Basal Ganglia Regulation of Motivated Behaviors

by

Mark Allen Rossi

Department of Psychology and Neuroscience

Duke University

Date: ______________________

Approved: ___________________________

Henry H. Yin, Supervisor

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Staci D. Bilbo

___________________________

Jennifer M. Groh

___________________________

Nicole Calakos

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Psychology and Neuroscience in the Graduate School
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ABSTRACT

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Abstract

Finding and consuming food and water are among the most critical functions for an animal's survival. Food seeking (e.g., exploration and approach) and consummatory (e.g., licking, chewing, swallowing) behaviors are usually highly controlled, resulting in stable food intake, body mass, and fat stores in humans and laboratory animals. These variables are thought to be governed by homeostatic control systems that closely regulate many aspects of feeding behavior. However, the homeostatic mechanisms underlying these processes are often disrupted in humans, resulting in either hyperphagia or hypophagia. Despite many decades of investigations into the regulatory circuits of animals and humans, the neural circuits that underlie voluntary feeding are unclear. There have been considerable advances into understanding how the brain is able to broadly regulate food consumption (e.g., the role of circulating hormones on food intake and body weight). As much work has focused on hypothalamic mechanisms, relatively little is known about how other neural systems contribute to specific aspects of food seeking and consumption.

The basal ganglia have been implicated in many aspects of motivated behavior including appetitive and consummatory processes. However, the precise role that basal ganglia pathways play in these motivated behaviors remain largely unknown. One reason for this is that the basal ganglia are functionally and anatomically heterogeneous, with distinct functional circuit elements being embedded within overlapping tissue. Until recently, tools permitting identification and manipulation of molecularly defined neuron populations were unavailable.

The following experiments were designed to assess the role of the basal ganglia in regulating appetitive and consummatory behavior in mice. The first experiment
(Chapter 2) examines the relationship between neural activity in the substantia nigra, a major output nucleus of the basal ganglia, and an animal’s motivational state. Both dopaminergic and GABAergic neurons show bursts of action potentials in response to a cue that predicts a food reward in hungry mice. The magnitude of this burst response is bidirectionally modulated by the animal’s motivational state. When mice are sated prior to testing, or when no pellets can be consumed, both motivational state and bidirectional modulation of the cue response are unchanging.

The second set of experiments (Chapter 3 and 4) utilizes a mouse model of hyperdopaminergia: Dopamine transporter knockout mice. These mice have persistently elevated synaptic dopamine. Consistent with a role of dopamine in motivation, hyperdopaminergic mice exhibit enhanced food seeking behavior that is dissociable from general hyperactivity. Lentiviral restoration of the dopamine transporter into either the dorsolateral striatum or the nucleus accumbens, but not the dorsomedial striatum, is sufficient to selectively reduce excessive food seeking. The dopamine transporter knockout model of hyperdopaminergia was then used to test the role of dopamine in consummatory processes, specifically, licking for sucrose solution. Hyperdopaminergic mice have higher rates of licking, which was due to increased perseveration of licking in a bout. By contrast, they have increased individual lick durations, and reduced inter-lick-intervals. During extinction, both knockout and control mice transiently increase variability in lick pattern generation while reducing licking rate. Yet they show very different behavioral patterns. Control mice gradually increase lick duration as well as variability in extinction. By contrast, dopamine transporter knockout mice exhibited more immediate (within 10 licks) adjustments—an immediate increase in lick duration variability, as well as more rapid extinction. These results suggest that the level of dopamine can modulate the persistence and pattern generation of a highly stereotyped
consummatory behavior like licking, as well as new learning in response to changes in environmental feedback.

The final set of experiments was designed to test the relationship between consummatory behavior and the activity of GABAergic basal ganglia output neurons projecting from the substantia nigra pars reticulata to the superior colliculus, an area that has been implicated in regulating orofacial behavior. Electrophysiological recording from mice during voluntary drinking showed that activity of GABAergic output neurons of the substantia nigra pars reticulata reflect the microstructure of consummatory licking. These neurons exhibit oscillatory bursts of activity, which are usually in phase with the lick cycle, peaking near the time of tongue protrusion. Dopaminergic neurons, in contrast, did not reflect lick microstructure, but instead signaled the boundaries of a bout of licking. Neurons located in the lateral part of the superior colliculus, a region that receives direct input from GABAergic projection neurons in the substantia nigra pars reticulata, also reflected the microstructure of licking with rhythmic oscillations. These neurons, however, showed a generally opposing pattern of activity relative to the substantia nigra neurons, pausing their firing when the tongue is extended. To test whether perturbation of the nigrotectal pathway could influence licking behavior, channelrhodopsin-2 was selectively expressed in GABAergic neurons of the substantia nigra and the axon terminals within the superior colliculus were targeted with optic fibers. Activation of nigrotectal neurons disrupted licking in a frequency-dependent manner. Using optrode recordings, I demonstrate that nigrotectal activation inhibits neurons in the superior colliculus to disrupt the pattern of licking.

Taken together, these results demonstrate that the basal ganglia are involved in both appetitive and consummatory behaviors. The present data argue for a role of striatonigral dopamine in regulating general appetitive responding: persistence of food-
seeking. Nigrotectal GABA projections appear to be critical for consummatory orofacial motor output.
Dedication

This thesis is dedicated to my family. I am especially grateful for the love and support of my parents, Dino and Cindy, and my brothers, Vince and Mario.
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Figure 1. Single unit recording in substantia nigra. (a) Representative photomicrograph of electrode tracks in the pars compacta (SNC; arrow; left). Schematic representation of the electrode placement (right; SNR, pars reticulata). Coordinates are relative to Bregma. Sample traces of DA (b) and GABA (c) neurons showing the narrower spike waveform of GABA neurons. Insets are inter-spike interval histograms. (d) Average waveforms (± s.e.m.) for all recorded DA and GABA neurons. We classified the neurons based on the waveforms of their action potentials. DA neurons, for example, are characterized by longer spike durations than GABA neurons. n is indicated by the numbers. (e) Full width half max (FWHM) values of classified DA and GABA neurons. (f) Schematic illustration of the FT60 task. One pellet was delivered into the food cup every 60 s (vertical black lines). An auditory cue preceded the pellet delivery by ~550 ms (red lines). Vertical scale bars represent 68 µV (b) and 23 µV (c), and horizontal scale bars represent 500 µm in a, and 200 µs in b and c (10 ms in insets).

