling to food stimuli and that the magnitude of dopamine signaling will correlate with the behavior. Glucose, the body's main energy substrate, is tightly regulated and monitored. Perturbations in glucose availability occur on an hourly basis and are of paramount significance when considering the factors that shape food-seeking behaviors. GLP-1 analogs are FDA approved to treat type 2 diabetes and obesity. Understanding how glucose deprivation and GLP-1R activation modulate dopamine signaling is an essential stepstone in paving a way toward bolstering their pharmacological efficacy.

Chapter II: Cytoglucopenia potentiates dopamine neuron activity evoked by food reward and food predictive cues

A. Introduction

Energy balance homeostasis initiates and maintains goal-directed behavior toward food in our daily lives. With the prevalence of readily available energy-dense foods (Lissner et al., 1987; Swinburn et al., 1999) and cues that predict them (e.g. billboards, advertisements, etc.) we are prone to overeating (Boswell & Kober, 2016; Harris et al., 2009; Johnson, 2013; Saelens et al., 2012; Zimmerman & Bell, 2010). Understanding neural encoding of food and food-cues is essential in curbing maladaptive behaviors directed toward food. Highly palatable foods and their associated cues evoke brief increases in dopamine release at the terminal regions and dopamine neuron activity in the midbrain, namely in the ventral tegmental area (VTA) (J. J. Cone et al., 2014; J. J. Day et al., 2007; Roitman et al., 2008; Schultz et al., 1993). While initially only responding to primary rewards, VTA dopamine neurons develop a response to the earliest cue that predicts reward (Schultz et al., 1993). If this dopamine response to cues is interrupted, the learning of cue-reward associations is impaired (Steinberg et al., 2013). Furthermore, phasic dopamine signaling is essential for driving approach behavior toward reward (J. J. Day et al., 2007; Engelhard et al., 2019; Hoffmann & Nicola, 2014; Roitman et al., 2004), reward reinforcement (K. M. Kim et al., 2012; Steinberg et al., 2014; Wise et al., 1978), and reward value (Hamid et al., 2015; Howe et al., 2013; Roitman et al., 2008). A change in physiological state (i.e. hunger) enhances both food seeking behavior (Wilson et al., 1995) as well as phasic dopamine responses toward rewarding stimuli and their associated predictors (J. J. Cone et al., 2014). However, physiological state can be communicated to and throughout the brain through a myriad of signals and circuits.

Glucose, the primary fuel for the brain and body, is very tightly regulated and perturbations in its availability result in restorative counterregulatory responses (Dunn-Meynell et al., 2009; Levin, 2006). In humans, cytoglucopenia, a lowering of available metabolizable glucose, increases hunger ratings and the rewarding value of sucrose (Thompson & Campbell, 1977). Indeed, cytoglucopenia is a potent driver of food directed behaviors (DiRocco & Grill, 1979; A. J. Li et al., 2014; Muller et al., 1972; S. Ritter et al., 2000; RR & AN, 1975; Slusser & Ritter, 1980). While modulation of glucose levels can recruit specific hindbrain (Hayes et al., 2009) and hypothalamic (S.-M. Han et al., 2005; Minokoshi et al., 2004) neurons to drive corrective behaviors, it remains unknown whether modulation of glucose levels alters dopamine signaling. Given that cytoglucopenia enhances the rewarding value of sucrose (Thompson & Campbell, 1977), I hypothesize that modulation of phasic dopamine signaling evoked by sucrose is a potential mechanism for this effect. In addition to signals associated with hunger, glucagon-like peptide 1 (GLP-1) is released in the brain to signal satiety and ingested calories (Holst, 2007). Central GLP-1R agonism decreases food consumption (Donahey et al., 1998; V. K. M. Han et al., 1986; Holst, 2007; Jin et al., 1988; W. E. Schmidt et al., 1985). However, it's unclear whether GLP-1 circuitry modulates VTA dopamine signaling to food itself. Similar to previous work (Konanur et al., 2020), I hypothesize that a GLP-1R agonist, Exendin-4 (Ex4), will suppress phasic dopamine signaling to intraoral sucrose.

I measured phasic dopamine neuronal activity in the VTA using *in vivo* fiber photometry while *ad libitum* rats received intraoral infusions of sucrose. I hypothesized that inducing cytoglucopenia via an antiglycolytic agent, 5-thio-d-glucose (5TG), would modulate phasic dopamine signaling to sucrose as well as cues associated with sucrose delivery. To probe a potential site of 5TG action for the modulation of dopamine signaling, I varied the timing and injection site. I found that 5TG modulated phasic dopamine signaling to intraoral sucrose and its associated cues in a timing and site-specific manner— favoring the interpretation that

cytoglucopenia in the hindbrain is effective in modulating phasic dopamine responses to food and food-cues. In contrast, Ex4 suppressed phasic dopamine signaling to intraoral sucrose—favoring the interpretation that signals that convey satiety also modulate phasic dopamine signaling to food.

B. Materials and methods

B. 1. Subjects

Male (n = 39) and female (n = 35; free cycling) Long Evans rats expressing Cre recombinase under the control of the tyrosine hydroxylase promoter [TH:Cre+(Witten et al., 2011); Rat Research Resource Center, RRRC#: 659] were individually housed after weaning within a temperature and humidity controlled room and on a 12:12 h light:dark schedule (lights on 0700 h). The TH:Cre+ phenotype was verified with a commercially available strain testing service (Transnetyx). Experiments occurred at approximately 1100 h, 4 hours after the onset of the light cycle. Rats were maintained on *ad libitum* food and water unless otherwise noted.

All rats were tested initially naïve to sucrose. Experimental manipulations and treatment order were counterbalanced across rats, with two intervening days between treatments. For experiments involving restriction, food or water (noted below) was removed for 20 hours before the onset of the experiment. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

B. 2. Surgeries

Rats were anesthetized with ketamine hydrochloride (100mg/kg, i.p.) and xylazine hydrochloride (10mg/kg, i.p.) for stereotaxic surgery. An intraoral catheter was inserted lateral to the first maxillary molar and exteriorized out of an incision at the top of the head and secured with skull screws and dental acrylic. The catheter was fashioned from ~6cm length of PE6 tubing (Scientific Commodities, Inc.). It was flanged at one end and passed through a Teflon

disk such that the disk sat against the flanged end. The disk was cut on one side and ultimately was positioned against the molar. During the same surgery, a Cre-dependent virus containing the construct for a genetically encoded Ca^{2+} indicator (AAV1.Syn.Flex.GCaMP6f.WPRE.SV40, Addgene) was unilaterally administered to the ventral tegmental area (VTA; 1 μL of 0.5e13 GC/ml: Anterior-Posterior (AP) −5.4mm, Medial-Lateral (ML) −0.7mm, Dorsal-Ventral (DV) −8.15mm relative to bregma) at 0.1 μL/min. After 5 min (to permit diffusion), the injector was removed. Then, an optic fiber (flat 400μm core, 0.48NA, Doric Lenses Inc.) was implanted in the VTA just above the injection site (AP −5.4mm, ML −0.7mm, DV −8.00mm relative to bregma). In some animals, an injection cannula (26Ga Cannula, PlasticsOne) was also implanted in either the lateral (LV; AP −0.9mm, ML −1.8mm, DV −2.6mm relative to bregma) or the fourth ventricle (4V; AP +2mm from occipital suture, midline, DV -4.5mm from dura). All animals received post-operative analgesia (0.1mL of 5 mg/ml meloxicam, s.c.) and were housed in their home cage for fourteen days to allow sufficient time for recovery and construct expression. During this time, rats had *ad libitum* access to food. Intraoral catheters were flushed daily with distilled, deionized water to ensure patency for the duration of the experiment.

B. 3. Intraoral infusion and photometry sessions

All habituation and experimental sessions took place during the light phase in standard operant chambers (ENV-009A-CT, Med Associates Inc.). A fluid line attached to a fluid reservoir was passed through a solenoid valve lying outside a sound attenuated chamber. From the valve, the fluid line then extended into the behavioral chamber and was attached to the intraoral catheter. Solutions were gravity fed and the height of the reservoir was adjusted such that opening the solenoid valve for 5s resulted in 200μl of fluid delivered to the rat. The opening of the solenoid valve was controlled by Med-Associates, Inc. hardware and software and timestamped. Rats were habituated to the intraoral infusions [2 sessions of 30 trials of intraoral water infusions; 5s infusion followed a randomly selected inter-trial interval (32-48s)] for two

sessions before the onset of experiments. Some experimental sessions utilized unsignaled intraoral infusions of 0.3M sucrose (n = 20 rats). To test whether experimental manipulations were specific to intraoral sucrose, some experimental sessions utilized unsignaled intraoral infusions of water (n = 8 rats).

For others, rats were first trained in a classical conditioning paradigm (cue→sucrose; n = 11 rats). Either a 4.5kHz tone or white noise (positive conditioning stimulus, CS+; 20 trials) was paired with intraoral sucrose in a counterbalanced manner, while the other tone/noise (negative conditioning stimulus, CS-; 20 trials) was paired with nothing. A CS+ trial consisted of an audio cue that lasted 1s, followed by 1s of silence, followed by a 5s infusion of 0.3M sucrose, followed by an inter-trial interval. A CS- trial consisted of an audio cue that lasted 1s, followed by 6 seconds of silence, followed by an intertrial interval. Rats were trained with the cue→sucrose paradigm for a total of 10 sessions before experimental manipulations. Fiber photometry recordings were made during all conditioning and test sessions.

B. 4. Drug injections

Because previous publications only report behavioral effects at approximately 1 hour after 5TG administration, I expanded the sampling rate to increase the likelihood of capturing the effects of pharmacological onset on dopamine signaling by administering 5TG either 15min or 45min before recording. To induce cytoglucopenia via a different mechanism than 5TG, I used peripheral injections of insulin. Experiments involving peripheral injections included 5TG (intraperitoneal, i.p.: 50mg/kg, 100mg/kg; either 15min or 45min before session onset; Sigma) or insulin (subcutaneous, s.c.: 0.5U/kg, 1U/kg, 2U/kg; 45min before session onset; Lilly, HumulinR). Drugs administered peripherally were dissolved in normal saline (0.9% NaCl). All animals were habituated to i.p. and s.c. injections with a single injection of normal saline the day before the first treatment.

To induce central cytoglucopenia, 5TG (135μg/3μL; either 15min or 45min before session onset; Cat. No. 88635, Sigma) was into injected either the LV or into the 4V in animals that received intraoral sucrose. To determine whether the effects of 5TG were nonspecific to the rewarding or caloric value of sucrose, 5TG (135μg/3μL; 45min before session onset) was also injected in the LV of *ad libitum* fed and watered animals that received intraoral water infusions. As a positive control for the effects of 5TG on dopamine responses to intraoral water, I either made rats thirsty with 20h water deprivation (but fed *ad libitum*) or centrally administered the thirst-associated hormone, angiotensin II (AngII). Previous work showed that AngII increases dopamine signaling to water stimuli (Hsu et al., 2020). AngII (10ng/1μL; immediately before session onset; Cat. No. H-1705, Bachem) was injected into the LV of *ad libitum* fed and watered rats. In contrast with cytoglucopenia as a hunger-associated signal, I tested whether a satiety-associated signal via the glucagon-like peptide 1 (GLP-1) system would modulate phasic dopamine signaling evoked by primary reward. The GLP-1 analog, Exendin-4 (Ex4; 0.1μg/1μL; 45min before session onset; Cat. No. 4044219, Bachem) was injected into the LV. Drugs administered centrally were dissolved in artificial cerebral spinal fluid (aCSF; Cat. No. 3525, Tocris). After habituation to intracranial cannula infusions in days preceding the experiment, drugs were manually administered with a 33-gauge microsyringe injector (Hamilton) that projected 2 mm beyond the guide cannula. All pharmacological treatments were performed in a counterbalanced, within-subjects design.

B. 5. <u>In Vivo Fiber Photometry</u>

In vivo fiber photometry was performed according to protocols previously described (Konanur et al., 2020). Briefly, LEDs (light-emitting diodes; Doric Lenses) administered 465nm (Ca^{2+} dependent) and 405nm (Ca^{2+} independent) excitation. Intensity of the 465nm and 405nm light was sinusoidally modulated at 211Hz and 531Hz, respectively, for all recording sessions. Light was coupled to a filter cube (FMC4, Doric Lenses) and converged into an optical fiber

patch cord mated to the fiber optic implant of the animal. Fluorescence was collected by the same fiber/patch cord and focused onto a photoreceiver (Visible Femtowatt Photoreceiver Model 2151, Newport). A lock-in amplifier and data acquisition system (RZ5P; Tucker Davis Technologies), was used to demodulate the fluorescence due to 465nm and 405nm excitation. Real-time events (e.g., cue, intraoral infusion) were sent as time-stamped TTL (transistor-transistor logic) to the same data acquisition system and recorded in software (Synapse Suite, Tucker Davis Technologies). A Fourier transformed subtraction was used to account for movement artifacts and fluorescence bleaching. The subtracted signal was smoothed using a custom fifth order bandpass Butterworth filter (cutoff frequencies: 0.05 Hz, 2.25 Hz). For Ca^{2+} transient analysis, a transient was defined as a point that exceeds 3 standard deviations of the overall signal above the previous point.

B. 6. <u>Signal normalization and processing</u>

For comparability of paradigm-related responses across recording sessions, the smoothed fourier subtracted Ca^{2+} specific signal of each session was normalized by each session's average fluorescence and SD to convert data to z-scores. The normalized signal was then aligned to events of interest (cues, intraoral infusion). All data processing was performed using custom MATLAB scripts (available upon request to corresponding author).

B. 7. <u>Immunohistochemistry and verification of recording sites</u>

Following completion of experiments, rats were deeply anesthetized with sodium pentobarbital (100mg/kg) and transcardially perfused with 0.9% saline followed by 10% buffered formalin solution (HT501320, Sigma Aldrich, Inc). Brains were removed and stored in formalin for 24h and then transferred to 20% sucrose in 0.01M KPBS. All brains were sectioned at 40µm on a freezing stage microtome (SM2010R, Leica Biosystems). VTA sections were collected and processed to label for GFP (as an indicator of GCaMP6f expression) and tyrosine hydroxylase (TH) via immunohistochemistry. Antibody incubations and washes were done at room

temperature. Tissues were permeabilized in 0.3% Triton-X 100 for 30min and were blocked in 2% normal donkey serum for 30min. Sections were incubated in 1:1000 rabbit anti-TH (AB152, Sigma Aldrich) and 1:1000 chicken anti-GFP (AB13907, Abcam) antibodies overnight (~18 h). Primary antibodies were diluted in the following solution: KPBS containing 2% normal donkey serum, followed by KPBS washes (8 changes, 10min each). Secondary antibody (1:500 Cy3 conjugated donkey anti-rabbit and 1:500 AF488 conjugated donkey anti-chicken; Jackson Immunoresearch) incubations were performed overnight. Sections were then mounted onto glass slides, air dried, and coverslipped with 50% glycerol in KPBS mountant. Only data from subjects with GCaMP6f expression and VTA fiber placement, verified using fluorescent microscopy in conjunction with the rat brain atlas of Paxinos and Watson (2006), were included in statistical analyses. All photometry recordings were made in the paranigral region of the VTA. Density distributions of fiber tip placements were estimated using a two-dimensional kernel as per Venables et. al., 2002.

B. 8. Statistical analyses

Dopamine signal during intraoral infusions or during cues was quantified by first averaging the signal during the period of interest and then using an analysis of variance (ANOVA) with treatment as a within-subjects variable. Sidak-corrected pairwise follow-up comparisons were used to compare each treatment (e.g. food restriction, 5TG, etc.) to control. Hierarchical-linear regression models (HLM) were used to analyze multifactorial relationships that required accounting for more than two independent variables. In such cases subjects were treated as a random factor term to account for inter-subject variance. The latency of a Ca^{2+} transient was defined as the time elapsed after the onset of an event (e.g. CS+ cue) to the peak of the Ca^{2+} transient. All statistical analyses were computed using the coding environment R (https://www.r-project.org/) with an α level for significance at 0.05.

C. Results

C. 1. Food restriction enhances phasic dopamine signaling in the ventral tegmental area to food

To measure the dopamine neural response to consummatory food reward, I made intraoral infusions (30 trials) of 0.3M sucrose while recording from dopamine cell bodies in the paranigral region of the VTA using *in vivo* fiber photometry (Figure II.1 A-C). Transients in phasic dopamine signaling occur throughout the recording session (Figure II.1 D) and intraoral infusions of sucrose evoke increases in phasic dopamine signaling (Figure II.1 E). To determine how sucrose-evoked dopamine transient neuronal activity compares with transients detected during inter-trial intervals, mean dopamine signal during sucrose infusion (5s) was compared to a “baseline” period of 5s prior to infusions onset and a “post-infusion” period of 5s after the infusion offset, in initially naïve rats (n = 19). Figure II.1 H depicts the average phasic dopamine signaling around intraoral sucrose across all trials and all subjects. I compared the mean phasic dopamine signaling in these time periods as a function of trial number in an HLM ($r^2 = 0.17$; Figure II.1 I). Mean phasic dopamine signaling during [$\beta = 0.45$, $p < 0.01$] and after [$\beta = 0.07$, $p = 0.02$] intraoral infusions was significantly elevated relative to baseline. A significant interaction revealed the mean phasic dopamine signaling increased as a function of trial only during the infusion period [$\beta = 0.009$, $p = 0.01$] and not during the post-infusion period [$\beta = 0.003$, $p = 0.40$] relative to the baseline period. This suggests that the VTA dopamine response to intraoral sucrose is being strengthened over exposure to the sucrose.

Previous reports show that dopamine neuron activity (Branch et al., 2013), dopamine release evoked by food (J. J. Cone et al., 2014; Wilson et al., 1995), and dopamine receptor function (Carr et al., 2003) increase with food restriction. Therefore, I hypothesized that dopamine responses to intraoral sucrose would be greater in food restricted relative to *ad libitum* fed rats. I tested rats under food deprived (20h) or *ad libitum* fed conditions (n = 6 rats) in

a within subject design with counterbalanced order for feeding conditions (Figure II.1 J). The mean dopamine response during intraoral infusion was significantly enhanced by food restriction relative to *ad libitum* feeding [$t(5) = 0.32$, $p = 0.01$] (Figure II.1 J, inset). There was no difference in the baseline period between feeding conditions [$t(5) = -0.64$, $p = 0.55$]. Taken together, these data support signals and circuits responsive to food deprivation modulate the dopamine response specifically to intraoral infusions of sucrose.

C. 2. <u>5TG induced cytoglucopenia potentiates food evoked phasic dopamine signaling</u>

In humans, antiglycolytic agents increase hunger ratings and the subjective value of sucrose (Thompson & Campbell, 1977). Antiglycolytic agents potently drive food intake within an hour of administration (R. C. Ritter et al., 1978; Slusser & Ritter, 1980). Since I established that food deprivation potentiates the dopamine response to intraoral sucrose, I hypothesized that administration of 5TG, an antiglycolytic agent, to *ad libitum* fed rats would recapitulate the effect. I injected 5TG (n = 13) either 15min or 45min before recording in a within subject design with counterbalanced order. Intraperitoneal (i.p.) administration of 5TG 15 minutes before testing failed to modulate dopamine signaling to intraoral sucrose at any dose [$F(2, 24) = 0.45$, $p = 0.64$] (Figure II.2 A). However, when 5TG was injected 45 minutes before testing, it significantly potentiated dopamine signaling to intraoral sucrose [$F(2, 24) = 7.87$, $p < 0.01$] at 50mg/kg [$t(12) = 2.67$, $p < 0.01$] and at 100mg/kg [$t(12) = 3.45$, $p = 0.47$] (Figure II.2 B).

The latency for 5TG to modulate dopamine signaling suggests a site of action distal to the injection (i.p.). I hypothesized that 5TG was exerting its delayed effects via diffusion to the brain. In subsequent experiments, I therefore administered 5TG (135μg, n = 6) into the lateral ventricle (LV) either 15min or 45min before recording in a within subject design with counterbalanced order. Relative to vehicle, LV 5TG significantly increased dopamine signaling to intraoral sucrose at 15min post-injection [$t(5) = 3.13$, $p = 0.03$] (Figure II.2 C) and at 45min post-injection [$t(3) = 3.46$, $p = 0.04$] (Figure II.2 D).

Prior work supports the hindbrain as a key site of central 5TG effects to promote eating behavior (R. C. Ritter et al., 1981). To determine whether hindbrain cytoglucopenia was sufficient to modulate dopamine signaling, I administered 5TG (n = 11) into the fourth ventricle (4V) either 15min or 45min before recording in a within subject design with counterbalanced order. Relative to vehicle, 5TG infusions into 4V significantly increased phasic dopamine signaling to intraoral sucrose at both 15min [$t(9) = 5.06$, $p < 0.01$] (Figure II.2 E) and 45min post-injection [$t(10) = 3.74$, $p < 0.01$] (Figure II.2 F).

To compare the magnitude of the effects of 5TG on dopamine signaling as a function of injection site and delay, I computed Cohen's D – a quantitative measure of effect size. Results revealed that 4V sites were most effective (see Cohen's D measures in Figure II.2). To further determine whether 5TG in the hindbrain had a more potent effect, I analyzed the same data described above using an HLM which contained 5TG treatment (vehicle vs. 5TG) and administration site (LV vs 4V) as independent variables, $r^2 = 0.386$. I found a main effect of 5TG [$\beta = 0.36$, $p < 0.01$] and an interaction between 5TG and administration site, such that dopamine signaling to intraoral sucrose was significantly larger in animals that received 5TG in the 4V [$\beta = 0.15$, $p < 0.01$]. Collectively, these data support that 5TG is likely acting in the hindbrain to modulate dopamine signaling to intraoral sucrose.

C. 3. <u>Insulin induced cytoglucopenia potentiates food evoked phasic dopamine signaling</u>

5TG induces cytoglucopenia that results in counterregulatory responses including, increase in eating and increase in blood glucose (R. C. Ritter & Slusser, 1980). It is therefore unclear whether the dopamine modulatory effects of 5TG are due to cytoglucopenia or are secondary to compensatory changes in blood glucose. To address this issue, I administered insulin subcutaneously (s.c., n = 12). Insulin, like 5TG, stimulates eating behavior and cytoglucopenia (R. C. Ritter et al., 1981). In sharp contrast to 5TG, though, insulin decreases blood glucose. I injected either vehicle or insulin (0.5U/kg, 1U/kg, or 2U/kg) 45min before

recording in a within subject design with counterbalanced order for insulin dose (Figure II.3 A). Each dose was accompanied by its own vehicle treatment. An analysis of the phasic dopamine signaling to intraoral sucrose revealed a significant main effect of treatment (vehicle vs. insulin) [$F(1,10) = 7.46$, $p = 0.02$], and a significant main effect of insulin dose (0.5, 1, 2U/kg) [$F(2,20) = 6.65$, $p < 0.01$], but no interaction between treatment and dose [$F(2,20) = 0.25$, $p = 0.78$]. Post-hoc contrasts showed no differences between vehicle and insulin at any dose (0.5U/kg insulin, [$t(29.2) = 0.93$, $p = 0.36$]; 1U/kg insulin, [$t(29.2) = 1.26$, $p = 0.22$]; 2U/kg insulin, [$t(29.2) = 1.96$, $p = 0.06$]). To further explore the significant main effects in the ANOVA, I used an HLM at each dose with insulin treatment (vehicle vs. insulin) and trial number as independent variables and subjects as a random factor to account for inter-subject variance (Figure II.3 B). I found a significant main effect of insulin treatment in the 0.5U/kg dose [$r^2 = 0.32$, $\beta = 0.10$, $p = 0.02$], the 1U/kg dose [$r^2 = 0.41$, $\beta = 0.10$, $p = 0.02$] and the 2U/kg dose [$r^2 = 0.51$, $\beta = 0.17$, $p < 0.01$]. Interestingly, I also found an interaction between insulin treatment at the 2U/kg dose and trial number, such that insulin potentiated phasic dopamine signaling to intraoral sucrose at the early trials [$\beta = -0.01$, $p < 0.01$]. In other words, the 2.0U/kg dose of insulin elicited a higher potentiation of dopamine signaling to sucrose [$\beta = 0.17$] than the 1.0U/kg [$\beta = 0.10$] or the 0.5U/kg [$\beta = 0.10$] doses, suggesting that dopamine signaling returns to near baseline levels at the end of the session for all doses. To verify whether insulin returned to baseline levels, I isolated the analysis to only the final ten trials. Indeed, we see that at the end of each session there is no effect of insulin on sucrose evoked dopamine at any dose (0.5U/kg: $\beta = 0.08$, $p = 0.26$; 1U/kg: $\beta = -0.03$, $p = 0.67$; 2U/kg: $\beta = 0.08$, $p = 0.10$). In general, these data further support that central cytoglucopenia is sufficient to augment dopamine signaling to primary reward and that it is unlikely that 5TG-induced increase in blood glucose modulates dopamine signaling.

C. 4. 5TG fails to potentiate phasic dopamine signaling to intraoral water

Conceivably, 5TG's augmentation of phasic dopamine signaling to intraoral sucrose may be nonspecific to the rewarding or caloric value of the intraoral solution. To address this question, I injected 5TG in the LV 45min before recording and measured VTA dopamine activity during intraoral infusions of water (Figure II.4 A) in *ad libitum* fed and watered rats (n = 8). 5TG failed to enhance phasic dopamine signaling to intraoral water [$t(7) = 0.40$, $p = 0.70$]. Previous work has shown that water restriction or the central delivery of hormone angiotensin II (AngII) potentiates the dopamine response to water (Hsu et al., 2020). Here, in the same rats in which 5TG failed to affect the dopamine response to intraoral water, I found that both water restriction [n = 10, $t(9) = 2.74$, $p = 0.02$] (Figure II.4 B) or LV AngII in *ad libitum* watered rats [n = 8, $t(7) = 3.67$, $p < 0.01$] (Figure II.4 C) potentiated phasic dopamine signaling to intraoral water. Thus, the failure of 5TG to alter dopamine signaling to water likely reflects a selective effect on caloric solutions.

C. 5. Central glucagon-like peptide 1 receptor (GLP-1R) stimulation suppresses phasic dopamine signaling to food

5TG, which increases food intake, increased the dopamine response to food in *ad libitum* fed animals. Collectively, the data suggest that hunger signals emanating from the brainstem can modulate dopamine signaling to food. GLP-1 is made and released by cells of the brainstem and operates, in part, as a satiety signal. I previously showed that GLP-1R signaling suppresses dopamine responses to food-cues (Konanur et al., 2020). To determine whether satiety signals modulate dopamine responses to primary reward, I administered LV GLP-1 analog, Exendin-4 (Ex4, n = 11) 45min prior to recording. While phasic dopamine signaling to intraoral sucrose did not shift after Ex4 administration (Figure II.5 A inset, ANOVA: [$F(2, 24) = 0.63$, $p = 0.54$]), there was a significant interaction between Ex4 dose and trial number (Figure II.5 B, HLM: [$\beta = -1.61$, $p < 0.01$]). Specifically, Ex4 more potently suppressed

dopamine signaling to intraoral sucrose during later infusions [0.05ug Ex4: β = -0.0076, $p < 0.01$; 0.1ug Ex4: β = -0.0156, $p < 0.01$] than vehicle [β = 0.0005, $p = 0.91$]. In addition to hunger-related signals, satiety-associated GLP-1R activation is also encoded in dopamine signaling evoked by intraoral sucrose.

C. 6. Food-cues evoke robust phasic dopamine signaling in the ventral tegmental area

The work described above focused on dopamine responses to a primary rewarding stimulus (intraoral sucrose). Given that the ubiquity of food advertisements increases food intake (Harris et al., 2009; Johnson, 2013; Saelens et al., 2012; Zimmerman & Bell, 2010), understanding factors that modulate the encoding of food-cues is critical. I conditioned *ad libitum* fed rats (n = 11) to associate an audio cue (CS+) to intraoral sucrose delivery and a different audio cue (CS-) to the absence of an intraoral infusion (Figure II.6 A). Over 10 days of conditioning, dopamine transient amplitude evoked by the CS+ significantly increased [β = 0.09, $p < 0.01$] while dopamine signaling during and after the CS- was unchanged [β = 0.0007, $p = 0.95$] (Figure II.6 B). Augmented VTA phasic dopamine signaling to cues over conditioning is consistent with a vast literature showing the development of time-locked dopamine responses to cues associated with primary reward (Coddington & Dudman, 2018; J. J. Day et al., 2007; Roitman et al., 2004; 2008; Waelti et al., 2001). I further characterized the dopamine response to the CS+/- by measuring the latency to the first dopamine transient after cue onset and computing the latency jitter (the period between earliest and latest evoked transient; Figure II.6 C). Over conditioning, CS+ latency jitter decreased [β = -25.49, $p < 0.01$], while CS- latency jitter did not change [β = 3.67, $p = 0.09$] (Figure II.6 D). Recapitulating previous work, a food-associated cue elicited a robust increase in phasic VTA dopamine signal.

C. 7. 5TG potentiates food-cue evoked and sucrose evoked dopamine signaling in a site-specific manner

Physiological state and learned reward cues interact to influence food seeking and intake (Weingarten, 1984). Since various signals of physiological state impact dopamine signaling evoked by food-cues (J. J. Cone et al., 2014; Hsu et al., 2020; Konanur et al., 2020), I hypothesized that cytoglucopenia would potentiate dopamine signaling to cues associated with sucrose reward. I injected 5TG into either the LV ($n = 5$) or into the 4V ($n = 6$) 45min before recording (Figure II.6 E). I found a significant increase in dopamine signaling to CS+ only when 5TG was administered in LV [$t(4) = 4.00$, $p = 0.02$] but not in subjects receiving 5TG in their 4V [$t(5) = 1.16$, $p = 0.30$] (Figure II.6 F, left). Interestingly, I found that 5TG augmented the dopamine response to sucrose only after 4V infusions [$t(5) = 3.40$, $p = 0.02$] but not after LV infusions [$t(4) = 1.78$, $p = 0.15$] (Figure II.6 F, right). Taken together, these data suggest that cytoglucopenia modulates food evoked dopamine signaling via the hindbrain, while forebrain structures interact with glucoprivation to modulate dopamine signaling to cues that predict food.

D. Discussion

Phasic activity of VTA dopamine neurons and NAc dopamine release are essential in driving motivated behaviors in an adaptive manner. Signals that convey hunger (J. J. Cone et al., 2014) and satiety (Konanur et al., 2020; Mietlicki-Baase et al., 2015) profoundly impact motivated behavior and phasic VTA activity evoked by primary reward and their associated cues. Arguably, glucose availability most faithfully reflects energy status and is monitored and regulated by various central sites. Notably, intracellular glucose is monitored and regulated by 5' AMP-activated protein kinase (AMPK), an energy sensor evolutionarily conserved in all cells (Hardie, 2018). However, very little is known regarding whether fluctuating brain glucose levels influence dopamine signaling to primary and appetitive food rewards. Here, we show that inducing cytoglucopenia potentiates phasic dopamine responses evoked by either primary

rewards (consummatory food reward) or by reward associated cues (known to initiate appetitive reward behaviors (Cleland & Davey, 1983; Robinson et al., 2014)). Recording real-time phasic signaling specific to dopamine neurons in the VTA provided us unique insight into how signals that relate physiological state modulate phasic VTA dopamine signaling evoked by primary rewarding taste stimuli.

Intraoral infusion of sucrose in ad libitum fed rats evokes a phasic increase in VTA dopamine signaling (Hsu et al., 2020). Furthermore, *ex vivo* electrophysiological recordings show that food restriction makes dopamine neurons more excitable (Branch et al., 2013). Here we show that food restriction in awake and behaving animals potentiates phasic dopamine signaling to intraoral sucrose. This food evoked dopamine response is consistent with previous reports that show an increase in food evoked dopamine release in the terminal regions in food restricted animals (Carr et al., 2003; J. J. Cone et al., 2014; Wilson et al., 1995). Here we see that food restriction amplifies phasic dopamine signaling only during intraoral sucrose infusions but not during the baseline period. Along with previous work, the present data suggest that food restriction does not modulate all dopamine signaling nonspecifically, but rather that food rewards are the trigger through which food restriction augments dopamine signaling.

5TG induced cytoglucopenia potentiated intraoral sucrose evoked dopamine signaling similar to that seen by food deprivation. We also showed that the proximity of 5TG administration to the hindbrain increased the potency of the modulation of dopamine signaling. These results are supported by previous reports that show there are glucose sensing structures in the hindbrain that are necessary for eating in response to cytoglucopenia (Flynn & Grill, 1983; S. Ritter et al., 2000; 2001) and that 5TG isolated to the forebrain fails to produce glucoprivic eating (R. C. Ritter et al., 1981). However, we do see that there is an augmentation of sucrose evoked dopamine signaling when 5TG is administered to the LV. Although peripheral and LV administration of 5TG does influence hindbrain glucosensors, due to the broad nature of this

pharmacological administration, it is possible that LV 5TG may also engage glucosensors in the forebrain (Claret et al., 2007; Oomura et al., 1974) and possibly VTA dopamine neurons themselves (Sheng et al., 2014). It is important to also note that 5TG delivered directly to most subregions of the lateral hypothalamus (LH) does not produce glucoprivic eating (S. Ritter et al., 2000). Current studies cannot completely parse the influence of the hindbrain versus the forebrain on food evoked dopamine signaling. Therefore, future studies that employ region-specific administration of 5TG will be required to determine whether regions such as the ventromedial hypothalamus in the forebrain or the nucleus of the solitary tract (NTS) in the hindbrain are sufficient to modulate food evoked dopamine signaling in response to cytoglucopenia.

5TG administration induces cytoglucopenia – which drives both eating and a counter-regulatory increase in blood glucose. It was therefore possible that changes in dopamine signaling related to the blood glucose response. To address this potential confound, we administered insulin – which, like 5TG, causes cytoglucopenia but, in sharp contrast to 5TG causes a decrease in blood glucose. In general, we found that insulin-induced cytoglucopenia potentiates dopamine signaling to consummatory food reward. Specifically, across the whole session we observed only trend at each dose but a main effect for insulin to increase dopamine signaling to intraoral sucrose. However, when the data were analyzed on a trial-by-trial basis, we observed a clear increase in dopamine signaling toward the beginning of the session, which then waned over the course of the session. The data support the idea that insulin lowers blood glucose to induce central cytoglucopenia, likely sensed by hindbrain neurons to then potentiate dopamine response to food reward. However, the dynamics of how insulin and 5TG modulate dopamine signaling, are clearly different. One plausible explanation is that insulin's effect on mesolimbic dopamine signaling is much more transient than the antiglycolytic effects of 5TG. For example, the transient time course of insulin is demonstrated in the behavior— although

insulin lowers blood glucose and increases food intake, eating behavior ceases before blood glucose recovers to baseline levels (R. C. Ritter et al., 1978). The brevity in modulating eating behavior suggests that insulin-induced cytoglucopenia's influence on dopaminergic drive is short-lived. The attenuation of dopamine signaling might be explained by inhibitory nociceptin neuron innervation. Paranigral VTA nociceptin neurons suppress motivated behaviors (Parker et al., 2019), suggesting that insulin-induced enhancement of dopamine signaling might be countered by nociceptin signaling toward the end of the session. Future studies might consider the interaction between nociceptin and insulin to better understand the dynamics of insulin-induced drive on mesolimbic dopamine responses to taste.

In keeping with our previous work (Fortin & Roitman, 2018; Hsu et al., 2020), we found that phasic dopamine signaling is modulated only to restorative stimuli that are relevant to the induced need state. For example, while thirst and thirst mimetics potentiated dopamine signaling to intraoral water, 5TG elicited no such enhancement. Conversely, a satiety signal via GLP-1R stimulation suppressed dopamine signaling to intraoral sucrose, consistent with previous reports (Alhadeff et al., 2012; Konanur et al., 2020). It's important to note that the current study does not address whether 5TG induced potentiation of dopamine signaling is specific to carbohydrates. However, given that 5TG modulates appetitive behavior toward sucrose but not toward fats (Altizer & Davidson, 1998), it is likely that 5TG induced cytoglucopenia orients goal-direction toward carbohydrates. Further work employing the intraoral delivery of other macronutrients (fats, proteins, etc.) will be necessary to determine whether cytoglucopenia impacts dopamine signaling in a carbohydrate specific manner.

In addition to consummatory food rewards, appetitive food rewards also elicit dopamine neuron activity (Schultz, 1998; Schultz & Romo, 1990; Waelti et al., 2001). Although we did not directly measure the learning performance of the cue-sucrose association, previous work indicates learning within the first exposure session via an increased anticipatory motor output to

food cues, where motor output is correlated with activity of nucleus accumbens medium spiny neurons (Roitman et al., 2005). In the present study cue evoked phasic dopamine signaling continues to develop over conditioning, suggesting that the cue-sucrose association is strengthened with repeated exposures. Further work would be necessary to determine whether physiological state enhances the rate of the development of evoked cue. Unlike modulation of the dopamine response to primary food reward, we found that cue evoked dopamine signaling is enhanced only when 5TG was administered to the forebrain but not the hindbrain. Hindbrain structures, such as the NTS, encode essential information pertaining to the taste of carbohydrates (W. W. Liu & Bohórquez, 2022; Roussin et al., 2012; Travers & Norgren, 1995). However, it seems that more anterior structures, such as the LH, may convey to the mesolimbic system information regarding learning and learned associations (Petrovich et al., 2005; Sharpe et al., 2017). Future work will use a more targeted approach to recording from VTA dopamine neurons that receive input from the LH or from the NTS to determine whether encoding of appetitive and consummatory stimuli are indeed modulated by physiological state in an anatomically distinct manner.