Figure 2. Effects of quinpirole on dopaminergic and GABAergic neurons. To confirm the classification of cell types, we performed additional pharmacological experiments. Quinpirole (1mg/kg), a D2 receptor agonist, was injected intraperitoneally during a recording session (red arrows). Example responses of DA (a-c) and GABA (d-f) neurons to quinpirole treatment are shown. Immediately following injection, the rate of dopaminergic firing decreased (b), whereas GABAergic neurons were unaffected (e). (c, f) Inter-spike interval histograms for the neurons in a and d. In a and d, vertical scale bars represent 76 µV and 137 µV, respectively. Horizontal scale bars represent 200µs. Scale bars in c and f represent 25 ms.

Figure 3. Dopaminergic and GABAergic neurons showed a phasic response to reward cue. (a) Activity of neurons that increased following reward cue normalized to the maximum firing rate; sorted according to increasing cumulative maximum values. Each row represents one neuron. X axis is time from reward cue onset (s). n is indicated in parentheses. (b) Representative peri-event histograms and rasters for individual DA (top) and GABA (bottom) neurons showing phasic responses following the reward cue (10 ms bins).

Figure 4. Synchrony is enhanced immediately after the reward cue. Examples of synchrony between pairs of SN neurons following the reward cue. (a) Example of a corrected joint peri-stimulus time histograms (JPSTH) in the left column, corresponding predictor matrix (middle) and histogram of JPSTH diagonal (right; 500 ms width) for a pair of simultaneously recorded DA neurons (a), GABA neurons (b), and DA-GABA pairs (c); DA activity is plotted on the horizontal axis. Axes are time relative to the reward cue. The predictor matrix is subtracted bin-by-bin from the raw JPSTH, and then normalized by the standard deviation of the predictor matrix (100 ms x 100 ms bins).
Figure 5. Population JPSTH analysis. (a) Normalized population JPSTH (pJPSTH) of all DA-DA pairs ($n = 185$ pairs). Synchrony was enhanced immediately after the onset of the cue. (b) Synchrony among GABA-GABA pairs ($n = 475$ pairs) was not affected by the reward cue. (c) Synchrony between DA and GABA neurons ($n = 394$ pairs) was enhanced following the reward cue. pJPSTH bins are 100 ms x 100 ms. Values are normalized by the standard deviation of predictor matrices. (d) The diagonal of each corrected pJPSTH is plotted (width = 500 ms, bins = 100 ms). DA-DA and DA-GABA pairs showed enhanced synchrony following the cue, whereas cue-elicited synchrony was not observed among GABA-GABA pairs. Only those neurons showing significant excitation following the reward cue were included in the analysis.

Figure 6. Motivational modulation of phasic responses to reward cue. Motivational modulation of DA and GABA activity in SN. (a) Sample PSTH illustrating the motivational shift in phasic cue response of decreasing (a) and increasing (b) DA neurons separated into four 30 min blocks (bin size = 30 ms). GABA neurons also showed similar decreasing (c) and increasing (d) motivational modulation.

Figure 7. Modulation is not due to changes in neuron isolation. To test whether changes in neuronal isolation could account for the modulation observed in single unit activity, waveform characteristics were calculated for all modulated neurons for each half of the recording session. Neither FWHM (a) nor the peak of the inter-spike interval (ISI; b) changed during the two-hour recording sessions (paired $t$-tests, $p > 0.05$). (c) Sample traces from the first and second half of the session for modulated neurons. The range of ISI values shown is 0-30 ms.

Figure 8. Food-seeking behavior gradually decreases with satiety. (a) Following reward delivery, mice entered the food cup to collect the pellet. Representative data from one mouse are shown. (b) The rapid increase in food cup entries after the reward cue was eliminated during extinction and following pre-feeding. Traces are from the same mouse. FT60 data is the average from all four blocks in a. (c) Rate of entries decreased as mice became sated during FT60 sessions. It was also reduced during extinction and following pre-feeding (average of all mice on all testing days; 20 min bins). (d) The latency to enter the food cup following reward delivery increased during extinction and following pre-feeding treatment. (e) Latency to enter the food cup for all mice on all testing days was pooled to yield population averages. (f) The proportion of DA and GABA neurons that exhibited significant inhibitions following the reward cue was unaffected by pre-feeding and extinction. (g) The number of excitatory responses varied as a function of motivational state for both DA and GABA neurons. * $p < 0.05$.
Figure 9. Average response of all excited neurons, including those that do not show motivational modulation during the session. To compare the basal activity and mean responses between the classes we examined the responses of all neurons that were excited following the cue. The activity (mean ± s.e.m.; 20 ms bins) of all neurons with excitatory responses to the reward cue, regardless of motivational modulation, is plotted for each half of FT60 (a), pre-feeding (b) and extinction (c) sessions. Overall, there was a reduction in the magnitude of the phasic response as mice became sated. During extinction (c) the phasic response is nearly abolished for both DA and GABA neurons. The response to the cue (d) and to reward receipt (e) are dissociable. The population responses are shown for DA (n = 128) and GABA (n = 272) neurons recorded during FT60 sessions. (f-g) Baseline firing rates do not vary between FT60, pre-feed, or extinction sessions for DA or GABA neurons presented in a-c (one-way ANOVAs, F < 1.0, p > 0.05).

Figure 10. Motivational modulation is absent following pre-feeding and during extinction. Example of the cue response of a DA neuron in FT60 (a), pre-feed (b), and extinction (c) sessions. Each line represents the average response from a 30-trial block. 10 ms bins, 100 ms Gaussian smooth. Corresponding waveforms are shown below.

Figure 11. Summary of phasic response in DA neurons. To compare the magnitude of the satiety effect to the magnitude of the mean response, we plotted the responses of all DA neurons showing a cue-elicited phasic burst. (a) The phasic response to the cue of increasing DA neurons (n = 19) was higher for the second half of FT60 sessions than it was for the first half. Decreasing DA neurons (n = 74) showed the opposite pattern. The phasic response of unmodulated neurons (n = 35) did not change over time. Modulation of the phasic response was reduced by pre-feeding (b; n = 5 increasing, 15 decreasing, 19 unmodulated) and eliminated during extinction (c; n = 2 increasing, 2 decreasing, 18 unmodulated). The phasic response of increasing neurons was nearly abolished following pre-feeding and during extinction, and the phasic response of decreasing neurons was abolished during extinction.