The current study underscores the interaction between signals that convey physiological state and the neural response to sapid sucrose and its associated cues. Although glucose sensing occurs in multiple regions in the periphery as well as in the central nervous system, the mesolimbic system is one of the few structures in the brain that integrate interoceptive and exteroceptive information. Mesolimbic dopamine output is critical in producing appropriate behaviors toward stimuli that restore homeostatic balance. A state of glucose deprivation clearly primes such dopamine circuits to respond more strongly to taste stimuli. However, it is yet unclear in what manner taste information arrives at the VTA. Future studies should consider whether taste information arrives at the VTA already modified by physiological state or whether the VTA is biased to respond differently to the same taste stimuli. Additionally, any studies

examining eating disorders should account for shifts in mesolimbic dopamine responses to food and food cues, as modulation of motivated behaviors are promising targets of therapeutic development. Shifts in glucose availability (relevant in various eating disorders) and GLP-1R signaling (relevant in clinical treatment of type 2 diabetes and obesity) have marked effects on circuits that encode motivational state toward food. Here, we show that physiological state (cytoglucopenia and GLP-1R signaling) modulates phasic VTA dopamine signaling specifically related to consummatory food rewards and to the cues that predict them.

Figure II.1: Intraoral sucrose evokes dopamine neuron activity in the VTA.

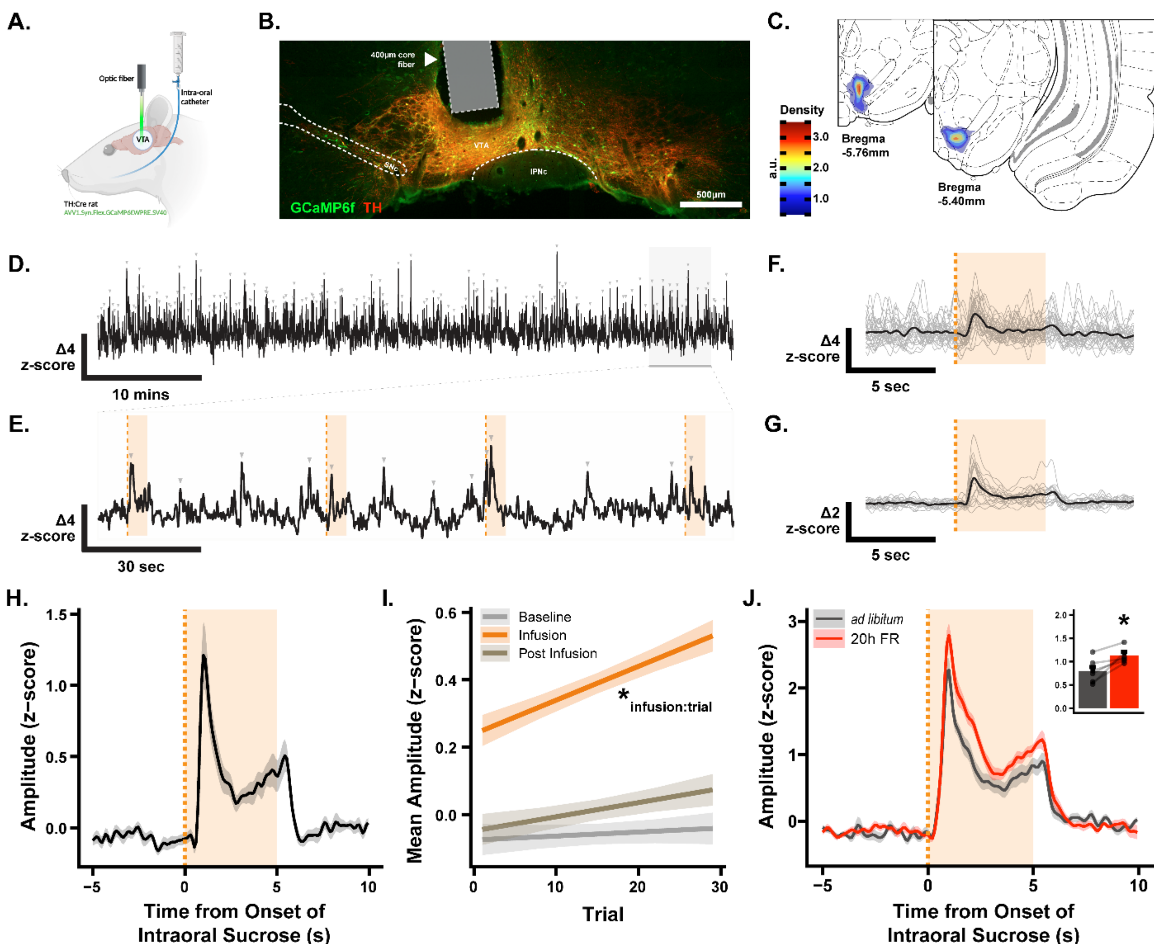

Figure II.1: Intraoral sucrose evokes dopamine neuron activity in the VTA. ***(A)*** A schematic of a rat prepared for *in vivo* fiber photometry recording. ***(B)*** Representative image of the VTA. ***(C)*** Location of optic fiber tips from all rats used in photometry recordings depicted as density distributions in arbitrary units determined by a 2-D kernel estimation of density. ***(D)*** Trace of dopamine neuron activity across the entire session during habituation to intraoral sucrose from a representative rat. Ca^{2+} transients are represented by light gray arrow tips. ***(E)*** A snippet from the session seen in ***D*** (shaded) containing four intraoral infusions (−5 to 10 s relative to the start [dotted vertical line] of the 5-s intraoral infusion [orange box]). ***(F)*** Average dopamine neuron activity (black) of 30 trials (gray) of one animal's session (seen in ***D***) aligned to the onset of intraoral sucrose. ***(G)*** Average dopamine neuron activity (black) of all animals' peri-sucrose averages (gray). ***(H)*** Same data as ***G***. ***(I)*** An HLM regression of the mean amplitude of dopamine neuron activity during the periods of baseline, intraoral sucrose infusion, and post-infusion, reveals a significant increase of dopamine signal only during infusion as a function of trial number. ***(J)*** Food restriction potentiates sucrose evoked dopamine neuron activity. Quantification in the inset represents the mean amplitude of dopamine neuron activity during the intraoral sucrose infusion. Asterisks denote $p < 0.05$ and "**:**" denotes an interaction. Solid lines represent the mean and error bars/ribbons represent SEM.

Figure II.2: 5TG induced cytoglucopenia modulates sucrose evoked dopamine neuron activity in a site- and time-dependent manner.

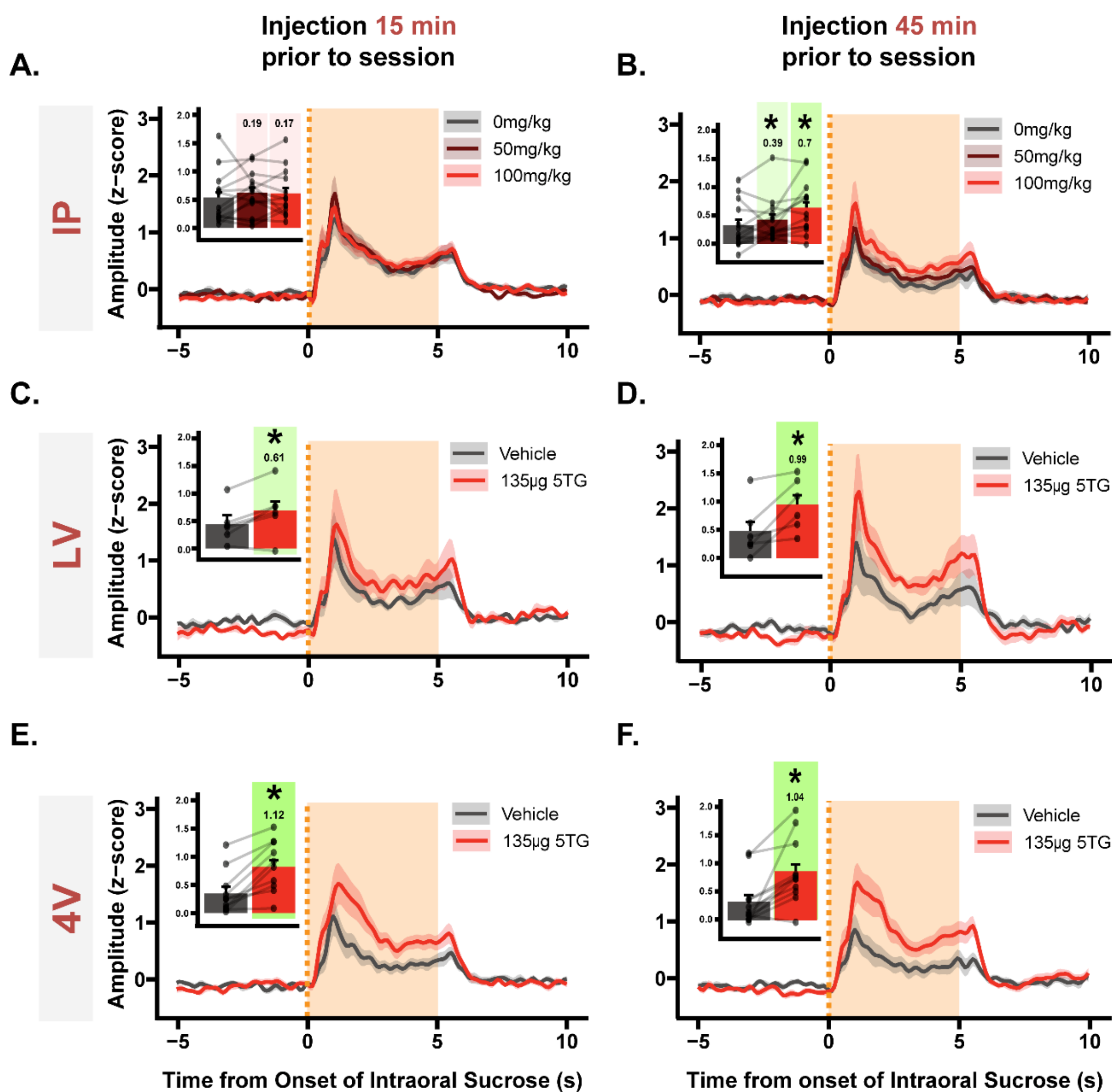

Figure II.2: 5TG induced cytoglucopenia modulates sucrose evoked dopamine neuron activity in a site- and time-dependent manner.Dopamine neuron activity 15 minutes ***(A,C,E)*** or 45 minutes ***(B,D,F)*** after IP ***(A,B)***, or LV ***(C,D)***, or 4V ***(E,F)*** 5TG injection, aligned to intraoral sucrose infusions (−5 to 10 s relative to the start [dotted vertical line] of the 5-s intraoral infusion [orange box]), quantification in the inset. Numbers above each bar within the insets represent effect size computed by Cohen's D. Red denotes $p > 0.05$, green denotes $p < 0.05$. Intensity of green color is proportional to effect size. Asterisks represent $p < 0.05$. Solid lines represent the mean and error bars/ribbons represent SEM.

Figure II.3: Peripheral insulin induced cytoglucopenia potentiates sucrose evoked dopamine.

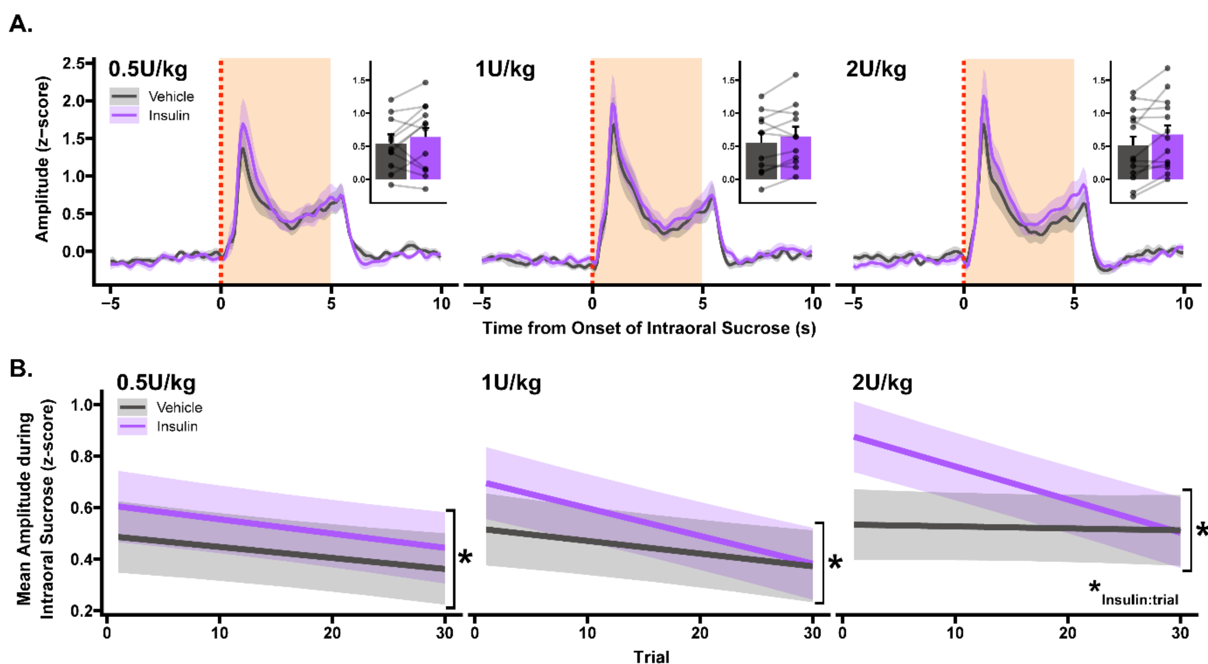

Figure II.3: Peripheral insulin induced cytoglucopenia potentiates sucrose evoked dopamine. ***(A)*** Dopamine neuron activity aligned to intraoral sucrose infusions 45 minutes after 0.5U/kg (left), 1U/kg (middle), and 2U/kg (right) s.c. insulin. Data are displayed in a format identical to Figure II.2. ***(B)*** HLM regressions of mean amplitude of dopamine neuron activity during intraoral sucrose infusion as a function of trial number at each dose of insulin represented in ***A***. Asterisks denote $p < 0.05$ and "**:**" denotes an interaction effect. Solid lines represent the mean and error bars/ribbons represent SEM.

Figure II.4: 5TG fails to potentiate water evoked dopamine neuron activity.

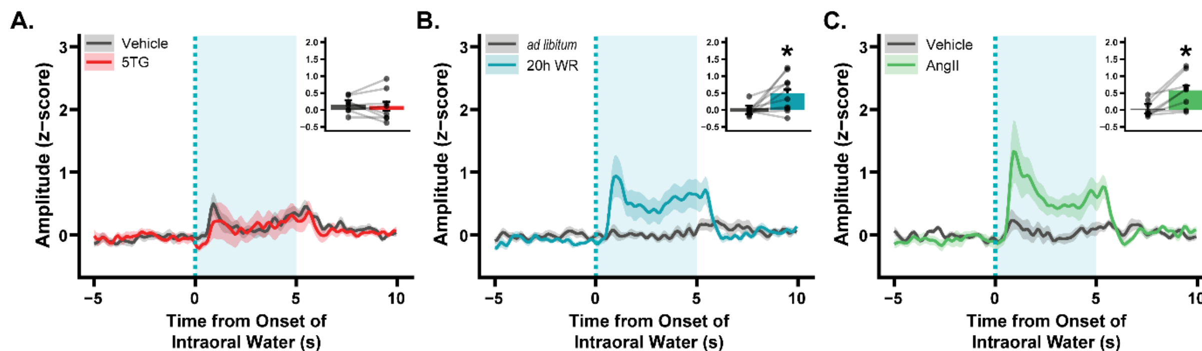

Figure II.4: 5TG fails to potentiate water evoked dopamine neuron activity. ***(A)*** Dopamine neuron activity to intraoral water (onset represented by vertical dotted line and infusion represented by blue box) is not enhanced 45 minutes after LV 5TG. However, 20 hour water restriction ***(B)*** and the thirst associated hormone, angiotensin II ***(C)***, potentiate dopamine neuron activity to intraoral water. Asterisks represent $p < 0.05$. Solid lines represent the mean and error bars/ribbons represent SEM.

Figure II.5: Satiety inducing glucagon-like 1 receptor (GLP-1R) stimulation suppresses sucrose evoked dopamine neuron activity.

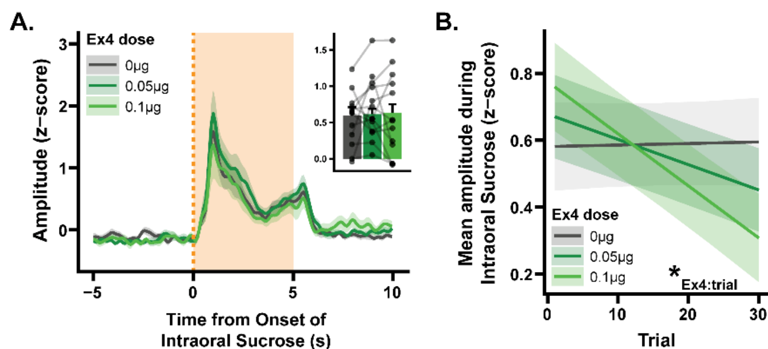

Figure II.5: Satiety inducing glucagon-like 1 receptor (GLP-1R) stimulation suppresses sucrose evoked dopamine neuron activity. ***(A)*** Dopamine neuron activity represented identically to Figure II.2, in response to LV injection of a GLP-1R agonist, Exendin-4 (Ex4). ***(B)*** Mean dopamine neuron activity during intraoral sucrose infusion (same data from ***A***) as a function of trial number. Asterisks denote $p < 0.05$ and "**:**" denotes an interaction effect. Solid lines represent the mean and error bars/ribbons represent SEM.

Figure II.6: 5TG potentiates CS+ and sucrose evoked dopamine signaling in a site-specific manner.

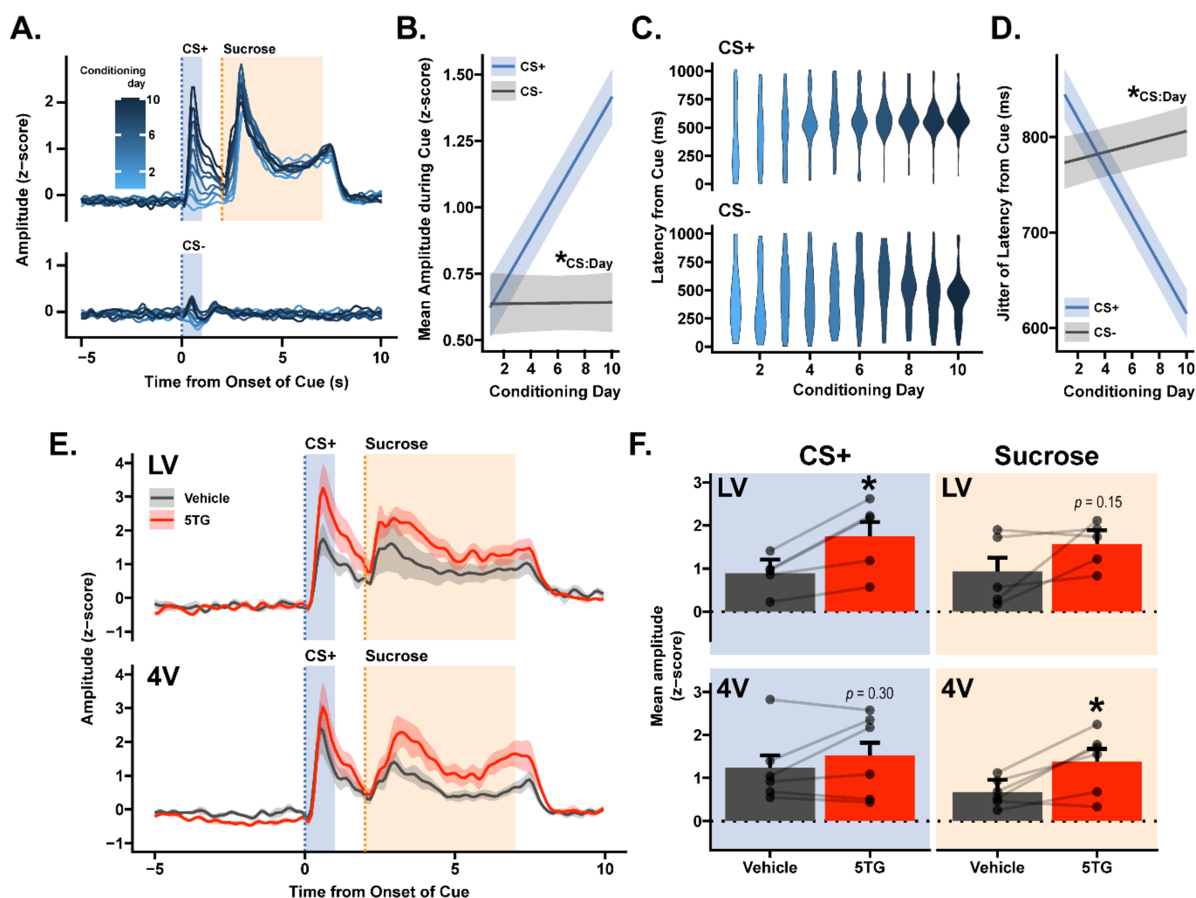

Figure II.6: 5TG potentiates CS+ and sucrose evoked dopamine signaling in a site-specific manner. ***(A)*** Dopamine neuron activity aligned to cue onset (blue vertical dotted line), with blue box representing the cue and orange box representing the sucrose. ***(B)*** Distribution of latencies from cue onset of Ca^{2+} transients that occur during the cue. ***(C)*** Jitter of latencies from cue onset decreases for CS+ but not for CS-, over conditioning. ***(D)*** Mean amplitude of dopamine neuron activity increases during CS+ but not during CS-, over conditioning. ***(E)*** Mean amplitude of dopamine neuron activity aligned to CS+ after an injection of either vehicle or 5TG in either the LV or in the 4V. ***(F)*** Quantification of data seen in ***E***. Asterisks denote $p < 0.05$ and ":" denotes an interaction effect. Ribbons and error bars around the mean represent SEM.

Chapter III: Phasic dopamine responses to a food-predictive cue are suppressed by the glucagon-like peptide-1 receptor agonist Exendin-4[1]

A. Introduction

Cues that are predictive of food hold powerful command over food-directed behavior, triggering craving and overeating in humans (Boswell & Kober, 2016), incentive motivation(Berridge, 2018), and increased appetitive behavior and overeating in rodents (Petrovich, 2013). While the neurocircuitry underlying cue-driven feeding is complex and distributed, there is substantial evidence that the mesolimbic dopamine system plays a critical role. Reliable predictors of food reward evoke brief (e.g. 1–2s, phasic) spikes in dopamine cell body activity (Coddington & Dudman, 2018; Lak et al., 2014; Schultz et al., 1997) and dopamine release in the nucleus accumbens (J. J. Day et al., 2007; Roitman et al., 2004; Stuber et al., 2008). Likewise, modulation of mesolimbic dopamine influences food-cue-evoked behavior (Halbout et al., 2019; Wyvell & Berridge, 2000). While considerable debate remains ((Berke, 2018), for example), recent work demonstrates that phasic responses of dopamine neurons and phasic dopamine release in the nucleus accumbens (NAc) are reinforcing (Steinberg et al., 2013; Tsai et al., 2009) and strongly promote goal-directed behaviors triggered by reward predictive cues (Coddington & Dudman, 2018; Fischbach-Weiss et al., 2018; Hamid et al., 2015; Hoffmann & Nicola, 2014; Medic et al., 2014). It is fundamentally advantageous for both humans and animals to utilize learned associations between external environmental cues and reinforcement in the service of obtaining substances required for homeostatic balance (e.g., food and water). It is also advantageous for these goal-directed behaviors to be regulated by

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changes in homeostatic states (e.g. hunger and satiety; see (Ferrario et al., 2016; Hsu et al., 2018; C. M. Liu & Kanoski, 2018; Rossi & Stuber, 2017) for review). Particularly in the context of feeding behaviors, deviations from homeostasis and the signals that relate them have potent modulatory effects on both the expression of goal-directed behaviors and on dopamine signaling in response to both primary food reward and cues associated with food reward (J. J. Cone et al., 2014; 2015; Roitman et al., 2004; Wilson et al., 1995).

Glucagon-like peptide-1 (GLP-1) is a neuropeptide and hormone derived from the nucleus of the solitary tract (NTS) and distal intestines, respectively. Peripheral and central activation of GLP-1 receptors (GLP-1R) strongly reduces food intake and body weight (For review see (Kanoski et al., 2016)), and GLP-1 analogs have been developed and approved for the treatment of Type II diabetes and obesity. GLP-1Rs act throughout the brain (Cork et al., 2015; Merchenthaler et al., 1999), including neural substrates within the mesolimbic pathway, to not only reduce food intake and body weight, but also to suppress goal-directed behaviors for food reinforcement (Alhadeff et al., 2012; Dossat et al., 2011; 2013; Mietlicki-Baase et al., 2013; 2014; Reiner et al., 2018; Richard et al., 2015). Thus, among the many gut- and brain-derived signals that relay hunger/satiety states, central GLP-1R signaling and its influence on the mesolimbic system provides one mechanism through which satiety factors might suppress goal-directed behaviors. While ongoing investigations have identified site-specific GLP-1R-mediated actions on goal-directed behaviors (Alhadeff et al., 2012; 2017; Dossat et al., 2011; 2013; Hsu et al., 2015; 2018; López-Ferreras et al., 2017; 2019; Mietlicki-Baase et al., 2013; 2014; Reiner et al., 2018; Richard et al., 2015) as well as putative roles of GLP-1Rs in modulating phasic dopamine signaling (Fortin et al., 2016; Fortin & Roitman, 2017; X. F. Wang et al., 2015), it remains unknown whether central GLP-1Rs modulate food-cue-evoked phasic dopamine signaling. This is a critical gap in the literature considering the importance of phasic dopamine signaling for cue-evoked approach behavior and reinforcement.

Here, I utilize in vivo fiber photometry in transgenic rats to measure calcium (Ca^{2+}) transients from VTA dopamine neurons in real-time. Food restricted male and female rats were trained to associate a 1s audio cue with brief access to a sucrose solution. Following training, rats received a central infusion of the GLP-1R agonist Exendin-4 (Ex4) just prior to test sessions. Rats were trained under food restriction to increase motivation to respond to sucrose and to mitigate central effects of endogenous GLP-1. Ca^{2+} transients occurred spontaneously but were also time-locked to key features of the behavioral paradigm. Regression analyses determined the interactions between measures of goal-directed behavior, sex, and event-related VTA phasic dopamine neuron activity. I found selective modulation of cue-evoked dopamine responses by central GLP-1R activation which was correlated with subsequent goal-directed behaviors.

B. Materials and methods

B. 1. Subjects

Male (n = 10) and female (n = 12; randomly cycling) Long Evans rats expressing Cre recombinase under the control of the tyrosine hydroxylase promoter [TH:Cre+; (Witten et al., 2011); Rat Research Resource Center, RRRC#: 659] were individually housed after weaning within a temperature and humidity controlled room and on a 12:12h light:dark schedule (lights on 0700h). Rats were maintained on *ad libitum* food and water unless otherwise noted, Four rats (n = 2 males and n = 2 randomly cycling females) were used to determine the penetrance and specificity of virally delivered constructs. Eight (n = 3 males and n = 5 randomly cycling females) were used to determine the relationship between Ca^{2+} transients and dopamine neuron excitability. Ten (n = 5 males and n = 5 randomly cycling females) were used to determine the effects of Ex4 on sucrose-directed behavior and dopamine transient activity. An additional 8 rats were initially and identically used but ultimately excluded due to criteria detailed in Section 7. Rats used in behavioral paradigms weighed >250g and were moderately food

restricted with 18g of food per day throughout the duration of their training and experiments. This modest amount of food restriction permitted gradual weight gain throughout training and testing. Animal care and use was in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

B. 2. Behavior

All training and experimental sessions took place during the light phase in standard operant chambers (ENV-009A-CT, Med Associates Inc.). Rats were trained to expect availability of a retractable sipper containing a 0.3M sucrose solution 1s after the onset of a tone (cue; 4.5 kHz, 1s duration). Licks at the sipper were timestamped using a contact lickometer and controller (ENV-252 M; ENV-250, Med Associates Inc.). A trial consisted of the 1s cue and 20s sipper availability followed by a randomly selected, variable inter-trial interval (32–48s). Daily sessions consisted of 30 trials for 10 consecutive days, after which, surgery was performed.

B. 3. Surgery

Male and female rats were anesthetized with ketamine hydrochloride (100mg/kg, i.p.) and xylazine hydrochloride (10mg/kg, i.p.) for stereotaxic surgery. First, a Cre-dependent virus containing the construct for a genetically encoded Ca^{2+} indicator AAV1.Syn.Flex.GCaMP6f.WPRE.SV40, University of Pennsylvania Vector Core) was unilaterally administered to the ventral tegmental area (VTA; 1μL of 0.5e13GC/ml: AP −5.4, ML −0.7, DV −8.15, mm relative to bregma) using a rate of 0.1μL/min and a 5min post infusion period to allow for diffusion before the injector was removed. Then, an optic fiber (flat 400μm core, 0.48NA, Doric Lenses Inc.) was implanted in the VTA just above the injection site (AP −5.4, ML −0.7, DV −8.00, mm relative to bregma). Finally, an infusion cannula (26Ga Cannula, PlasticsOne) was implanted above the lateral ventricle (LV; AP −0.9, ML −1.8, DV −2.6, mm relative to bregma). All animals received post-operative analgesia (0.1mL of 5mg/ml meloxicam,

s.c.) and were housed in their home cage for two weeks to allow sufficient time for recovery and construct expression. During this time, rats had *ad libitum* access to food. After two weeks, food restriction, for rats trained in the behavioral paradigm, resumed.

B. 4. Fiber photometry

LEDs (465, 405 nm, Doric Lenses) were used to excite GCaMP6f in order to measure Ca^{2+} activity. As Ca^{2+} binds GCaMP6f, the conformation of the GFP moiety changes to increase fluorescent efficiency and the fluorescence due to 465 nm light increases, but the fluorescence due to 405 nm (the isosbestic point) light remains unchanged (Lütcke et al., 2010). Intensity of the 465 nm and 405 nm light were sinusoidally modulated at 211 Hz and 531 Hz, respectively, for all recording sessions (Lerner et al., 2015), then were coupled to a filter cube (FMC4 contains excitation filters at 405 nm, 460–490 nm and emission filters at 500–550 nm, Doric Lenses) and converged into an optical fiber patch cord, which was mated to the fiber optic implant. GCaMP6f fluorescence was collected by the same fiber and focused onto a photoreceiver (Visible Femtowatt Photoreceiver Model 2151, Newport). A lock-in amplifier and data acquisition system (RZ5P; Tucker Davis Technologies), was used to control the LEDs and independently demodulate the fluorescence brightness due to 465 nm (Ca^{2+} dependent) and 405 nm (Ca^{2+} independent) excitation (recorded as mV arriving from the photoreceiver). Behavioral events (e.g. cue, licks) were timestamped and sent as digital inputs to the same data acquisition system and recorded in software (Synapse Suite, Tucker Davis Technologies). To calculate fluorescence due specifically to fluctuations in Ca^{2+}, corrected for bleaching and movement artifacts, a subtraction of the 465 nm signal by the 405 nm signal in the frequency domain was made and then inverted to recover a ratiometric time domain Ca^{2+} signal ($\Delta F/F$). The subtracted signal was smoothed using a custom fifth order bandpass butterworth filter (Figure III.1 D; cutoff frequencies: 0.05 Hz, 2.25 Hz).

B. 5. Signal normalization

For comparability of task-related responses across recording sessions, the smoothed fourier subtracted Ca^{2+} specific signal of each session was normalized by the mean transient Ca^{2+} amplitude of that session. A transient was defined as a point that exceeds 3 standard deviations of the overall signal above the previous point. The normalized signal was then aligned to cue onset in order to quantify the activity of dopamine neurons. All data processing was performed using custom MATLAB scripts (which are available upon request to corresponding author).

B. 6. Pharmacology

To determine the relationship between Ca^{2+} transients and dopamine neuron activity, a cohort of 8 (n = 3 males and n = 5 randomly cycling females) rats were prepared for photometry. Rats were injected, on 4 different days, 10min prior to a recording sessions (no behavioral paradigm; 20min of recording). Injections were of either the D2 receptor antagonist raclopride (Cat. No. R121, Sigma-Aldrich, Inc; 2mg/ kg i.p) or the D2 receptor agonist quinpirole (Cat. No. Q102, Sigma-Aldrich, Inc; 20µg/kg s.c.; D2 receptor agonist) or their vehicle (physiological saline). Injection order was counterbalanced across rats and each drug session had an accompanying saline control session. During test sessions, the frequency of spontaneous transients was measured. To determine the effects of GLP-1R activation on behavior and dopamine transient activity, Ex4 (Cat. No. 4044219, Bachem), was dissolved (0, 0.05, 0.1µg) in artificial cerebrospinal fluid (Cat. No. 3525, Tocris) and administered into the lateral ventricle in a 1µL volume (30Ga injector protruding 2mm past the guide, Plastics One Inc.) 45min prior to a behavioral test session using a counterbalanced, within-subjects design. The 0.1µg dose was chosen based on previous literature demonstrating robust suppression of food intake and food motivated behaviors (Dickson et al., 2012). Two days of no testing separated test sessions for all experiments.

B. 7. Immunohistochemistry and verification of recording sites

Following completion of experiments, rats were deeply anesthetized with sodium pentobarbital (100mg/kg) and transcardially perfused with 0.01M PBS followed by 10% buffered formalin solution (HT501320, Sigma Aldrich, Inc). Brains were removed and stored in formalin for 24 h and then transferred to 20% sucrose in 0.01M PBS. All brains were sectioned at 40μm on a freezing stage microtome (SM2010R, Leica Biosystems). VTA sections were collected and processed to label for GFP (as an indicator of GCaMP6f expression) and tyrosine hydroxylase (TH) via immunohistochemistry. Antibody incubations were done at 4°C (washes and other steps at room temperature). Tissues were permeabilized in 0.3% Triton-X 100 for 30min and were blocked in 2% normal donkey serum for 10min. Sections were incubated in rabbit anti-TH (AB152, Sigma Aldrich) and chicken anti-GFP (AB13907, Abcam) antibodies overnight (~18h). Primary antibodies were diluted in the following solution: KPBS containing 2% normal donkey serum, followed by KPBS washes (8 changes, 10min each). Secondary antibody (Cy3 conjugated donkey anti-rabbit and AF488 conjugated donkey anti-chicken; Jackson Immunoresearch) incubations were performed overnight. Sections were then mounted onto glass slides, air dried, and coverslipped with 50% glycerol in KPBS mountant. Only data from subjects with GCaMP6f expression and VTA fiber placement were included in statistical analyses. Rats (n = 8) were excluded because of missed placement of the optic fiber in regions other than the VTA (n = 2) or because of poor tissue quality and the inability to confirm construct expression and fiber placement (n = 6). Quantification of the specificity of Cre-dependent GCaMP6f expression were performed in a separate cohort of TH:Cre+ rats (n = 2 male and n = 2 female rats).

B. 8. Statistical analyses

The specificity of the GCaMP6f expression was quantified using descriptive statistics in the form of percent colocalization with TH. Influence of D2 receptor pharmacology on dopamine

neural activity (frequency of Ca^{2+} transients) were analyzed using paired t-tests against each drug's vehicle-paired session. Behavior was analyzed using two-way repeated measure analysis of variance (ANOVA) with Ex4 dose as a within-subjects variable and sex as a between-subjects variable. The magnitude of all transients and cue-evoked transients (mean of signal during 0 s to 1 s after cue onset) were analyzed via multiple linear regression with Ex4 dose as a within-subjects variable and sex as a between-subjects variable. The time course of the cue-aligned dopamine dynamics was analyzed by first averaging the signal into 1 s bins and then using two-way repeated measures analysis of variance (ANOVA) for each sex, separately, with time (−5 to +10 s relative to cue onset) and Ex4 dose as within-subjects variables. Sidak-corrected pairwise comparisons were used to compare 1 s bins for each dose of Ex4 relative to vehicle. I determined the first and last quartile for latency to first lick and compared dopamine responses from these two quartiles when data were aligned to cue onset or first lick using unpaired t-tests. To isolate the relationship between cue evoked transients and behavior, multiple linear regressions of the behavioral measures (lick latency and number of licks per trial) were performed with the magnitude of cue-evoked transient as a within-subjects variable and sex as a between-subjects variable, while accounting for the variance due to Ex4 dose. All statistical analyses were computed using the coding environment R (https://www.r-project.org/) with an α level for significance at 0.05.

C. Results

C. 1. Selective expression of calcium-dependent fluorescent construct captures dynamic fluctuations in dopamine signaling

In order to selectively measure activity in VTA dopamine neurons, I delivered an adeno-associated virus containing a Cre-dependent GCaMP6f construct followed by implant of a fiber optic into the VTA of TH:Cre+ rats. To quantify the specificity of GCaMP6f expression, sections containing the VTA from a cohort of rats (n = 2 males and 2 randomly cycling females) were

labeled with GFP and TH antibodies (Figure III.1 A). TH-positive [85.24 ± 17.7 mean ± SEM TH cells/section] and GFP-positive [52.1 ± 11.9 mean ± SEM GFP cells/section] cells were separately counted on each section and a merged image (Figure III.1 A, enlarged inset) to identify and quantify co-labeled cell bodies (Figure III.1 B). The viral construct had good penetrance [58.80 ± 1.40% of cells that labeled for TH were co-labeled for GCaMP6f] and selectivity [97.0 ± 0.90% of cells that labeled for GCaMP6f were co-labeled for TH; Figure III.1 B] similar to other reports (Decot et al., 2017). Placement of fiber optic tips from all rats included in analyses were verified in the VTA and shown in Figure III.1 C.