Figure 12. Summary of phasic response in GABA neurons. GABA neurons show the same pattern of modulation as DA neurons. (a) The phasic response to the reward cue of increasing GABA neurons (n = 68) was higher for the second half of FT60 sessions than it was for the first half. Decreasing GABA neurons (n = 138) showed the opposite pattern. The phasic response of unmodulated neurons (n = 66) did not change. Modulation of the phasic response was reduced by pre-feeding (b; n = 15 increasing, 24 decreasing, 33 unmodulated) and eliminated during extinction (c; n = 7 increasing, 4 decreasing, 73 unmodulated). The phasic response of increasing neurons was nearly abolished following pre-feeding and during extinction, and the phasic response of decreasing neurons was abolished during extinction.
Figure 13. Mice are active throughout the recording session. To ensure that motivational modulation was not caused by mice falling asleep or losing interest in the pellets, we used video tracking to record the position of mice during recording sessions. Mice spent most of their time near the food cup (a). Speed was calculated (pixels/frame) throughout the session. Mice remained active for the entire two-hour session (b).

Figure 14. Cue-elicited synchrony of neural activity is modulated by motivational state. pJPSTH diagonals (mean ± s.e.m.; 100 ms x 100 ms bins; 500 ms diagonal width) are shown for DA-DA (n = 185 FT60; n = 81 pre-feed; n = 45 extinction), GABA-GABA (n = 475 FT60; n = 346 pre-feed; n = 389 extinction), and DA-GABA (n = 394 FT60; n = 144 pre-feed; n = 138 extinction) pairs recorded during FT60 (a), pre-feeding (b), and extinction (c). The diagonals of the corrected and normalized pJPSTHs are in the top row, while the diagonals of the predictor matrices are in the bottom row. The predictor was defined as the cross-product of the JPSTH for each pair of neurons. Pre-feeding reduced cue-elicited synchrony for DA-DA and DA-GABA pairs while increasing overall synchrony between GABA-GABA pairs (b). Extinction reduced synchrony between DA-GABA and DA-DA pairs without affecting GABA-GABA pairs (c).

Figure 15. DAT^−/− mice are more motivated for food than controls. (a) DAT^−/− mice weigh less than DAT^+/+ mice. (b) DAT^−/− mice were more active than DAT^+/+ and DAT^+/− controls in an open field test. Average speed during 3min exposure. (c) Examples of open field tracking. (d) DAT^−/− mice show elevated rates of food cup entries during the inter-reward interval on FT60 tests. When sated, DAT^−/− mice (n=16) no longer entered the food cup more than DAT^+/+ (n=15) and DAT^+/− (n=5) mice. In extinction, when food was no longer delivered, all mice also stopped entering the food cup. Thus, enhanced rates of food cup entries cannot be attributed to general hyperactivity. (e) When normalized to baseline, all groups show similar levels of deprivation. (f) The rate of DAT^−/− food cup entries was elevated only when mice were hungry and pellets were being delivered, confirming that entries are food-directed. Values are mean and s.e.m. * p<0.05.

Figure 16. DAT^−/− mice show impaired instrumental learning. (a) DAT^−/− mice are severely impaired at learning to lever press for food pellets. (b) Inter-response intervals for the first eight rewards during initial acquisition are shown. After three presses, DAT^−/− mice show significantly longer inter-response intervals than DAT^+/+ and DAT^+/− mice. Values are mean ± s.e.m.
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Figure 20. Striatal rescue of DAT does not restore hyperactivity or instrumental responding in DAT−/− mice. (a) In an open field test, speed remained significantly elevated above DAT+/+ and DAT+/− controls for all DAT−/− groups. (b) DAT rescue does not cause mice to gain more weight than controls. (c) All DAT−/− mice were significantly impaired at learning to press a lever for food. Viral rescue did not restore instrumental learning. (d) Food cup entries during instrumental training were comparable between groups, indicating comparable Pavlovian approach responses (p>0.05).

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1. Introduction

The term 'homeostasis' refers to the constancy of physiological variables essential for survival (i.e., water content, blood oxygen, pH, temperature) that organisms are able to maintain (Cannon, 1932). One critical aspect of this constancy is energy balance, which is necessarily regulated by food intake. Animals are able to regulate their food intake, energy balance, and body weight with great precision across many different time scales (i.e., hours, days, months) (Adolph, 1947; Wayner and Carey, 1973; Keesey and Powley, 1986). Because food is not always readily available in the environment, animals must periodically seek out food to replenish energy stores. This may require exploration, learning instrumental actions, or Pavlovian associations that eventually lead to food consumption. Many animals eat in discrete bouts or meals (Collier and Johnson, 1990; Smith, 1996; Staddon, 1997). This requires mechanisms that closely monitor energy stores and regulate both food seeking and consummatory behavior, for without precise regulation, behavioral output would be sporadic and energy balance and body weight would be inconsistent. Although many attempts have been made to understand the substrates of motivated behavior, little is known about the precise neural mechanisms regulating food-directed output.

Feeding behavior is critical to an animal's survival, and proper regulation of this behavior is a major function of many bodily systems. While there has been extensive investigation of the physiological mechanisms regulating food intake and energy balance over the last century (Bernard et al., 1927; Cannon, 1932; Morgane and Panksepp, 1979; Staddon, 1983a), most efforts to understand how an organism initiates and terminates feeding have focused on either peripheral (i.e., hormones) or hypothalamic mechanisms. The central nervous system substrates of initiation, maintenance, and
termination of voluntary feeding, however, must extend beyond hormonal interactions with the hypothalamus. This is because energy deficits are sufficient to generate complex behaviors that must be orchestrated by other brain systems. Only recently have researchers begun to explore extra-hypothalamic regulation of feeding behavior (Morton et al., 2006; Palmiter, 2007).

The basal ganglia, a collection of subcortical nuclei, are necessary for the execution of many voluntary movements including reward seeking and goal-directed behavior (Yin and Knowlton, 2006) and are thought to contribute to effective feeding behavior (Palmiter, 2007; Salamone and Correa, 2012). Because much of the evidence used to support the notion that the basal ganglia are critical for reward-guided behaviors is based on studies that have used hungry animals performing tasks for food rewards, it is often difficult to dissociate the role that these structures play in reward and learning from food-directed behavior per se.