Fiber photometry was used to record activity from VTA dopamine neurons. To remove contributions to the fluorescent signal from photobleaching and movement artifacts in all fiber photometry experiments, the Ca^{2+} independent signal (in response to 405nm light; Figure III.1 D purple trace, for example) was subtracted from the Ca^{2+} dependent signal (in response to 465nm light; Figure III.1 D, light green, for example) in the frequency domain. The subtracted signal was returned to the time domain (Figure III.1 D, dark green, for example). To further validate fluctuations in the subtracted fluorescent signal as resulting from changes in dopamine activity, rats (n = 3 males and n = 5 randomly cycling females) were injected with dopamine D2 receptor drugs (representative traces shown in Figure III.1 E). D2 autoreceptor antagonism by raclopride increases burst firing in dopamine neurons (Andersson et al., 1995) and phasic dopamine release in the nucleus accumbens (Aragona et al., 2008). D2 receptor agonism by quinpirole suppresses dopamine neuron firing rate and inhibits phasic dopamine release in the nucleus accumbens (Anzalone et al., 2012). Thus, increasing or decreasing D2 autoreceptor activity, decreases or increases the firing rate of dopamine neurons (Gentet & Williams, 2007), respectively. Administration of raclopride significantly increased transient frequency [5.2 ± 0.4 min^{-1} versus 11.0 ± 1.8 min^{-1} for vehicle versus raclopride, respectively; $t(7) = 3.59$, $p < 0.01$; Figure III.1 F] whereas administration of quinpirole significantly attenuated transient frequency

[5.9 ± 0.6 min−1 versus 1.7 ± 0.6 min^{-1} for vehicle versus quinpirole, respectively; t(7) = 10.63, p < 0.01; Figure III.1 G]. Thus, fluorescent signals measured in the VTA were sensitive to drugs acting on the D2 receptor.

C. 2. <u>GLP-1R activation suppresses sucrose directed behavior</u>

I administered Ex4 via the lateral ventricle in male (n = 5) and female (n = 5 randomly cycling) rats and recorded several behavioral measures (latency to first lick, number of licks per trial, percent of trials with at least one lick) in response to cue-predicted sucrose availability (Figure III.2). As previous work indicated sex differences with respect to GLP-1R signaling and food reward (Richard et al., 2016), I used sex as a between-subjects variable in our analyses. For latency to first lick (Figure III.2 A), I found a main effect of Ex4 dose [F(2,16) = 12.63; p < 0.001], but no main effect of sex [F(1,8) = 0.25; p = 0.63] nor an interaction [F(2,16) = 0.26; p = 0.77]. There was no difference in lick latency between vehicle and 0.05µg Ex4 [p = 0.11]. However, the 0.1µg dose of Ex4 caused a significant increase in latency relative to vehicle [2.7 ± 1.4 versus 12.8 ± 1.4s for vehicle versus 0.1µg, respectively; t(16) = 5.0, p = 0.003]. For number of licks per trial (Figure III.2 B), there was a trend for an effect of Ex4 dose [F(2,16) = 3.11; p = 0.07], no main effect of sex [F(1,8) = 0.15; p = 0.71] and no interaction [F(2, 16) = 0.83; p = 0.45]. On some trials, rats failed to engage in any licking behavior following cued spout availability. I therefore examined the relationship between Ex4 dose and sex on the percentage of trials in which at least one lick was emitted (Figure III.2 C) and found a main effect of Ex4 dose [F(2,16) = 13.22; p < 0.001], no main effect of sex [F(1,8) = 0.32; *p* = 0.59] and no interaction [F(2,16) = 0.3; *p* = 0.75]. There was no difference in the percent of trials with licks emitted between vehicle and 0.05µg Ex4 [*p* = 0.14]. However the 0.1µg dose significantly decreased the percent of trials with licks relative to vehicle than in the 0.1µg Ex4 dose [94.7 ± 7.6 versus 43.7 ± 7.6 percent for vehicle and 0.1µg dose, respectively; t(16) = 5.09, *p* = 0.002].

Thus, Ex4 suppressed latency to begin licking and the percent of trials with a lick similarly in both male and female rats.

C. 3. <u>Central GLP-1R activation selectively suppresses cue evoked dopamine activity in the VTA</u>

Dopamine transients occur throughout the recording session (Figure III.3 A, for example). To determine if Ex4 modulated dopamine transients, regardless of when they occur, I measured the magnitude of all transients recorded across behavioral sessions. Linear regression [$r^2 < 0.01$, $F(34,992) = 0.04$, $p = 0.99$, Figure III.3 B] indicated no relationship with increasing dose of Ex4 [$p = 0.77$], no main effect of sex [$p = 0.94$], and no interaction between Ex4 dose and sex [$p = 0.75$]. Dopamine transients are specifically evoked by the sucrose-predictive cue (Figure III.4 A, for example). To determine if Ex4 specifically modulated cue-evoked phasic dopamine activity, I isolated transients that occurred during the 1 s cue period predicting sucrose availability. Here, regression [$r^2 = 0.15$, $F(3896) = 52.19$, $p < 0.001$; Figure III.4 B] of cue evoked transients identified a significant decrease in transient magnitude by dose [$\beta = -0.16$, $p < 0.001$] and that cue-evoked transients, in general, were larger in males [$\beta = 0.08$, $p < 0.001$]. There was no significant interaction between Ex4 dose and sex [$p = 0.36$].

Given the difference between cue-evoked transient magnitude between males and females was modest and there was no interaction with Ex4 dose, I combined data from male and female rats and plotted dopamine dynamics for the 5s before and 10s after cue onset (Figure III.4 C). For analysis, I averaged data across 1s bins. A two-way repeated measures ANOVA showed that there was a significant interaction between time and dose of Ex4 [$F(28,252) = 4.49$, $p < 0.001$]. Post-hoc analysis indicated that dopamine activity after the 0.1 µg dose was significantly suppressed relative to vehicle during the first [0.39 ± 0.03 versus 0.04 ± 0.03 ΔF/F for vehicle and 0.1µg dose, respectively; $t(9) = 7.74$, $p < 0.001$], second [0.36 ± 0.03 versus 0.09 ± 0.03 ΔF/F for vehicle and 0.1µg dose, respectively; $t(9) = 6.14$, $p < 0.001$] and

third bins [0.33 ± 0.03 versus 0.17 ± 0.03 ΔF/F for vehicle and 0.1μg dose, respectively; $t(9) = 3.58$, $p = 0.013$] after cue onset. There were no significant differences in dopamine activity between vehicle and 0.05 μg dose.

C. 4. Magnitude of cue-evoked dopamine is correlated with indices of behavior

To determine if the magnitude of cue-evoked dopamine activity was related to the onset of licking, I compared cue-evoked dopamine activity on short (defined by the first quartile of all latencies; Figure III.5 A, left) versus long (defined by the last quartile of all latencies, Figure III.5 A, right) latency trials. For these subsets of trials, the dopamine activity was aligned to either cue (Figure III.5 B, left) or first lick (Figure III.5 B, right) onset. Dopamine activity during the 1s cue was significantly larger for short [0.5 ± 0.03 ΔF/F] versus long [0.2 ± 0.03 ΔF/F] latency trials [$t(310) = 7.81$, $p < 0.001$; Figure III.5 B left inset]. In contrast, dopamine activity in the 1s after first lick did not differ [0.4 ± 0.03 versus 0.5 ± 0.04 ΔF/F for short versus long latency trials, respectively; $t(310) = -1.62$, $p = 0.11$; Figure III.5 B right inset]. These results suggest that large increases in dopamine activity are correlated with rapid approach behavior and that suppression of cue-evoked responses may weaken subsequent goal-directed action. To further explore this possibility, I examined the relationship between the magnitude of cue-evoked dopamine transients and lick latency across all trials (Figure III.5 C) and found a significant negative correlation [$r^2 = 0.37$, $F(2897) = 262.9$, $p < 0.001$; $\beta = -8.52$, $p < 0.001$]. I found a positive relationship between the magnitude of cue-evoked dopamine activity and number of licks emitted per trial [$r^2 = 0.26$, $F(2897) = 158$, $p < 0.001$; $\beta = 39.86$, $p < 0.001$; Figure III.5 D]. Together these results suggest that the magnitude of cue evoked dopamine activity biases approach and licking behavior.

D. Discussion

Phasic activity of VTA dopamine neurons and NAc dopamine release are critical neurophysiological substrates underlying goal-directed behaviors, particularly behaviors that

become invigorated based on cue-reward relationships. Importantly, physiological states like hunger and satiety have robust effects on phasic dopamine signaling (reviewed in (Hsu et al., 2018)). In the current manuscript I focused on central GLP-1R signaling, a potent inhibitor of food intake and food-motivated behaviors (For review see (Kanoski et al., 2016)), to determine its modulatory impact on cue-driven phasic dopamine signaling. I captured phasic dopamine signaling in awake and behaving male and female rats by utilizing in vivo fiber photometry while they performed a Pavlovian conditioning task and revealed a selective, dose-dependent suppression of cue-evoked phasic dopamine signaling that correlated with reductions in approach and licking behavior.

The VTA is a heterogeneous structure with respect to both cell types (e.g. dopamine, GABA) and projection targets (e.g. prefrontal cortex; dorsomedial shell, lateral shell, core of the nucleus accumbens) (Jong et al., 2018; Lammel et al., 2012; 2015; Morales & Margolis, 2017). With respect to cell types, I am confident that I am recording only from dopamine neurons given our use of transgenic TH-Cre rats (Decot et al., 2017; Witten et al., 2011) and Cre-dependent expression of GCaMP (Figure III.1 B). With respect to projection targets, our optical fiber placements were within the paranigral/parabrachial pigmented region in the posterior half of rostrocaudal extent of the VTA ((Ikemoto, 2007); see Figure III.1 A and C for placements). Dopamine cell bodies in this VTA territory project to the dorsomedial nucleus accumbens shell subregion (Ikemoto, 2007) – where phasic dopamine plays a role in encoding reward value (Sackett et al., 2017). However, the present work cannot resolve the responses of individual dopamine neurons based on their target. Future studies will combine photometry using fluorescent dopamine sensors (e.g. dLight1,2; (Patriarchi et al., 2018)) and projection-site specific recording. It will be especially important to compare the effects of GLP1-R on modulation of phasic dopamine in different striatal regions. Indeed, while dopamine signaling in the nucleus accumbens relates to reward, substantia nigra (W. Han et al., 2018) and dorsal

striatal (Tellez et al., 2016) dopamine signaling may be more closely linked with post-ingestive feedback and satiety.

I examined the effects of Ex4 on dopamine transients and, when analyzing all transients regardless of when they occur, found no effect. Assuming that cell body transient activity is well correlated with dopamine concentration fluctuations in terminal regions, these data are consistent with microdialysis studies where Ex4 alone had no impact on dopamine levels in the nucleus accumbens but instead suppressed drug-stimulated dopamine levels (Egecioglu et al., 2013; 2013; 2013). As shown previously (Roitman et al., 2004; Schultz et al., 1997) and recapitulated here, phasic dopamine activity was evoked by a reliable predictor of food reward. When analysis was restricted to transient activity during the cue, Ex4 caused a dose-dependent suppression. The specificity of central Ex4 effects on cue-evoked activity suggests that GLP-1R activation modulates VTA dopamine neuron excitability in response to cue-related inputs. Indeed, GLP-1 producing neurons of the hindbrain nucleus tractus solitarius (NTS) directly project to the VTA (Alhadeff et al., 2012) and GLP-1R is expressed in the VTA (Cork et al., 2015; Merchenthaler et al., 1999) – suggesting the potential for direct effects of central Ex4 on VTA receptors. Indeed, GLP-1R activation in the VTA decreases chow intake (Dickson et al., 2012), high-fat diet intake (Alhadeff et al., 2012), and sugar pellet rewards earned in a progressive ratio operant test (Dickson et al., 2012). Recent work has suggested that the effects of VTA GLP-1R activation on behavior may depend on an anterior-posterior gradient. For example, effects of Ex4 on alcohol-induced locomotion were restricted to the posterior VTA (Jerlhag, 2020; Vallöf et al., 2019). Moreover, Ex4 administration in the posterior VTA suppresses cocaine-seeking behaviors (Hernandez et al., 2018; H. D. Schmidt et al., 2016). However, a systematic investigation of the impact of anterior-posterior VTA GLP-1R activation on cocaine-seeking has not been performed. While our optic fiber placements were in the

posterior VTA, systematically varying the anterior-posterior placement may further elucidate a gradient for GLP-1R modulation of dopamine cell bodies.

The impact of GLP-1R on cue-evoked phasic dopamine signaling may also be mediated by action at cell bodies with afferent projections to the VTA (Mietlicki-Baase et al., 2013). A promising candidate is the lateral dorsal tegmental nucleus (LDTg) - which directly projects to the VTA (Cornwall et al., 1990; Lodge & Grace, 2006) and modulates VTA dopamine neuron activity (Cornwall et al., 1990; Steidl et al., 2017; 2017; Steidl & Veverka, 2015). Most importantly, peripherally administered fluorescent Ex4 accumulates in the LDTg (Reiner et al., 2018), is a site of robust GLP-1R expression (Merchenthaler et al., 1999) and Ex4 modulation of feeding behaviors (Reiner et al., 2018). Future site-specific GLP-1R manipulation will be needed to map circuit level mechanisms for Ex4 modulation of phasic dopamine signaling during goal-directed behaviors.

Cue-evoked VTA dopamine activity was strongly correlated with sucrose-directed behavior. The magnitude of dopamine activity was significantly higher in response to the cue when rats rapidly approached the spout relative to long latency trials. Importantly, even on long latency trials, there was a sharp increase in dopamine activity when rats finally approached the spout. This is consistent with prior work from our lab (Roitman et al., 2004) and others (Mohebi et al., 2019; Wassum et al., 2012) where phasic dopamine release in the nucleus accumbens was correlated with the initiation of approach behavior. Here I extend this work to clearly implicate dopamine cell body activity in cue-evoked approach behavior as well (see also (Engelhard et al., 2019; Silva et al., 2018)). Indeed, using regression analyses, I found further support for critical association between VTA phasic dopamine signaling and food-directed behavior (correlations with lick latency and number of licks).

It is important to note that the rats in the present experiments were food restricted. While this is a common practice in behavioral neuroscience to facilitate responding for food

reinforcement, it has critical implications for both phasic dopamine activity and putative GLP-1R signaling. Hunger enhances phasic firing of VTA dopamine neurons and phasic dopamine responses to cues that predict food reward (Branch et al., 2013; J. J. Cone et al., 2014; 2015). Moreover, GLP-1R blockade and chronic pre-pro-glucagon (GLP-1 precursor) knockdown is associated with hyperphagia (Barrera et al., 2011), suggesting endogenous roles of GLP-1 in suppressing hunger. Thus, one interpretation of the findings that is consistent with previous literature (J. J. Cone et al., 2014; 2015) is that in the hungry state, phasic dopamine activity exhibits heightened sensitivity to cues that predict food. Our data suggest that this sensitivity can be then attenuated by central GLP-1R signaling – similar to effects of leptin that have been reported (Plasse et al., 2015). Food deprivation also silences endogenous GLP-1 neural activity (Maniscalco et al., 2015; Maniscalco & Rinaman, 2013). Thus, a physiological role of GLP-1R activity in modulating cue-evoked phasic dopamine signaling remains a critical area for exploration. Besides loss-of-function experiments, other approaches that modulate GLP-1R signaling (i.e. modulations of meal size, gastric distension, etc. (Hayes et al., 2009; Kreisler & Rinaman, 2016; Maniscalco et al., 2015)) will provide valuable insight.

Central Ex4 administration has been linked with nausea, malaise, and interoceptive stress (Kanoski et al., 2012; Kinzig et al., 2002), and highlights an important caveat in the data described here. Malaise and negative affect have been shown to suppress phasic dopamine signaling, an effect that can be mediated by central GLP-1R signaling (Fortin et al., 2016). Moreover, central GLP-1R signaling at select brain regions (e.g. the medial NTS (Jonghe et al., 2016; Kanoski et al., 2012)) produces indices of nausea and malaise in rodents. Ventricular doses of Ex4 similar to those used in the current study are sufficient to induce reductions in food intake and body weight, but also increase measures of malaise (Kanoski et al., 2012). Thus, it remains possible that Ex4 effects on phasic dopamine signaling could be secondary to malaise or negative affect. Likewise, systemic (Erreger et al., 2012) or central (Anderberg et al., 2016)

Ex4 reduce indices of general locomotor behavior. Site specific delivery of Ex4 at doses that are sufficient to reduce motivation but subthreshold for malaise or motor impairment (Alhadeff et al., 2012) will be a critical area for future investigation. Still, the power of using a cue in the present study – and measuring the dopamine response to it – is that the dopamine response is evoked by a salient sensory stimulus that occurs regardless of the animal's behavior. I additionally found that the magnitude of the dopamine response predicts subsequent behavior even after accounting for the variance in the dopamine response due to dose of Ex4. Thus, current findings provide crucial insight into the mechanisms through which interoceptive stress might be relayed to mesolimbic pathways and ultimately affect goal-directed behaviors.

Recent data has identified sex as a modifier of GLP-1R regulation of feeding behaviors (López-Ferreras et al., 2017; 2019; Maske et al., 2017; Richard et al., 2016; Vogel et al., 2016). For example, lateral ventricular infusion of Ex4 more potently suppressed responding for food reward under a progressive ratio but not consumption of food, in females (Richard et al., 2016). Thus, I included sex as a biological variable in our analyses. I found that Ex4 delivered to the lateral ventricle suppressed cue-triggered approach (latency to first lick) as well as consumption of sucrose (number of licks per trial) to similar degrees in female and male rats. On the surface, this result is surprising given previous reports. However, sex differences in behavioral responses to Ex4 are dependent on site of action. For example, GLP-1R signaling in the lateral hypothalamic area (LHA) plays endogenous roles in feeding behaviors, and acute blockade of LHA GLP-1Rs only has an effect in males and not in females during lever pressing tasks (López-Ferreras et al., 2017). Similarly, GLP-1R activation in the supra-mammillary nucleus has more potent effects on food motivated behaviors in males compared to females (López-Ferreras et al., 2019). In contrast, GLP-1R activation in the VTA had more potent effects on motivated behaviors in females compared to males (López-Ferreras et al., 2019). Estrous cycle stage also plays an important role in modulating goal-directed behaviors. Intra-LHA administration of Ex4

has more potent effects on food-motivated behaviors in the estrus phase, whereas there is no effect in the metestrus/diestrus stage (López-Ferreras et al., 2017), highlighting the importance of sex hormones and their interactions with food-motivated behaviors. Indeed, central estrogen blockade attenuates the anorexigenic effects of Ex4 in both female and male rats (Richard et al., 2016). Importantly, the studies here were performed in males and randomly cycling females with Ex4 delivery to the lateral ventricle. Thus, future studies examining the interactions between estrus phase, sex hormones, phasic dopamine signaling, and selective sites of central GLP-1R signaling are warranted.

There are ample reports of sex differences with respect to dopamine signaling generally ((Becker & Chartoff, 2018), for review) and phasic dopamine signaling in particular (Conway et al., 2019; Cummings et al., 2014; Shams et al., 2016; Yoest et al., 2019). I and others have previously reported enhanced electrically-evoked dopamine release in females relative to males (Conway et al., 2019; Q. D. Walker et al., 1999). Yet, in the context of cue-evoked VTA dopamine activity, here I observed a lower magnitude dopamine response in females. Resolving these conflicting findings can be challenging given that comparisons are being made between recordings from cell bodies in the VTA with release in terminal regions, like the nucleus accumbens and dorsal striatum. While cell body and release dynamics relative to food-predictive cues are very similar, mechanisms at dopamine terminals capable of modulating the magnitude of dopamine release independent of cell body effects have been identified (Mateo et al., 2017; Mohebi et al., 2019; Threlfell et al., 2012). Concluding whether females have greater or smaller dopamine responses relative to males is also dependent on comparing results from studies where dopamine signaling is electrically-evoked, when release probability is high, versus dopamine signaling that is cue-evoked– which is presumably being driven by a subset of inputs to dopamine cell bodies (Tian et al., 2016). Thus, it is important to highlight that sex as a

biological variable in the context of the magnitude of cue-driven phasic dopamine signaling remains understudied.

5. Conclusions

Physiological states, such as hunger and satiety, have potent effects on goal-directed behaviors. The clinically relevant long-acting GLP-1 analog Ex4 suppressed the phasic response of VTA dopamine neurons to sucrose-predictive cues. The suppressed response, in turn, was correlated with decreased sucrose-directed behaviors. Central GLP-1R activation thus holds potential for tuning a form of dopamine signaling that biases approach in response to environmental cues that are associated with food reinforcement.

Figure III.1: Selective expression of calcium-dependent fluorescent construct in dopamine neurons captures dynamic fluctuations in dopamine signaling.

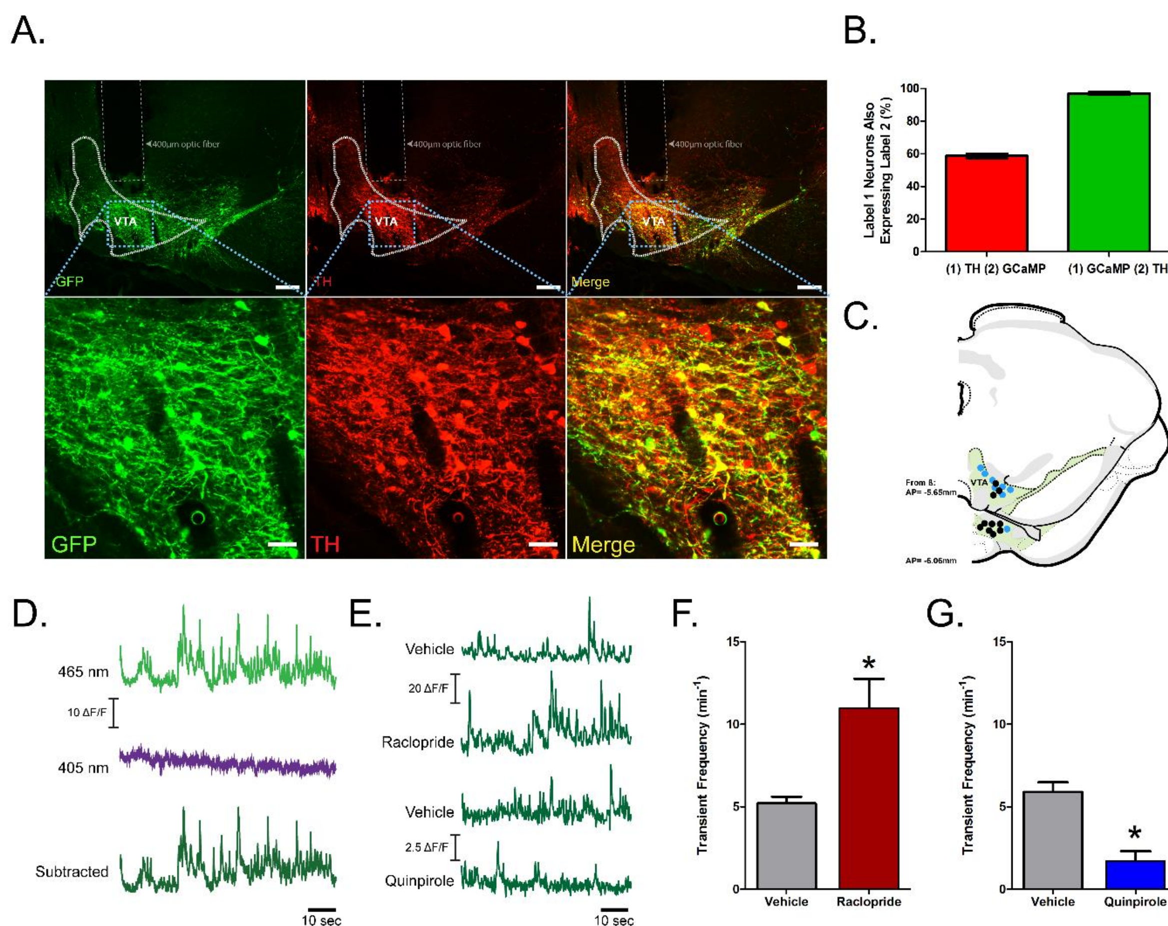

Figure III.1: Selective expression of calcium-dependent fluorescent construct in dopamine neurons captures dynamic fluctuations in dopamine signaling. ***(A)*** Representative images (top panels; scale bar = 200μm) with a high magnification of the VTA (bottom panels; scale bar = 100 μm) with GFP label in green, TH label in red, and a merge. ***(B)*** Quantification of neurons expressing TH that also labeled for GFP [(1) TH (2) GCaMP] and neurons labeling for GFP that also expressed TH [(1) GCaMP (2) TH] (n=4 rats). ***(C)*** Location of optic fiber tips from all rats used in photometry recordings. Solid blue and black circles superimposed on coronal sections modified from the Swanson brain atlas [83] represent optic fiber placements for rats used in D2 receptor and Ex4 pharmacology experiments, respectively. ***(D)*** Representative traces from the VTA of real-time Ca^{2+} dependent signal (465nm), Ca^{2+} independent signal (405nm), and fourier subtracted, dopamine activity signal. ***(E)*** Representative traces of dopamine activity after vehicle, raclopride or quinpirole. Quantification of transient frequency after vehicle versus raclopride ***(F)*** and vehicle versus quinpirole ***(G)***; n=8 rats. Data in panels B, F & G are mean ± SEM, *: $p < 0.01$.

Figure III.2: Ex4 dose-dependently suppresses indices of sucrose-directed behavior (n=5 male and n=5 female rats).

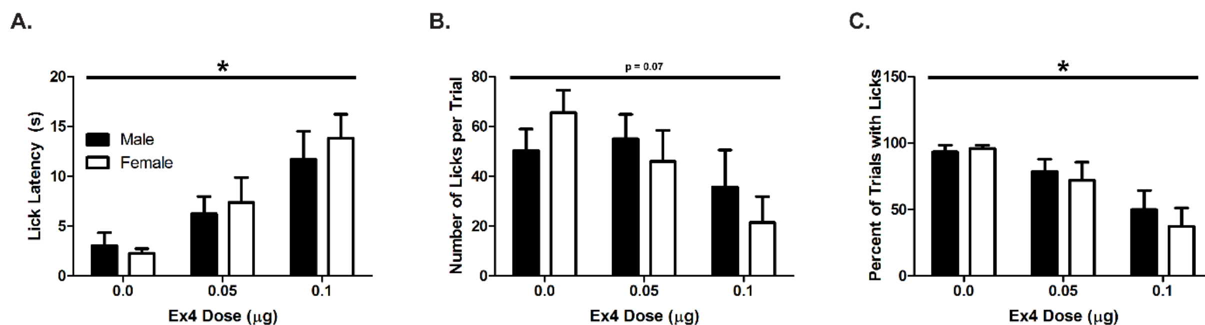

Figure III.2: Ex4 dose-dependently suppresses indices of sucrose-directed behavior (n=5 male and n=5 female rats). ***(A)*** Latency to begin licking increases with Ex4. ***(B)*** Number of licks per trial trend towards a decrease with increasing doses of Ex4. ***(C)*** Percent of trials with licks decreases with increasing doses of Ex4. All effects were independent of sex. Data are mean ± SEM, *: $p < 0.01$ and $p = 0.07$ represent the main effect of Ex4 dose.

Figure III.3: Spontaneous VTA dopamine transients are not modulated by central Ex4.

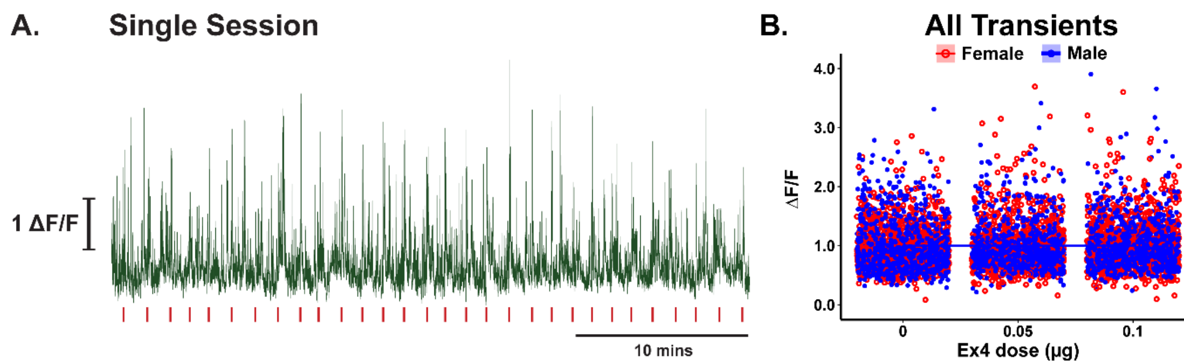

Figure III.3: Spontaneous VTA dopamine transients are not modulated by central Ex4. ***(A)*** Trace of dopamine activity across the entire behavioral session from a representative rat (vehicle session; red vertical ticks represent the time of cue administration). ***(B)*** Multiple linear regression of the magnitude of all dopamine transients (symbols) reveals no effect of Ex4 for either males (n=5 rats; blue symbols) or females (n=5 rats; red symbols).

Figure III.4: Central Ex4 suppresses cue evoked dopamine activity.

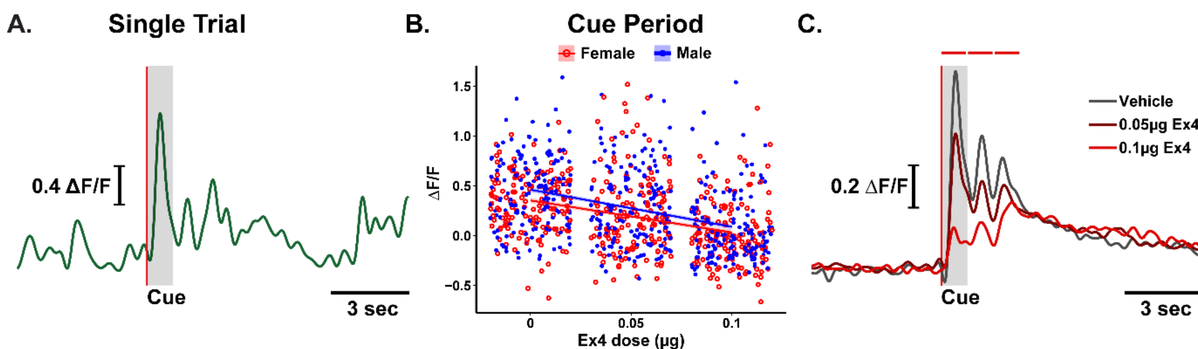

Figure III.4: Central Ex4 suppresses cue evoked dopamine activity. ***(A)*** Dopamine activity aligned to cue onset (vertical red line) in a representative trial. ***(B)*** Multiple linear regression of each cue response (symbols) reveals a dose-dependent suppression of cue-evoked transient magnitude for both males (n=5; blue symbols) and females (n=5; red symbols). ***(C)*** Ex4 dose-dependently suppresses averaged dopamine activity aligned to cue onset (vertical red line) collapsed across sex (n=10 rats). Horizontal red bars above the trace represent times when dopamine activity following 0.1 μg Ex4 were significantly different from vehicle, $p < 0.01$. Lines represent group means.

Figure III.5: Magnitude of cue evoked dopamine activity is correlated with indices of sucrose-directed behavior.

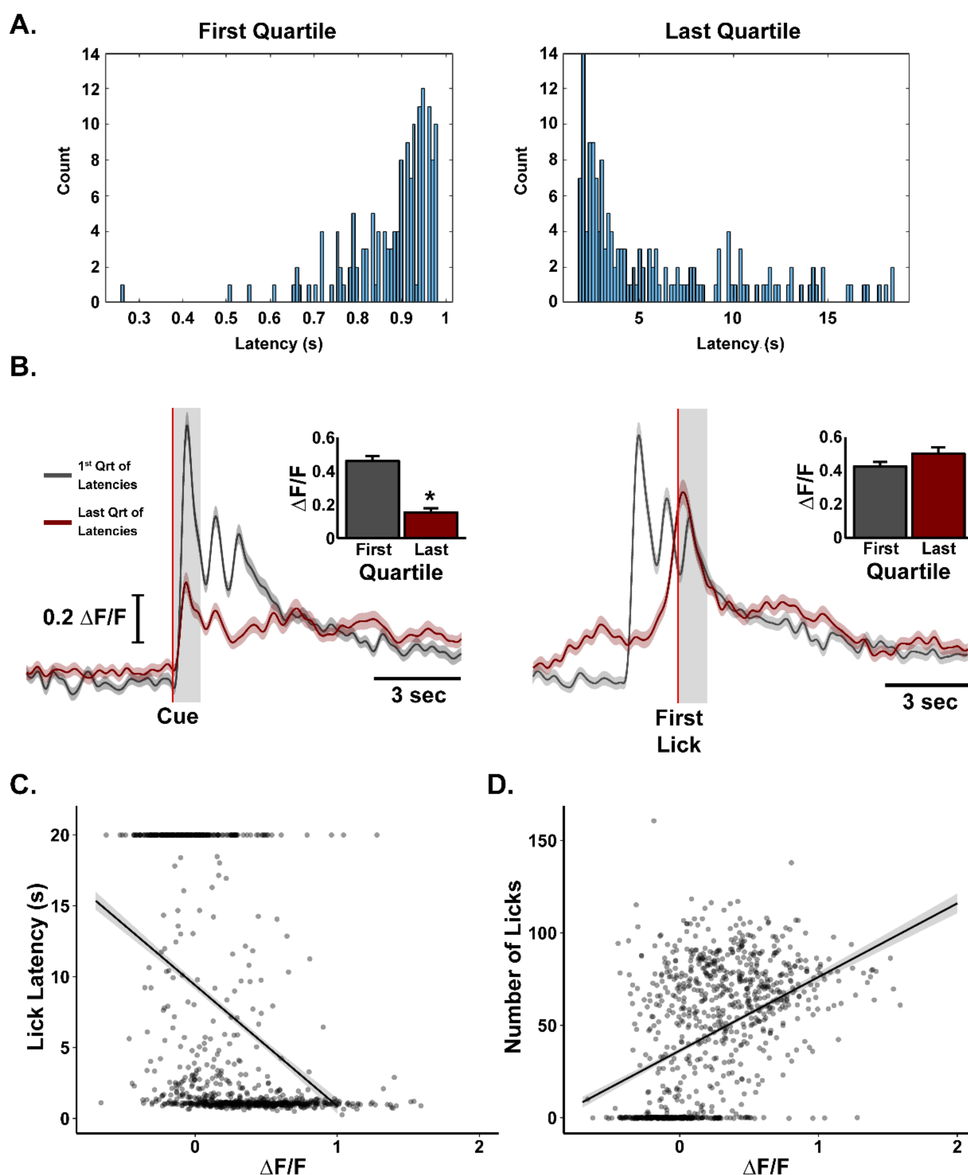

Figure III.5: Magnitude of cue evoked dopamine activity is correlated with indices of sucrose-directed behavior. ***(A)*** Distributions of short (n = 152 trials) versus long (n = 160 trials) first-lick latencies. ***(B)*** Cue-evoked (left) but not first-lick-evoked (right) average dopamine activity is significantly greater for short (grey) versus long (red) latency trials. Vertical red lines indicate the onset of the behavioral event. Insets are quantifications of the averaged dopamine activity in the first second after the behavioral event as denoted by the grey shaded areas on the traces. Data are mean ± SEM, *: $p < 0.01$. ***(C)*** Multiple linear regression shows a negative relationship between lick latency and cue-evoked dopamine activity and a ***(D)*** positive relationship between number of licks per trial and cue-evoked dopamine activity for every trial across all behavioral sessions (n=900 trials).

Chapter IV: General Discussion

Our daily survival requires the preservation of homeostasis through various internal regulatory mechanisms and restorative behaviors. Physiological states, whether we are hungry or sated, heavily influence our eating decisions and our experiences with food. One part of how food motivated behaviors are shaped is the manner through which taste stimuli are received and evaluated. Associations between food and cues that predict them are learned and then integrated with various internal cues to generate motivated behaviors. Hunger and satiety profoundly modulate food-directed motivated behaviors, however the neural basis of motivation is poorly understood and still being elucidated. I'm interested in how taste stimuli are neurally encoded based on physiological state.

One node in the brain where such integration of physiological state and food value occurs is in midbrain dopamine neurons of the ventral tegmental area (VTA). Food and food associated cues evoke brief increases in dopamine release in the striatum and dopamine firing rate in the VTA, that is termed phasic activity. Such phasic activity is essential in invigorating approach behavior toward food, the reinforcing aspects of the food, and food reward value. Understanding how physiological state may change phasic dopamine activity in non-diseased states is central to understanding how this system is reconfigured in diseased states where behaviors toward food are maladaptive.

Summary of findings: In chapter II, I found that food restriction and cytoglucopenia both potentiate VTA dopamine signaling to food (Figure II.1; Figure II.2; Figure II.3). However, cytoglucopenia fails to modulate VTA dopamine signaling to water (Figure II.4), suggesting that dopamine signaling is tuned only toward stimuli that are restorative to physiological status. Interestingly, cytoglucopenia potentiates VTA dopamine to food cues differently based on the site of administration of the antiglycolytic agent (5-thio-d-glucose, 5TG). Forebrain 5TG potentiates food-cue evoked VTA dopamine signaling, while hindbrain 5TG potentiates food

evoked VTA dopamine signaling (Figure II.6). This suggests that the forebrain contributes information to the VTA regarding learned cues, while the hindbrain provides information tuned more toward the taste of the primary reward. In chapter III, I found that the satiety signal, glucagon-like peptide 1 (GLP-1), suppresses food-seeking behaviors (Figure III.2) via VTA dopamine signaling to food-cues (Figure III.4; Figure III.5). Chapter II elucidates a possible mechanism through which eating behaviors are initiated, while chapter III provides a mechanism through which eating behaviors may cease.

A. Physiological state modulates the neural representation of taste

We and others have shown that food evokes phasic dopamine signaling (Roitman et al., 2008; Schultz et al., 1993). In the present studies, I show that intraoral sucrose evokes robust phasic dopamine signaling and that changes in physiological state modulates that dopamine response (Figure II.1). How is it that the taste of sucrose comes to evoke a robust response from a set of neurons that are critical in motivated behaviors? How does taste information arrive at the VTA? How might physiological state be poised to change that information? Does taste information arrive at the VTA already modulated by physiological state? Alternatively, does physiological state impact the VTA dopamine neurons such that their activity is biased according to whether the animal is hungry or sated?