In fact, many studies have demonstrated the intermingled nature of 'reward' systems and 'feeding' systems. Electrical and optogenetic self stimulation the basal ganglia and related pathways, for example, are sufficient for animals to learn instrumental actions and approach behaviors (Wyrwicka and Doty, 1966; Wise, 1981; White and Hiroi, 1998; Adamantidis et al., 2011; Kravitz et al., 2012; Rossi et al., 2013b). In many cases, electrical stimulation of these same sites induces complex appetitive behaviors such as eating or drinking (Andersson et al., 1960; Wyrwicka and Doty, 1966). To further complicate the distinction between reward and feeding, the rewarding effects of self stimulation are often dampened by satiety and potentiated by fasting (Brady et al., 1957; Hoebel and Teitelbaum, 1962; Wilkinson and Peele, 1962; Hoebel, 1969; Wyrwicka, 1976; Katz et al., 1978). For example, electrical self stimulation of the
substantia nigra in rats is potentiated by food deprivation, and withholding electrical stimulation facilitates weight gain (Katz et al., 1978).

The fact that the same brain areas are involved in both feeding behavior and more abstract 'reward' related behaviors poses a problem for the current understanding of these systems. The same pathways that have been studied in the context of self stimulation are thought to by dysfunctional in people with disorders of motivation, such as addiction, anorexia, and obesity (Volkow and Wise, 2005). However, specific contributions of the basal ganglia to particular motivated behaviors, such as feeding, lack clear understanding. Without careful analysis of behavior and the underlying circuits, the neural substrates of motivated behavior will remain unclear. The goal of this dissertation is to understand how the basal ganglia contribute to motivated feeding behavior.

The following chapters will focus on two distinct aspects of motivated feeding behavior. First, I will discuss how neurons in the basal ganglia contribute to preparatory or appetitive behaviors (Chapters 2 and 3). These are the actions that precede physical contact with the food, e.g., exploration and approach (Konorski, 1967). Chapters 4 and 5 will focus on how the basal ganglia and immediate downstream targets contribute to the execution and maintenance of consummatory behaviors.

1.1 Feeding Behavior

To understand how the brain orchestrates the complex behaviors that circumscribe feeding, it is imperative to first understand the behavior itself. In many animals, voluntary feeding has two major phases: appetitive and consummatory. During the appetitive phase, an animal may explore its environment, perform instrumental actions, and approach a possible food source. When successful, this appetitive behavior leads directly to contact with the goal (i.e., food). The consummatory behavior
occurs when the animal is in contact with the food and frequently consists of rhythmic orofacial movements (e.g., licking, chewing, suckling, lapping, swallowing). These will be discussed below in the order in which they typically occur during natural feeding.

1.1.1 Appetitive Behavior

Appetitive behavior refers to some activity that can increase the probability of satisfying an organism's need. While appetitive behavior for food is a common area of study and will be the focus of much of the work presented here, appetitive processes can be used to describe many different behaviors, including those related to feeding, reproduction, and social interaction. For the purposes of the present discussion, I will use the term 'appetitive' to refer to food seeking behavior: the processes of exploring and approaching a known food source. I will attempt to restrict the discussion of appetitive behavior below to studies that have investigated the neural substrates of food-directed behavior.

Appetitive behavior is generally thought to rely on the limbic system. The hypothalamus, ventral striatum (including the nucleus accumbens), hippocampus, amygdala, and related cortical areas have been implicated in both human and animal studies of reward-guided appetitive behavior (Robbins and Everitt, 1996). Frequently, the overlap among reward modalities (i.e., food, drugs of abuse, money, electrical stimulation) makes it difficult to disentangle the contributions of defined neural populations to specific behaviors. It is likely that these brain regions work in concert to allow an animal to learn about particular rewards and related stimuli and subsequently guide appetitive behavior toward some goal. However, since the idea of a limbic 'reward system' is often based on ambiguous or conflated notions of what a reward is, resulting in an all-encompassing notion of Reward, much of the data are difficult to interpret.
Furthermore, the neural pathways thought to contribute to reward-guided behaviors are often not as anatomically distinct as they are sometimes assumed to be. For example, several excellent reviews have highlighted the interactions between limbic 'reward systems' and 'motor systems' (Mogenson et al., 1980; Groenewegen et al., 1996; Kelley, 2004; Yin et al., 2008). As a result, much of the literature has neglected the contributions of the dorsal striatum and the substantia nigra to appetitive processes. However, I will discuss below how classic motor systems may contribute to appetitive behavior.

1.1.2 Consummatory Behavior

Consummatory behavior refers not to the act of consuming food, but rather of consummation, or the completion of some goal. Consummatory behavior occurs as the culmination of appetitive behavior. That is, once the goal is reached, appetitive behavior ceases or pauses, and consummatory behavior commences. In the chapters that follow, I will use the term 'consummatory' to refer specifically to the orofacial behaviors concerned with ingestion of food or water.

1.1.2.1 Voluntary licking

Many animals eat in discrete meals, or prolonged (on the order of minutes) periods of appetitive and consummatory behavior (Collier and Johnson, 1990; Smith, 1996; Staddon, 1997). Within a meal, consummatory behavior occurs in bouts, or periods (seconds to minutes) of patterned orofacial movements that result in ingestion. In rodents, these orofacial movements are commonly categorized as licking, lapping, sucking, or chewing (Spector et al., 1998a). Within a consummatory bout, periodic oscillatory orofacial movements guide ingestion of nutrients into the body. One
repertoire of behaviors that is particularly useful in studying how neural circuits contribute to behavior is consummatory drinking.