To be able to answer any of these questions, one must understand the neuroanatomy of how taste is processed by the brain. First, substances on the tongue, larynx, and pharynx are sensed by taste receptor cells and information transduced through the facial, glossopharyngeal, and vagus nerves (Lindemann, 1996). Taste stimuli activate taste receptor cells and first-order neurons in a concentration dependent manner (Ganchrow & Erickson, 1970). The majority of carbohydrate sensation is carried by a branch of the facial nerve called chorda tympani (Oakley, 1985), which then directly innervates the nucleus of the solitary tract (NTS). In fact, all nerves carrying taste afferents terminate at the NTS. The taste information then ascends to the

thalamus and then to the insular cortex. However, taste information does not flow linearly through to the cortex, but rather is distributed divergently to many other structures, such as the hippocampus, hypothalamus, and the amygdala (Breslin, & Huang, 2006; Sewards, 2004; Simon et al., 2006). Although the NTS sends projections to the VTA (Alhadeff et al., 2012), it is unclear whether taste information arrives at the VTA directly from the NTS. An alternate afferent pathway to the VTA could be from the amygdala (Araujo & Simon, 2009), as dense projections to the VTA arise from the central amygdala (Watabe-Uchida et al., 2012). Regardless of the pathway, it is clear that taste information arrives at the VTA and has profound implications for food directed behaviors.

In the present studies I show that VTA dopamine responses sapid taste stimuli are modulated by physiological state. However, it is unclear where in the taste sensing pathway the neural representation of taste is modified by hunger or satiety such that VTA dopamine neurons differentially respond to sucrose. First-order taste neurons that carry information from the taste buds via the chorda tympani to the NTS are modulated by physiological state. For examples, physiological state signals such as glucagon-like peptide 1, stomach distension, and leptin modulate taste sensitivity transduction in the chorda tympani (Hellekant, 1971; Kawai et al., 2000; Niki et al., 2015; Takai et al., 2015; Yoshida et al., 2013). Interestingly, not all interoceptive signals modulate activity in first-order neurons. For example, fibroblast growth factor 21, a satiety signal released by the liver, does not modulate taste sensitivity, but does modulate sweet-seeking behaviors upon acting in the hypothalamus a node distal to taste reception (von Holstein-Rathlou et al., 2016). Although stimulus sensitivity can be modulated at the first-order neurons, such modulation should not be conflated with the affect elicited by the taste stimulus. For example, animals reject saline solutions at high concentrations while chorda tympani responses monotonically increase with increasing concentrations (H. Grill & Norgren, 1978). However disruptions in sodium balance does modulate chorda tympani responses to

saline solutions (Contreras, 1977; Contreras & Frank, 1979). Evidently, taste stimulus reception itself can be modulated by specific signals that convey physiological state.

Similar to first-order responses, physiological state and signals that convey it modulate neural responses to taste stimuli in second-order NTS neurons (Giza & Scott, 1983; 1987; Glenn & Erickson, 1976). Although the NTS activity to taste stimuli is only linearly amplified relative to chorda tympani activity (Ganchrow & Erickson, 1970), the NTS functions as more than just a relay of taste information. For example, intra-NTS injections of leptin modulate food intake, suggesting that physiological state controls information flow at the NTS. Therefore, it may seem that taste information is modified prior to being received by the VTA at both first-order and second-order neurons. However, there is evidence to support that physiological state modulates gustatory signals even at the level of the dopamine neurons in the VTA (J. J. Cone et al., 2014; Mietlicki-Baase et al., 2015). It is possible that the dopamine responses to intraoral sucrose in the present studies are a consequence of taste information converging from multiple pathways including the NTS, the amygdala, and prefrontal cortex (Yamamoto, 2006). Further functional tracing studies are necessary to understand exactly by what circuit taste information arrives at the VTA and at what stages the gustatory information is modulated by physiological state. To date, data suggest that physiological state modulates gustatory signals at multiple nodes within the gustatory sensing pathway, potentially aiding the amplification of motivational drive toward food during times of negative energy balance.

B. Dopamine responses to food predictive cues are modulated by physiological state

A hungry organism is driven to find food for its survival. In order to be successful in finding the appropriate nutrients, the organism uses its catalog of learned associations to guide its way to the nearest source of nutrition. Cues such as the smell or the color of a ripe fruit that predict successful nutrient acquisition are critical in an organism's repertoire for survival. Such cues that predict reward evoke dopamine neuron responses and provide the organism the

incentive to approach and consume food (Robinson & Berridge, 1993). Dopamine responses in the VTA develop to the earliest predictor of reward (Schultz, 2013; Schultz et al., 1993; 1997). In fact, when a reward-associated cue is presented repeatedly over numerous trials, dopamine responses are elicited to the cue rather than to the expected reward (Fiorillo et al., 2003; Schultz et al., 1993; Waelti et al., 2001). Inhibiting dopamine signaling during the cue presentation decelerates the development of the cue-reward association while stimulation accelerates it (Morrens et al., 2020; Steinberg et al., 2013; 2014). Cue evoked dopamine signaling is essential in learning associations between cues and food.

In the present studies, dopamine responses developed to the cues that predicted food reward delivery (Figure II.6). In order for the audio cue to elicit a dopamine response in such a short latency (~100ms), the VTA must receive input from the auditory system via a relatively short pathway, similar to the visual system (Comoli et al., 2003). Unlike the visual system, the auditory system's connectivity to the mesolimbic dopamine system is not well studied. Sound information is received from the spiral ganglion and delivered into the central nervous system by the vestibulocochlear nerve (Malmierca, 2015). Upon entry into the brain, the vestibulocochlear nerve synapses onto the cochlear nucleus, from where multiple branches innervate the nucleus of the lateral lemniscus, superior olivary nucleus (a.k.a. superior olivary complex), and the inferior colliculus. From here, information is carried to the primary auditory cortex where much of the higher-order processing of sound occurs. The pathway through which the VTA receives the alerting auditory stimulus is poorly understood. Retrograde tracing studies show that VTA receives very poor innervation from the inferior colliculus (IC) (Geisler et al., 2007; Geisler & Zahm, 2005). It is possible that the sound information responsible for causing the short latency onset of phasic dopamine signaling arises from the innervation from the IC because the IC has been shown to increase in activity over learning conditioning stimuli (Gonzalez-Lima & Scheich, 1984; 1984). However, the VTA also receives a higher density but spacially diffuse innervation

from the so called “reticular formation,” which is thought to be a system for generating wakefulness and arousal (Lecea, 2012). Most importantly, a component of the reticular activating system, the laterodorsal tegmental nucleus (LDTg) receives sound information (Koyama et al., 1994) and is the nucleus that provides the drive for phasic firing in dopamine neurons and allows for cue reinforcement (Lodge & Grace, 2006; Steidl & Veverka, 2015). Therefore, I posit that sound information can be incident on the VTA via multiple pathways—via the LDTg, the reticular formation, and the inferior colliculus. Further targeted studies are necessary to determine whether each of these nodes contribute to the ability of audio cues to elicit phasic dopamine signaling in Pavlovian conditioning paradigms, such as the one employed in the present studies.

Regardless of the circuit that is responsible for audio cues inducing such a short-latency response in dopamine neurons, the present study showed that in general, food-cue-evoked phasic dopamine signaling was modulated by signals that conveyed physiological state (Figure II.6; Figure III.4). These findings are consistent with the literature since, in humans, ghrelin (hunger-associated hormone) levels correlates with neural activity responses in the ventral tegmental area (Kroemer et al., 2013). Furthermore, both decreasing ghrelin receptor activity (A. K. Walker et al., 2012) and inducing satiety (Corbit et al., 2007) suppresses cue-evoked food seeking. Collectively, data suggest that physiological state and the signals that convey it set the gain on phasic dopamine activity to cues that predict food. Therefore, cues prompt the mesolimbic dopamine system to appropriately set the drive toward the primary food stimulus based on physiological need.

C. <u>Cytoglucopenia potentiates dopamine signaling to food and food-cues</u>

I found that cytoglucopenia, induced by either antiglycolytic agents or by peripheral insulin, potentiates dopamine signaling to food stimuli and food associated cues (Figure II.2; Figure II.3; Figure II.6). I used the antiglycolytic agent 5-thio-D-glucose (5TG), similar to 2-

deoxy-D-glucose (2DG), which is an unmetabolizable glucose analog. These antiglycolytic agents work to decrease metabolizable intracellular glucose by inhibiting glycolysis at key stages, namely by inhibiting glucose uptake (Betz et al., 1975; 2013) and by blocking hexokinase (Bachelard et al., 1971; M. Chen & Whistler, 1975; Horton et al., 1973). The macroscopic effect of administering such antiglycolytic agents is that they increase hunger ratings and the "tastiness" of sugar in humans (Thompson & Campbell, 1977), as well as a potent increase in food intake in both humans and rats (R. C. Ritter & Slusser, 1980; Slusser & Ritter, 1980; Thompson et al., 1984; Thompson & Campbell, 1977). Interestingly, 2DG is used therapeutically in humans to treat cancer (Berthe et al., 2018; Pajak et al., 2020) and COVID-19 (Bhatt et al., 2022) because of the efficacy of antiglycolytic agents to slow glucose metabolism preferentially in diseased cells. Given the ubiquity of type 2 diabetes causing obesity and the advent of antiglycolytic agents in the treatment of cancer and COVID-19, it is more important than ever to understand the mechanisms through which glucose levels are regulated and how they govern energy balance.

In the present studies, 5TG doses were determined based on the minimum dose to increase food intake in previous studies (Kamatchi et al., 1986; A. J. Li et al., 2014; Tordoff et al., 1988). 2DG also increases eating in rats but only at much higher doses (Slusser & Ritter, 1980). It is important to note that the present studies do not measure effective glucose utilization. It is unclear whether changes in glucose availability in these experiments is within the physiological bounds of approximately 0.2mmol/L in the brain (Campfield et al., 1985; 1996; Silver & Erecinska, 1994; Vries et al., 2003). Some researchers are of the opinion that glucose utilization at the neuronal level may only play a role in inducing food intake during extreme deficits, but not during a typical day or a short fast (Levin et al., 2006). Furthermore, if decreased incrementally, subjects feel hungry at blood glucose concentrations almost at levels that cause cognitive impairment (D. Kerr et al., 1993; Mitrakou et al., 1991). However, glucose

sensing neurons can detect very small changes in ambient concentrations (R. Wang et al., 2004). This suggests that glucose levels are controlled very dynamically and hunger is based on factors other than just the absolute blood glucose concentration. AMPK activity, sensitive to fluctuations in intracellular glucose, can be suppressed to decrease food seeking behaviors (Hayes et al., 2009; Minokoshi et al., 2004), suggesting that endogenous glucose detection by AMPK does influence physiological eating. Although the administration of anti-glycolytic agents is potentially pushing glucose utilization to supraphysiological limits, understanding how intrinsic cellular mechanisms compensate, for the deficit of glucose is essential in understanding, compensation mechanisms carried out by hormones, such as ghrelin, insulin, and other signals, that convey physiological state to the brain to modulate food seeking behavior. Future studies might consider experimentally changing brain and blood glucose concentrations at varying rates within physiological levels that induce hunger to determine whether such manipulations modulate mesolimbic dopamine activity to food and food-cues.

I found that peripherally administered insulin potentiated dopamine responses to intraoral sucrose (Figure II.3). Interestingly, the potentiation of dopamine waned over time, suggesting that insulin may act directly on the central nervous system (CNS). Insulin indeed crosses the blood-brain-barrier and enters the CNS through a passive saturable transport at physiological ranges of blood insulin levels (Banks et al., 1997; Schwartz et al., 1991). Currently, it is widely agreed that the source of central insulin is via pancreas derived means. Although many regions of the brain contain insulin, the only evidence of CNS insulin synthesis in mammals is in the olfactory mucosa (Lacroix et al., 2008), but at too low of a level to account for the presence of insulin throughout the brain (Havrankova et al., 1981). Unlike the effects of insulin in the periphery, insulin in the CNS primarily does not promote glucose uptake as most glucose transporters in the brain are insulin independent (McEwen & Reagan, 2004). Behavioral effects of central insulin are well documented and shown to have opposite effects on behavior

as it does in the periphery. Central insulin reduces food intake, body weight, serum insulin levels, and increases blood glucose concentrations (Ajaya & Haranath, 1982; Brief & Davis, 1984; Debons et al., 1970; Florant et al., 1991; Hatfield et al., 1974; Schwartz et al., 1992; Strubbe & Mein, 1977; Woods & Porte, 1983). However, the cellular mechanisms of how insulin modulates eating behavior is largely unclear. Most current models suggest that insulin acts in the hypothalamus on Agouti-related peptide (AgRP) and proopiomelanocortin/cocaine-and-amphetamine-regulated transcript (POMC/CaRT) neurons to suppress food intake via melanin concentrating hormone (MCH) neurons (Kleinridders et al., 2014). This is further complicated by the fact that insulin receptors are found in high densities in other regions of the brain, including the olfactory bulb, cortex, hippocampus, striatum, thalamus, midbrain, brainstem, and the cerebellum (Dou et al., 2005; Fernandez & Torres-Alemán, 2012; Havrankova et al., 1981; Zhao et al., 2004). Although the insulin doses in this study were likely supraphysiological (Aparicio et al., 1974; Shih et al., 2007), loss-of-function studies show endogenous activity does protect from hyperphagia (Obici et al., 2002). Thus, effects of central insulin in the present thesis are likely working through parallel, overlapping mechanisms, and even directly on VTA dopamine neurons to modulate their activity (Figlewicz et al., 2003).

Cytoglucopenia enhanced dopamine responses to intraoral sucrose (Figure II.2; Figure II.3) and to cues that predicted intraoral sucrose (Figure II.6). However, the enhancement varied based on where 5TG was administered. Due to the nature of cerebroventricular flow (Bothwell et al., 2019), 5TG administered in the lateral ventricle (LV) likely influenced more forebrain structures and 5TG administered in the fourth ventricle (4V) likely influenced more hindbrain structures. Forebrain 5TG potentiated cue evoked dopamine while hindbrain 5TG potentiated dopamine responses to the primary sucrose reward. There are likely parallel but separate mechanisms that govern this discrepancy. Firstly, there are separate populations of glucose sensitive neurons both in the hypothalamus, i.e. forebrain (Oomura et al., 1969), and in the

NTS, i.e. the hindbrain (Dallaporta et al., 1999). The glucose-sensitive neurons in the NTS are not only necessary for glucoprivic eating (S. Ritter et al., 2001), but blood glucose directly modulates their response to taste stimuli (Giza & Scott, 1983). Additionally, NPY producing epinephrine neurons in the NTS increase food intake (J. Chen et al., 2020). Since NTS neurons directly project to the VTA (Alhadeff et al., 2012) and modulate VTA dopamine neuron activity (X. F. Wang et al., 2015), it is likely that the hindbrain NTS glucose-sensitive neurons send specific information to the VTA regarding the taste and the receipt of the primary reward. The glucose-sensitive neurons in the hypothalamus, on the other hand, might send the VTA information regarding the cues associated with the primary reward rather than the reward itself. Firstly, we know that the lateral hypothalamus sends direct GABAergic (LH^{GABA}) projections to the VTA that directly inhibit VTA GABAergic neurons (Nieh et al., 2016). This results in a disinhibition pathway that potentiates food directed behaviors. Furthermore, LH^{GABA} are accompanied by glutamate projections (LH^{glut}) to the VTA, where the combination of projections stimulates compulsive sucrose seeking even at the cost of a punishing electrical shock (Nieh et al., 2015). Most interestingly, it seems that this LH^{GABA} neurons encode reward predictions and regulate learning (Sharpe et al., 2017). Collectively, these data shed light on the discrepancy in the potentiation of dopamine responses between 5TG administered in the LV and in the 4V. 5TG in the LV likely stimulated the LH to VTA pathway to preferentially modulate the dopamine responses to the cue. On the other hand, 5TG in the 4V likely potentiated sucrose evoked dopamine via the NTS.

D. <u>Glucagon-like peptide 1 suppresses dopamine signaling to food and food-cues</u>

Food intake is inherently stress-inducing. For example, an excessively large meal can make one feel quite uncomfortable. The absorption of food causes large physiological changes in blood osmotic pressure, blood glucose, gastrointestinal distension, etc., that the body tolerates and manages with various housekeeping mechanisms (Woods, 1991). Therefore,

satiety is a process through which the body protects itself against the burden of food-induced changes in physiology. One satiety-inducing molecule, called glucagon-like peptide 1 (GLP-1), is released by a set of hindbrain neurons that express mRNA for preproglucagon (PPG). I demonstrated that GLP-1 suppresses food-cue evoked and food evoked dopamine neuron activity (Figure III.4; Figure II.5). It is important to consider that GLP-1 signaling in the brain is not just used to convey satiety but is also used to signal interoceptive and psychogenic stress (Holt & Rinaman, 2022). The idea that satiety is a stress-inducing process and that common circuits/neurons might encode both satiety and stress mechanisms is explored by many groups (Calvez et al., 2011; Maniscalco et al., 2013; R. C. Ritter et al., 1999; RJ et al., 2000). In the context of the current studies, it is not possible to infer that only circuits pertaining to satiety are engaged. It is likely that Ex4 administration in the LV engaged a subset of GLP-1R that signal stress, since dopamine signaling is suppressed to unpleasant or aversive stimuli and signals that convey aversion (Roitman et al., 2005; Verharen et al., 2020). For example, lithium chloride induced aversion suppresses phasic dopamine release and is mediated by GLP-1 signaling (Fortin et al., 2016). However, endogenous GLP-1 signaling decreases synaptic drive to the dopamine neurons in the VTA, decreases synaptic dopamine release in the NAc, and subsequently decreases high-fat diet intake (X. F. Wang et al., 2015).

Although the doses of GLP-1 agonist, Exendin-4 (Ex4), in these studies were potentially above levels of GLP-1 in the brain (Hsu et al., 2015), preclinical (Donahey et al., 1998; Turton et al., 1996) and clinical data (Finan et al., 2015) show that the Ex4 doses used are effective in dampening motivated behavior toward food and drugs of abuse without inducing malaise. Additionally, NTS neurons project directly to the VTA to suppress food intake, supporting the relevance of the role of GLP-1 signaling in modulation dopamine neuron activity to food and food associated stimuli (Alhadeff et al., 2012). Further investigation in obese subjects is

warranted since GLP-1 signaling is impaired in obesity (Knop et al., 2012; Madsbad, 2014), suggesting that dopamine signaling would also be dysregulated as a function of GLP-1.

GLP-1R is a cell surface G protein-coupled receptor (GPCR) which activates a heteromeric Gs protein (Graaf et al., 2016; Gromada et al., 1995; 1998; 2004). Gs in turn activates adenylate cyclase activity, producing cyclic adenosine monophosphate (cAMP), which then activates protein kinase A (PKA) (Graaf et al., 2016). In agreement with expected Gs GPCR function, GLP-1R agonism does increase PKA activity (Hayes et al., 2011). Although it is known that GLP-1R activation suppresses food intake by upregulating the mitogen-activated protein kinase pathway and downregulating the activity of AMPK (Hayes et al., 2011), it is still unclear how GLP-1R signaling (via a stimulatory GPCR pathway) suppresses dopamine neuron activity. It is possible that NTS PPG neurons innervate VTA GABA neurons to disinhibit VTA dopamine neurons, similar to the innervation by LHA^{GABA} neurons (Nieh et al., 2016). Intra-VTA administration of Ex4 does decrease food intake without inducing malaise (Alhadeff et al., 2012; Dickson et al., 2012) and this effect is abolished with a pretreatment of the GLP-1R antagonist (Mietlicki-Baase et al., 2013). Interestingly, an *ex vivo* study shows that GLP-1R activation in the VTA increases spontaneous excitatory postsynaptic current (sEPSC) frequency and this effect was shown to be mediated by AMPA/kainate receptors (Mietlicki-Baase et al., 2013). This is seemingly contradictory to the findings in this thesis since I showed that dopamine activity is suppressed with central GLP-1R activation. Additionally, endogenous GLP-1R activation suppresses VTA dopamine neuron activity (X. F. Wang et al., 2015). The inherent nature of *ex vivo* experiments removes the possibility of observing the effects of endogenous GLP-1R signaling in circuits that might otherwise be functional in an intact organism. It is also important to note that the recording of activity in the present studies is an aggregate from the paranigral subregion of the VTA and does not capture the intricacies of single-cell interactions. Because presynaptic dopamine D2 receptors have a inhibitory effect on dopamine signaling (Adell &

Artigas, 2004; Gentet & Williams, 2007), it is possible that GLP-1 release activates dopamine neurons that then activate dopamine D2 receptors to suppress the net output of the dopamine neurons in the VTA. Further investigation is required to determine how an excitation via GLP-1R agonism ultimately yields a net suppression of VTA dopamine neuron activity seen in the present studies and others (Fortin & Roitman, 2017).

E. The implications of a multiplexed dopamine signaling for obesity

Understanding how the modulation of a dopamine signal is relevant to the function of day-to-day behavior toward food, is a monumental task. Firstly, the neural mechanisms that govern our behavior no longer function in the same context in which they evolutionarily developed. Rather, these adaptive mechanisms have a higher likelihood to turn maladaptive in an environment where the ready availability of caloric dense foods prime our bodies for obesity. Although dopamine signaling is crucial in the search for and the consumption of food in harsh natural settings where food availability is sparse, the potent invigoration of behavior toward the aforementioned obesogenic foods can contribute to overconsumption and one of many reasons that we find ourselves with an obesity pandemic. Secondly, to forge a path toward curbing the obesity crisis, it is essential that we understand how dopamine neurons adaptively integrate interoceptive and exteroceptive cues to produce appropriate behavior.

To this day, a debate persists regarding the true meaning and function of dopamine signaling in the mesolimbic circuitry. While some interpret dopamine signaling to convey reward value (Hamid et al., 2015; Howe et al., 2013; Mohebi et al., 2019; Phillips et al., 2003; Roitman et al., 2004; Syed et al., 2015; Wassum et al., 2012), some argue that the dopamine signal represents reward prediction error (Saunders et al., 2018; Schultz, 1986; 1998; Steinberg et al., 2013; Usypchuk et al., 2022; Waelti et al., 2001). Reward prediction error (RPE) is the error between perceived and predicted rewards (Schultz, 2016). Under the RPE framework, the dopamine output is represented as a learning signal to update future reward value. In other

words, current reward evaluation updates the expectation of reward in the future and that current reward value is influenced by previous reward experience. For example, dopamine release in the NAc correlates with current reward value but not with RPE (Mohebi et al., 2019). However, when dopamine neurons are artificially stimulated in a task that requires action to receive reward, the optogenetic stimulation during a decision point in a previous trial reinforces that action for the subsequent trial regardless of whether a reward was received (Hamid et al., 2015). This experiment supports the idea that dopamine encodes learning that a specific action results in the reward, rather than the absolute value of the reward.

Considering this debate regarding the function of dopamine neuron activity, I posit that my work provides further insight to the fact that dopamine neurons encode a multiplexed signal and not necessarily just reward value or just learned associations. I found that dopamine neuronal signaling to both food-cues as well as food itself scaled with signals that convey physiological state. A signal of hunger, cytoglucopenia, potentiated dopamine neuron activity to cues, consistent with literature that shows dopamine role is to invigorate behavior in the face of cues that predict reward (J. J. Day et al., 2007; Hoffmann & Nicola, 2014; Roitman et al., 2004; Saunders et al., 2018; Steinberg et al., 2014). On the other hand, a signal of satiety, GLP-1R stimulation, suppressed such dopamine signals. Dopamine neuron responses were modulated by these hunger and satiety signals even when rewards were unexpected. This latter fact suggests that reward value is modulated not only at the level of behavior, but also at the level of dopamine signaling. Dopamine modulation toward caloric stimuli is especially important to consider because dopamine scales with caloric density (physical value) (Geary & Smith, 1985; Hajnal et al., 2004). Therefore, the physical value of a stimulus, the current physiological context, and prior learned associations may all be encoded in dopamine activity as multiplexed simultaneous signals. The physical value of densely caloric foods may overlay the encoding of

physiological state, resulting in a potentiation of dopamine invigoration toward food overconsumption.

Interestingly, the physical value of a food stimulus might be encoded differently in different regions of the dopamine circuitry. Tellez et. al. showed that ventral striatal dopamine release correlated with the taste (hedonic), whereas dorsal striatal dopamine release correlated with the caloric content of the reward (Tellez et al., 2016). This suggests that the function of dopamine varies with location of release, which is supported by the literature. Firstly, dopamine neurons are widely heterogeneous both in where the project but also in what they corelease (Ikemoto, 2007; Morales & Margolis, 2017). Secondly, in addition to the functions mentioned above, dopamine has been linked to spatial reward discrimination (Takikawa et al., 2004), response inhibition (Ogasawara et al., 2018), long term memory (Bromberg-Martin et al., 2010; H. F. Kim et al., 2015), economic common currency and formal utility (Lak et al., 2014; 2016; Stauffer et al., 2014), and many other constructs. Rather than elaborating on each of these constructs, I note them here to illustrate the heterogeneity of dopamine function. Although the data in the present thesis cannot account for the absolute hedonic value of the food stimulus, I did show that dopamine neurons integrate physiological status with the appropriate oral stimulus— only thirst, not 5TG-induced cytoglucopenia, potentiates dopamine responses to water. This suggests that dopamine neurons in the paranigral VTA encode caloric value as a function of physiological state. This interaction between the taste and physiological state set the gain on a system that is responsible for converting drive (hunger) into action (eating).

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Author's note: The third chapter of this thesis has been published in the journal Physiology and Behavior. The article is titled "Phasic dopamine responses to a food-predictive cue are suppressed by the glucagon-like peptide-1 receptor agonist Exendin-4" and is cited in the Citations section (PMID#: 31821815). Below is the current copyright information from Physiology and Behavior that states that I do not need permission to republish that content here.

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Curriculum Vitae

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Education

Neuroscience Ph.D., University of Illinois at Chicago (UIC), 2016-present
Biological Sciences, M.S., University of California, San Diego (UCSD), June 2012
Biological Sciences, B.S., UCSD, December 2010
Human Development, B.A., UCSD, December 2010

Awards and Honors

New Investigator Travel Award, Society for the Study of Ingestive Behavior, 2022
Laboratory of Integrative Neuroscience Travel Award, UIC, 2018
Graduate Student Council Travel Award, UIC, 2018, 2019
Eugene and Ruth Roberts Summer Student Academy Fellowship, City of Hope, 2008

Leadership Activities

• New Investigator Advisory Board, Society for the Study of Ingestive Behavior, 2019-2022.
• External Relations Chair: Secured sponsorship and organized the biotechnology job fair, career development workshops, and biotech information panels. Biological Sciences Student Association in UCSD, 2008, 2009, 2010.

Research Experience

Graduate Student, Department of Psychology, UIC, 2016-present (Laboratory of Dr. Mitch Roitman). Investigating the neural basis of motivated ingestive behavior, including the involvement of the mesolimbic dopamine system in hunger/satiety. Using immunohistochemistry, pharmacology, behavior, and fiber photometry to characterize these mechanisms.
Laboratory Manager/Technician, Department of Human and Evolutionary Biology, USC, 2013-2016 (Laboratory of Dr. Scott Kanoski). Explored the neuronal control of "higher-order" aspects of eating behavior, including decisions about whether to feed or not to feed, what to consume, and how much of it to consume. Used immunohistochemistry, pharmacology, behavior, and gene expression to characterize these mechanisms.
Master's Student Researcher, Department of Biology, UCSD, 2010-2012 (Laboratory of Dr. Kathleen French). Identified novel inhibitory cell type in the central nervous system of the medicinal leech. Characterized the cell using immunohistochemistry and electrophysiology leading to further investigation of its role in leech behavior.
Undergraduate Research Assistant, Department of Virology, City of Hope, 2008 (research advisor: Dr. Aprille Seidel). Investigated a potential biomarker protein overexpressed in carcinogenic melanocytes. Used molecular biology and immunology to produce candidate melanoma vaccines.

Peer-reviewed Journal Publications (reverse chronological order)

Hsu TM, Bazzino P, Hurh SJ, **Konanur VR**, Roitman JD, Roitman MF. Thirst recruits phasic dopamine signaling through subfornical organ neurons. *PNAS*. 2020.
Konanur VR, Hsu TM, Kanoski SE, Hayes MR, Roitman MF. Phasic dopamine responses to a food-predictive cue are suppressed by the glucagon-like peptide-1 receptor agonist Exendin-4. *Phys & Behav*. 2020.
Noble EE, Hahn JD, **Konanur VR**, Hsu TM, Page SJ, Cortella AM, Liu CM, Song MY, Suarez AN, Szujewski CC, Rider D, Clarke JE, Darvas M, Appleyard SM, Kanoski SE. Control of Feeding Behavior by Cerebral Ventricular Volume Transmission of Melanin-Concentrating Hormone. *Cell Metab*. 2018.

Hsu TM, Noble EE, Liu CM, Cortella AM, **Konanur VR**, Suarez AN, Reiner DJ, Hahn JD, Hayes MR, Kanoski SE. A hippocampus to prefrontal cortex neural pathway inhibits food motivation through glucagon-like peptide-1 signaling. *Mol Psychiatry*. 2018.
Hsu TM, Noble EE, Reiner DJ, Liu CM, Suarez AN, **Konanur VR**, Hayes MR, Kanoski SE. Hippocampus ghrelin receptor signaling promotes socially-mediated learned food preference. *Neuropharmacology*. 2018.
Reiner DJ, Mietlicki-Baase EG, McGrath LE, Zimmer DJ, Bence KK, Sousa GL, **Konanur VR**, Krawczyk J, Burk DH, Kanoski SE, Hermann GE, Rogers RC, Hayes MR. Astrocytes Regulate GLP-1 Receptor-Mediated Effects on Energy *Balance. J Neurosci*. 2016.
Hsu TM, Hahn JD, **Konanur VR**, Noble EE, Suarez AN, Thai J, Nakamoto EM, Kanoski SE. Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. *eLife*. 2015.
Hsu TM, **Konanur VR**, Taing L, Usui R, Kayser BD, Goran MI, Kanoski SE. Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus*. 2015.
Hsu TM, Hahn JD, **Konanur VR**, Lam A, Kanoski SE. Hippocampal GLP-1 receptors influence food intake, meal size, and effort-based responding for food through volume transmission. *Neuropyschopharmacology*. 2015.

Presentations / Abstracts **(# presenting author, reverse chronological order)**

#**Konanur VR**, Roitman MF. (Jul. 2022) Cytoglucopenia Potentiates Sucrose- and Sucrose Cue-Evoked Dopamine Signaling in the Ventral Tegmental Area. The Society for the Study of Ingestive Behavior (annual meeting). Porto, Portugal (**oral presentation; New Investigator Travel Award**).
#Konanur VR, Roitman MF. (May 2022) Cytoglucopenia Potentiates Sucrose Evoked Dopamine Signaling in the Ventral Tegmental Area. UIC Graduate Program in Neuroscience Annual Symposium. Chicago, IL (**poster**).
#Konanur VR, Roitman MF. (Oct. 2021) Glucose dynamics modulate dopamine signaling: implications for food-motivated behavior. Center for Alcohol Research in Epigenetics: Emerging techniques in Neuroscience. Chicago, IL (**oral presentation**).
#Konanur VR, Roitman MF. (Oct. 2021) Glucose dynamics modulate dopamine signaling: implications for food-motivated behavior. UIC Neuroscience Graduate Research Symposium (annual meeting). Chicago, IL (**oral presentation**).
#Konanur VR, Roitman MF. (Jul. 2021) Blocking Glucose Utilization via 5-thio-D-glucose Potentiates Sucrose-Evoked Dopamine Signaling in the Ventral Tegmental Area. The Society for the Study of Ingestive Behavior (annual meeting). Virtual meeting (**poster**).
#Konanur VR, Roitman MF. (Apr. 2021) Blocking glucose utilization via 5-thio-D-glucose enhances sucrose evoked dopamine signaling. UIC Graduate Program in Neuroscience Annual Symposium. Chicago, IL (**poster**).
#Konanur VR, Hsu TM, Roitman MF. (Dec 2020) Modulation of Food-Driven Phasic Dopamine Signaling by Glucagon-like Peptide 1. Center for Alcohol Research in Epigenetics Junior Scholar Seminar Series. Chicago, IL (**oral presentation**).
#Konanur VR, Hsu TM, Roitman MF. (Jan 2020) Modulation of Food-Driven Phasic Dopamine Signaling by Glucagon-like Peptide 1. UIC Neuroscience Graduate Research Symposium (annual meeting). Chicago, IL (**oral presentation**).
#Konanur VR, Hsu TM, Roitman MF. (Nov. 2019) Central Exendin-4 selectively suppresses cue-evoked phasic dopamine spikes and resultant behavior. The Society for Neuroscience (annual meeting). Chicago, IL (**poster**).
#Konanur VR, Hsu TM, Roitman MF. (Jul. 2019) Central Exendin-4 selectively suppresses cue-evoked phasic dopamine spikes and resultant behavior. The Society for the Study of Ingestive Behavior (annual meeting). Utrecht, Netherlands. (**poster**).
#Konanur VR, Hsu TM, Roitman MF. (May 2019) Central Exendin-4 Selectively Suppresses Cue-Evoked Phasic Dopamine Spikes and Resultant Behavior. Center for Alcohol Research in Epigenetics (annual meeting). Chicago, IL (**poster**).
#Konanur VR, Hsu TM, Roitman MF. (Apr. 2019) Central Exendin-4 Selectively Suppresses Cue-Evoked Phasic Dopamine Spikes and Resultant Behavior. The Society for Neuroscience Chicago Chapter (annual meeting). Chicago, IL (**poster**).
#Konanur VR, Hsu TM, Roitman MF. (Feb. 2019) The satiety factor GLP-1 modulates phasic dopamine signaling and behavior. UIC Neuroscience Graduate Student Symposium (annual meeting). Chicago, IL (**oral presentation**).
#Hsu TM, **Konanur VR**, Bazzino P, Roitman MF. (Nov. 2018) Homeostatic need states differentially recruit cue evoked VTA phasic dopamine signaling. The Obesity Society (annual meeting). Nashville, Tennessee (oral presentation).

#Hsu TM, **Konanur VR**, Roitman MF. (Jul. 2018) Thirst and the hormone Angiotensin II recruit VTA dopamine signaling to water availability. The Society for the Study of Ingestive Behavior (annual meeting). Bonita Springs, Florida (oral presentation).
#Konanur VR, Roitman MF. (Mar. 2018) Using in vivo Fiber Photometry to Further Understand Mechanisms of Amphetamine Action. Monitoring Molecules in Neuroscience (Biannual meeting). Oxford, UK (**poster**).
#Hsu TM, **Konanur VR**, Roitman MF. (Mar. 2018) Thirst and the hormone Angiotensin II recruit VTA dopamine signalling to water consumption. Monitoring Molecules in Neuroscience (Biannual meeting). Oxford, UK (poster).
#Konanur VR, Roitman MF. (Oct. 2017) Using in vivo Fiber Photometry to Further Understand Mechanisms of Amphetamine Action. UIC Neuroscience Symposium (annual meeting). Chicago, IL (**poster**).
#Noble EE, Song MY, **Konanur VR**, Hsu TM, Suarez AN, Hahn JD, Kanoski SE. (Jul. 2016) Evidence for “bulk flow” neurohumoral transmission by the orexigenic neuropeptide, melanin-concentrating hormone. The Society for the Study of Ingestive Behavior (Annual Meeting) Porto, Portugal (oral presentation).
#Suarez AN, Hsu TM, **Konanur VR**, Noble EE, Kanoski SE. (Jul. 2016) The role of vagus nerve signaling in hippocampal-dependent memory function. The Society for the Study of Ingestive Behavior (annual meeting) Porto, Portugal (oral presentation).
#Konanur VR, Noble EE, Hsu TM, Kanoski SE, Hahn JD. (Nov. 2015) Neuroanatomical Evidence for Neurohumoral Transmission by Melanin-Concentrating Hormone Neurons in the Rat. The Society for Neuroscience (annual meeting) Chicago, IL (**poster**).
#Hsu TM, Hahn JD, **Konanur VR**, Kanoski SE. (Jul. 2015) A novel hippocampal-hypothalamic neural circuit mediating appetite through ghrelin receptor signaling. The Society for the Study of Ingestive Behavior (annual meeting). Denver, CO (poster).
#Hsu TM, **Konanur VR**, Kanoski SE. (Aug. 2014) Adolescent consumption of sugar-sweetened beverages impairs hippocampal-dependent learning. The Society for the Study of Ingestive Behavior (annual meeting). Seattle, WA (oral presentation).
#Konanur VR, Todd KL, Kristan WB, French K. (Nov. 2011) Identifying and characterizing leech neurons labeling for GABA. The Society for Neuroscience (annual meeting). San Diego, CA (**poster**).

Teaching Experience

Teaching Assistant:
Neuroanatomy – BIOS 483. Spring 2019, 20, 21, 22
Laboratory in Behavioral Neuroscience – PSCH 363. Fall 2018, 2019, 2020, 2021; Spring 2018
Laboratory in Cognitive Neuroscience – PSCH 367. Fall 2017
Biological Techniques – BIBC 103. Fall 2011; Winter 2011, 2012; Spring 2011, 2012
Metabolic Biochemistry – BIBC 102. Fall 2009, 2010, 2012; Winter 2010; Spring 2010
Guest Lecturer:
Neuroanatomy – BIOS 483. Spring 2022
Seminal on Neurobiology – BIOS 386. Fall 2017
Mentored Undergraduate Research Students:
University of Illinois at Chicago:
- Paula Bazzino (2018-2019); Post-baccalaureate researcher
- Beto Araiza (2021-2022); Post-baccalaureate researcher

University of Southern California:
- Lilly Taing (2013-2016); Health and Humanity/ Health care studies.
- Joanna Liang (2013-2015); Psychology/Natural Science.
- Mehul Trivedi (2013-2015); Biological Sciences/ Psychology.
- Ryan Usui (2013-2015); Human Biology.
- Emily Nakamoto (2013-2016); Neuroscience/ Art.
- Jessica Thai (2013-2016); Biological Sciences.
- Agustina Kim (2013-2016); Human Biology.
- Allison Apfel (2014-2016); Health Promotion and Disease Prevention.
- Natalie Demirjian (2014-2016); Neuroscience.
- Kaitlin Sontag (2014-2016); Human Biology.

Affiliations/Memberships

- The Society for the Study of Ingestive Behavior (2019, 2021, 2022)

• The Society for Neuroscience Chicago Chapter (2019)
• The Society for Neuroscience (2011, 2015, 2019)
• Monitoring Molecules in Neuroscience (2018)