Researchers have been interested in drinking behavior for many decades. In rodents, drinking takes the form of licking, which occurs in a relatively stereotyped manner, consisting of alternating tongue protrusions and retractions in which the tongue cycles in and out of the mouth at approximately 6-9 Hz (Halpern, 1977; Spector et al., 1998a; Weijnen, 1998). Careful analysis of the structure of licking behavior can be an informative tool for understanding the neural circuits that contribute to consummatory behavior. A variety of devices have been designed to study the movements associated with such fast oscillations of the tongue. They can be called drinkometers, lickometers, lick detectors, or lick sensors, interchangeably. The first electronic ‘drinkometer’ was developed by Stellar and Hill in which a circuit was completed when rats that were standing on a ground plate contacted a metal drinking tube (Stellar and Hill, 1952). When the circuit is completed, the contact is registered as a voltage change, which can be recorded by analog or digital means. This form of contact lickometer is useful because it can yield extremely precise temporal resolution of behavior and allows researchers to additionally measure the duration of individual contacts with the drinking tube. Many variations on this design have been implemented since its inception which pass very little current into the animal making contact lickometers useful for electrophysiological recording studies (Hayar et al., 2006; Slotnick, 2009).

A common alternative to contact lick detectors is the optical lick sensor. This type of lickometer usually relies on a photobeam, which is broken with each tongue protrusion (Weijnen, 1998; Schoenbaum et al., 2001). Though this type of sensor is less likely to interfere with electrophysiological recordings, the time of each lick is highly dependent on the exact placement of the photobeam relative to the drinking tube.
Optical lick sensors also only give a proxy to the time that the tongue is actually in contact with the drinking tube (the duration of the beam break, or the time between two consecutive beam breaks) and are usually thought to yield less accurate measures of licking.

The brainstem circuitry involved in consummatory licking has been studied in great detail. The muscles that are involved in protruding (e.g., Genioglossus) and retracting (e.g., Styloglossus and Hyoglossus) the tongue are innervated by cranial nerves, particularly cranial nerve XII (Halpern, 1977; Travers et al., 1997b). Because of the pattern of innervation from the hypoglossal nerve, early researchers performed electrophysiological recordings from the hypoglossal nucleus—which gives rise to the neurons of the hypoglossal nerve—in an attempt to understand how rhythmic tongue protrusions and retractions observed during licking were controlled by the brainstem. These researchers found that neurons within the hypoglossal nucleus displayed oscillatory activity that was tightly coupled to the lick cycle in rats (Wiesenfeld et al., 1977). Electrical stimulation in and around the hypoglossal nucleus in cats produced tongue protrusions that were coupled with the stimulation at frequencies up to the natural lick frequency (~5 Hz). At higher frequencies, the tongue remained protruded or retracted for the duration of the stimulation (Morimoto et al., 1966).

Premotor neurons that are thought to contribute to hypoglossal regulation of tongue and jaw movements are found throughout the reticular formation at the levels of the pons and medulla (Brozek et al., 1996; Travers et al., 1997b, 2000; Moore et al., 2014; Stanek et al., 2014). These neurons also exhibit oscillatory activity that is tightly coupled with the lick cycle (Nakamura et al., 1980; Travers et al., 1997b, 2000). Injection of TTX into the gigantocellularis region of the pontine reticular formation blocked consummatory licking in rats (Brozek et al., 1996), and electrical stimulation was
able to elicit tongue movements (Morimoto et al., 1966; Brozek et al., 1996). These results lead to the hypothesis that together, neurons in the hypoglossal nucleus and reticular formation are responsible for generating rhythmic licking.

Because consummatory licking is relatively stereotyped, it is reasonable to think that licking merely reflects the output of a central pattern generator and is therefore invariant (Corbit and Luschei, 1969). However, the pattern and rate of licking can be altered by many variables including taste, viscosity, motivational state, and lick sensor configuration (Cone, 1974; Cone et al., 1975b; Weijnen, 1998). Because of the dramatic effects that internal states (e.g., the level of deprivation) and perceptual feedback can have on determining the pattern of tongue oscillations, higher brain centers must contribute to this behavior.

Although the brainstem circuitry that regulates tongue and jaw movements has been thoroughly investigated, much less is known about how more rostral regions are able to affect consummatory motor output. Cortical control of orofacial movements has been established in adult animals. Ablation of the frontal cortex in rats disrupted stress-induced oral behavior (Shipley et al., 1980; Whishaw and Tompkins, 1988). Neural activity in the frontal cortex reflects licking behavior (Gutierrez et al., 2006; Gutierrez et al., 2010; Komiyama et al., 2010; Li et al., 2015), and stimulation of frontal cortical regions can produce orofacial movements (Magoun et al., 1933; Morimoto and Kawamura, 1973). These regions project directly to deep brain regions, including the hypoglossal nucleus and reticular formation, but they also send projections to the basal ganglia. The functions of these different projections remains unknown, but it is likely that projections to the basal ganglia are important for consummatory licking. Cats and rats that have been decerebrated early in life, and thus lack intact basal ganglia, fail to show appropriate voluntary licking for milk and water even though they are able to orient their
heads and move toward auditory, olfactory, and tactile stimuli. These animals only show reflexive licking and chewing when food or milk are placed into contact with the lips (Bignall and Schramm, 1974; Grill, 1980). Interestingly, voluntary licking behavior is relatively normal in cats that lack only the cerebral cortex (Bjursten et al., 1976), and ablation of the entire frontal cortex fails to affect tongue usage in rats (Castro, 1972). These results suggest that descending cortical pathways can affect orofacial behavior, but they are not necessary for the basic motor output. The basal ganglia, on the other hand, appear to be necessary for consummatory behavior.

1.2 The Basal Ganglia

1.2.1 Anatomical organization of the basal ganglia

The basal ganglia are a set of interconnected subcortical nuclei that are critical for voluntary behavior. The input nucleus, the striatum, receives heavy, roughly topographic cortical input (Alexander et al., 1986; Albin et al., 1989; Alexander and Crutcher, 1990). Cortical pyramidal neurons synapse with spines of the projection neurons of the striatum: the medium spiny neurons. The medium spiny neurons are GABAergic and make up more than 90% of the striatal neurons. They fall into two major categories that can be distinguished based on their gene expression profiles and connectivity.

The striatonigral (or 'Direct') pathway is composed of medium spiny neurons that express dopamine D\textsubscript{1}-like receptors and project directly to the output nuclei of the basal ganglia. The striatopallidal (or 'Indirect') pathway is composed of medium spiny neurons that project to the external segment of the globus pallidus and express dopamine D\textsubscript{2}-like and adenosine A2A receptors (Alexander and Crutcher, 1990; Gerfen et al., 1990; Fuxe et al., 2005). Neurons from both pathways receive neuromodulatory input from
dopamine neurons located in the ventral midbrain (the ventral tegmental area and substantia nigra pars compacta). The direct and indirect pathways are thought to regulate motor output in an opposing manner. It has been argued that activation of direct pathway medium spiny neurons facilitates movement, whereas activation of indirect pathway medium spiny neurons inhibits movement (Kravitz et al., 2010).

The major output nucleus of the basal ganglia in rodents is the substantia nigra pars reticulata (SNR). SNR neurons receive inhibitory afferent projections from striatal projection neurons (direct pathway) as well as from globus pallidus neurons (indirect pathway). They also receive glutamatergic input from the subthalamic nucleus and the thalamus. SNR neurons are GABAergic and project to the thalamus and brainstem and are critical for movement (Faull and Mehler, 1978; Parent and Hazrati, 1995). Of particular interest to the data presented below are the projections from the SNR to the midbrain tectum, specifically, to the superior colliculus (SC). The SNR neurons that project to the SC tend to be restricted to the more deep and lateral regions. That is, neurons located more laterally in the SNR tend to project to the SC, and more superficial and medial neurons target the thalamus (Faull and Mehler, 1978; Redgrave et al., 1992). A more detailed discussion of the function of nigrotectal projections will be included below.

The last major anatomical feature of the basal ganglia that is relevant to the following chapters is that of the dopamine (DA) cell groups located in the ventral midbrain. The two DA groups are those found in the ventral tegmental area (VTA) and those found in the substantia nigra pars compacta (SNC). These represent a roughly contiguous group of cells in which the more medial are classified as belonging to the VTA and the more lateral are classified as belonging to the SNC. In some places along the rostro-caudal axis, the medial lemniscus separates the two groups (Paxinos and
Franklin, 2003). Both SNC and VTA DA neurons receive input from many regions. Generally, the VTA receives input from limbic structures (e.g., limbic cortex, amygdala, hypothalamus), while the SNC tends to receive input from motor related structures (e.g., motor cortices) (Watabe-Uchida et al., 2012). These distinctions, however, are not absolute, and considerable overlap between their afferent connections exists. Recently it was also demonstrated that DA neurons receive input from medium spiny neurons located in striatal patch regions as well. The SNC receives more input from the dorsal striatum (caudate and putamen in primates), while the VTA receives predominant input from the nucleus accumbens (Watabe-Uchida et al., 2012). VTA DA neurons project most prominently to the nucleus accumbens, whereas SNC DA neurons project heavily to the dorsal striatum, though this is only a rough distinction (Beckstead et al., 1979; Simon et al., 1979).

1.2.2 Functions of the basal ganglia

1.2.2.1 Basal ganglia control of movement

The function of the basal ganglia has been hotly debated (Wichmann and DeLong, 1996; Nelson and Kreitzer, 2014; Yin, 2014a). However, there is apparent consensus that the basal ganglia are critical for control of movement. As noted above, the medium spiny neurons of the striatum receive both glutamatergic input from diffuse cortical regions as well as neuromodulatory input from dopamine cells located in the ventral midbrain. Dopamine signaling on medium spiny neurons is thought to underlie synaptic plasticity that accompanies changes in behavior during learning via opposing actions of D_{1}-like and D_{2}-like receptors (Nicola et al., 2000; Shen et al., 2008). These opposing actions are thought to lead to long term potentiation or depression of medium spiny neuron activity, which are thought to underlie learning.
However, the function of dopamine in continuous regulation of motor behavior is less clear than is role in synaptic modification. In patients with Parkinson's disease, in which nigrostriatal dopamine neurons die, voluntary movement is severely impaired: PD patients become unable to produce voluntary movements. Similarly, when striatal dopamine is neurochemically depleted in primates and rodents, movement is disrupted, producing symptoms that include tremors and akinesia (Sabol et al., 1985; Bergman et al., 1990). Conversely agonizing dopamine receptors within the striatum can potentiate locomotion (Essman et al., 1993). As a result of this pattern of results and because dopamine cannot directly activate neurons but instead modulates the existing signals, it is proposed that dopamine acts as a gain function on the descending cortico-striatal signals (Servan-Schreiber et al., 1990).

Given the effects of striatal dopaminergic receptor manipulations on movement, it is unsurprising that striatal projection neurons have been implicated in the control of movement. The direct and indirect pathways are thought to act in opposition to one another (Kravitz et al., 2010). It is thought that activation of the direct, striatonigral, pathway facilitates movement, whereas activation of the indirect, striatopallidal, pathway inhibits movement. While this schematic of basal ganglia function in which the direct pathway acts as a accelerator for movement and the indirect pathway acts as a break provides a convenient functional characterization, the actual functions of these pathways are probably much less dichotomous. For example, recent studies have shown that neurons in both pathways are activated during action initiation (Cui et al., 2013; Isomura et al., 2013), and that activation of both pathways is necessary for movement (Tecuapetla et al., 2014). Together, these results suggest that binary activation of one pathway is insufficient to explain movement. Rather, dynamic, balanced activity between the two likely determines motor output.
The SNR is a major output nucleus of the basal ganglia that is downstream of both the direct and indirect pathways. SNR neurons have been widely thought to act as a gate, facilitating movement when they are inhibited and preventing movement when they are active (Hikosaka and Wurtz, 1983a, b). This has proven a particularly attractive hypothesis in light of the supposed antagonistic nature of the direct and indirect pathways. This 'model' was largely based on electrophysiological recordings from neurons in the SNR and downstream targets as restrained monkeys performed discrete eye movements. Because of the discrete nature of these tasks, it was difficult to understand how SNR neurons contribute to natural, unrestrained, continuous movements. However, accumulating evidence suggests that SNR neurons do not regulate movement in such a binary manner. Selective activation of medium spiny neurons belonging to either the direct or indirect pathways has both inhibitory and excitatory effects on SNR output neurons (Freeze et al., 2013). Furthermore, a recent set of studies have argued that the activity of SNR neurons have a continuous relationship with movement (Fan et al., 2012; Barter et al., 2014; Barter et al., 2015). During free movement, putative GABAergic output neurons recorded from the SNR of mice show consistently opposing activity. During postural disturbances and goal-directed head movements, subsets of SNR neurons exhibit opposing activity patterns that are associated with the specific movement of the animal. These results are inconsistent with the proposed binary model in which nigral neurons are active during rest and quiescent during movement. It has been proposed that the SNR output neurons serve as a position reference signal to direct downstream behavioral control systems rather than a gate that simply permits or withholds movement (Barter et al., 2014; Yin, 2014c).
1.2.2.2 Basal ganglia control of appetitive behavior

The basal ganglia have been strongly implicated in goal-directed behavior in general (Yin et al., 2008). Specifically, much evidence has suggested that properly functioning basal ganglia are necessary for executing food-directed behaviors. Cats with bilateral striatal ablation fail to spontaneously eat or drink for many days after surgery, although their consummatory behavior is largely intact (Villablanca et al., 1976). Mice with selective disruption of glutamatergic signaling in striatal neurons eat ~20% of control mice while exhibiting limited deficits in other voluntary movements (Ohtsuka et al., 2008).

Lesions of the substantia nigra are difficult to interpret because they usually involve destruction of both the SNR output neurons and the ascending SNC dopamine neurons (DeLong and Georgopoulos, 2011). While destruction or inactivation of SNR neurons results in profound motor deficits, the specific effects on appetitive behavior are less clear. However, the role of ascending nigrostriatal dopaminergic neurons has been thoroughly examined.

Feeding was originally thought to be independent of dopamine signaling (Koob et al., 1978; Ikemoto and Panksepp, 1996). However, in human volunteers, pictures of food and food-related stimuli potentiate BOLD signaling in the VTA and related structures (Stoeckel et al., 2008). Systemic injections of low doses of \( d \)-Amphetamine, which acts as a potent dopamine receptor agonist, stimulates feeding but not drinking in rats (Winn et al., 1982).

Recent studies utilizing genetic manipulations and targeted pharmacological approaches have also argued that dopamine is indeed critical for feeding behavior. For example, dopamine deficient mice that have tyrosine hydroxylase—a rate limiting enzyme involved in the production of dopamine—genetically ablated in dopamine neurons, and therefore cannot produce dopamine, are born normal but quickly became aphagic and
adipsic and do not seek out food (Zhou and Palmiter, 1995; Szczypka et al., 1999b). They eventually die of starvation, but with 3,4-dihydroxy-l-phenylalanine (L-DOPA) treatment, which restores dopamine production, they voluntarily eat and can survive (Szczypka et al., 1999b). Furthermore, leptin knockout mice (ob/ob), which are typically hyperphagic and obese, fail to eat when dopamine is concurrently eliminated (Szczypka et al., 2000).

Striatal dopamine controls appetitive behavior. Extracellular dopamine levels in the nucleus accumbens (NAC) and dorsal striatum rise immediately following cues that signal the availability of food rewards (Roitman et al., 2004; Natori et al., 2009) and, in the NAC, peak during instrumental responding for sucrose (Roitman et al., 2004). Extracellular dopamine levels in the striatum also reflect caloric intake, and pharmacologically blocking dopamine signaling disrupts the ability of mice to maintain constant caloric intake (Ferreira et al., 2012). Viral-mediated restoration of dopamine production in the entire striatum is sufficient to rescue food seeking and consumption, but not all movement dysfunction, in dopamine deficient mice (Szczypka et al., 1999a). Furthermore, disruption of dopamine signaling in the dorsal striatum inhibits feeding in wild-type mice (Sotak et al., 2005), and dopamine depletion with 6-Hydroxydopamine (6-OHDA) in the ventrolateral striatum causes hypophagia and reduces appetite (Jicha and Salamone, 1991; Salamone et al., 1993). Conversely, amphetamine injection into the ventral striatum potentiates feeding in rats (Winn et al., 1982; Kelley et al., 1989). Additionally, viral-mediated rescue of dopamine signaling in the central portion of the dorsal striatum of dopamine deficient mice is sufficient to restore food seeking and consumption but not all locomotor deficits (Szczypka et al., 2001; Hnasko et al., 2006). This suggests that appetitive approach behavior is dissociable from other forms of locomotion and that dorsal striatal—specifically, in the central and lateral regions—
dopamine is a critical mediator of appetitive behavior that functions independently of general locomotion. Collectively, these studies demonstrate that when dorsal striatal dopamine is reduced, food seeking, approach, and consumption are reduced, whereas when dopamine is increased in this area, these behaviors are potentiated.

Much work has focused on the role of nucleus accumbens dopamine in food-directed behavior. However, a critical functional distinction exists between mesolimbic and nigrostriatal dopamine. While nigrostriatal dopamine is necessary for feeding behavior—contributing to appetite, food seeking, and consumption—it has been argued that nucleus accumbens dopamine is more involved in invigorating goal-directed behavior (Salamone and Correa, 2012).

When accumbens dopamine is depleted with 6-OHDA, general feeding behavior is not impaired (Jicha and Salamone, 1991; Salamone et al., 1993). In addition, local depletions of dopamine in the nucleus accumbens fail to alter rates of lever pressing for food at low ratio schedules in rats. At high ratios (>15), however, rats reduce the rate of lever pressing relative to sham controls (Aberman and Salamone, 1999). The effects of accumbens dopamine depletions are most apparent when the depletion is centered in the NAC core rather than the shell (Sokolowski and Salamone, 1998). When rats with accumbens dopamine depletions are given the choice of responding for a preferred food on a fixed-ratio 5 (FR5) schedule of reinforcement while non-preferred food is freely available, they press the lever fewer times and eat significantly more non-preferred food than controls. This suggests that when accumbens dopamine is reduced, appetite is left intact, but motivation to work for food is disrupted (Salamone et al., 1991; Sokolowski and Salamone, 1998). In agreement with this, genetically hyperdopaminergic dopamine transporter knockdown mice have enhanced motivation for food (Cagniard et al., 2006a; Cagniard et al., 2006b). They show elevated lever pressing on a progressive ratio task
and on a concurrent choice task in which non-preferred food is freely available but the preferred food requires a high rate of responding (Cagniard et al., 2006b).

These data illustrate an important dissociation between mesolimbic and nigrostriatal dopamine in appetitive behavior and provide additional support to the argument that these areas contribute independently to motivated behavior. Taken together, the evidence suggests that nigrostriatal dopamine is required for appetitive aspects of feeding, including exploration, approach, and instrumental responding for food rewards, whereas dopamine in the nucleus accumbens acts to selectively enhance the willingness to work for food. That is, dorsal striatal dopamine determines whether an animal will eat, whereas nucleus accumbens dopamine determines how much effort an animal is willing to expend in order to eat.

While many studies have demonstrated the efficacy of basal ganglia manipulations in changing net food consumption and body weight, relatively few have examined the effects of these manipulations on the specific behavioral repertoires that are necessary for the food consumption to occur. The following section will examine the evidence for a role of basal ganglia pathways in controlling consummatory behaviors.

1.2.2.3 Basal ganglia control of consummatory behavior

Cats and rats that have been decerebrated early in life, and thus lack intact basal ganglia, fail to show appropriate voluntary licking for milk and water even though they are able to orient their heads and move toward auditory, olfactory, and tactile stimuli. These animals only show reflexive licking and chewing when food or milk are placed into contact with the lips (Bignall and Schramm, 1974; Grill, 1980). Thus, the basal ganglia are necessary for voluntary, goal-directed licking.
As discussed above, the medullary circuitry that regulates orofacial behavior is well established (Travers et al., 1997b). However, the ways in which higher level pathways contribute to consummatory feeding behavior is much less clear. Although there have been numerous studies that have examined cortical control of licking and chewing, there is a dearth of information regarding basal ganglia contribution to this behavioral repertoire. The basal ganglia represent an intermediate level—between the cortex and reticular formation—that are certainly able to regulate consummatory behavior. However, the mechanisms and specific pathways that are involved are unclear. The following section will discuss what is known about basal ganglia regulation of consummatory behavior.

The basal ganglia are an anatomically and functionally heterogeneous group of nuclei. To further refine our understanding of the basal ganglia pathways that contribute to consummatory behavior, researchers performed discrete, targeted manipulations of nuclei within the basal ganglia and measured the effects on orofacial behavior.

The contribution of basal ganglia dopamine systems to orofacial behaviors has also been extensively investigated (Gunne et al., 1988; van Zessen et al., 2012; Nieh et al., 2015). Systemic injections of dopamine receptor agonists produce oral stereotypy, which includes vacuous tongue and jaw movements (Redgrave et al., 1980; Arnt et al., 1988). Optogenetic stimulation of the GABAergic projections from the lateral hypothalamic area to the VTA increased oral stereotypy and food consumption in well-fed mice (Nieh et al., 2015). Conversely, optogenetic activation of GABAergic cell bodies within the VTA reduces dopamine release and simultaneously disrupts consummatory licking (van Zessen et al., 2012).

The striatum receives extensive dopaminergic innervation and is thought to be the site of action for dopamine-induced oral behavior. In support of this, excitotoxic
lesions of the dorsal striatum with kainic acid, which spare dopaminergic fibers, causes a brief (2-4 days) reduction in food intake in rats, which subsequently rebounds to control levels (Pettibone et al., 1978; Sanberg and Fibiger, 1979). Interestingly, rats with kainic acid lesions of the striatum spill more food during meals, suggesting that their consummatory behavior may be less precisely patterned (Pettibone et al., 1978). A disruption of the typical consummatory rhythm may account for less efficient eating because the muscles controlling the tongue and jaws are not properly coordinated following such lesions. Local infusion of the GABA antagonist, picrotoxin, into the dorsal striatum causes rhythmic tongue and jaw movements, and the activity of individual striatal neurons is tightly coupled with these movements (Nakamura et al., 1990b). Moreover, injection of d-amphetamine or dopamine into the ventrolateral striatum produces oral stereotypy (Kelley et al., 1988; Dickson et al., 1994), which can be blocked by antagonizing either D1 or D2 receptors (Delfs and Kelley, 1990). Neurons found throughout the striatum (nucleus accumbens and dorsal striatum) exhibit lick-related activity when rats and monkeys drink (Kimura et al., 1990; Krause et al., 2010), and electrical stimulation within the striatum disrupts consummatory licking (Krause et al., 2010). Taken together, these results implicate the striatum—specifically the ventrolateral striatum—in controlling orofacial movements.

A major downstream target of striatal neurons that are thought to control oral behavior is the SNR. SNR neurons fire bursts of action potentials during orofacial behavior associated with syntactic grooming sequences in rats (Meyer-Luehmann et al., 2002) as well as drinking (Joseph et al., 1985). When the GABA receptor agonist, muscimol, or GABA are injected directly into the SNR, effectively shutting down the firing of SNR output neurons (Westby et al., 1994), voracious licking is observed (Taha et al., 1982). Furthermore, inactivation of the SNR with lidocaine significantly reduces oral
stereotypies induced by injections of amphetamine into the ventrolateral striatum (Canales et al., 2000).

SNR output neurons send efferent projections to many targets, including the thalamus, reticular formation, and midbrain tectum. One pathway that has been specifically implicated in regulating orofacial behavior is the nigrotectal pathway. Electrophysiological (Chevalier et al., 1981; Hikosaka and Wurtz, 1983b; Westby et al., 1994) and anatomical tracing (Graybiel, 1978; Redgrave et al., 1992) studies have confirmed that the SNR sends projections to the deep layers of the lateral region of the superior colliculus. The GABAergic output neurons of the SNR are known to tonically inhibit their downstream targets within the superior colliculus, and release of this inhibition has been argued to be required for licking. It has been proposed that projections from the lateral SNR to the lateral regions of the superior colliculus are critical for the control of orofacial movements, specifically licking (Wang and Redgrave, 1997). Compulsive licking can be induced by systemic injection of the non-selective dopamine agonist, apomorphine. This aberrant licking can be disrupted by permanent lesions or temporary inactivation with muscimol in the lateral superior colliculus (Taha et al., 1982; Wang and Redgrave, 1997). Thus, there is strong evidence to suggest that the projections from SNR to superior colliculus are critical for regulating voluntary licking.

The results of decades of research into basal ganglia regulation of orofacial behavior have implicated a relatively specific pathway in controlling consummatory licking. Namely, the ventrolateral striatum receives descending cortical input that is modulated by nigrostriatal dopamine. These neurons, in turn, project to the lateral SNR, which somehow orchestrates orofacial movements via projections to the lateral superior colliculus. Although there is general support for nigrotectal regulation of consummatory behavior, the details are still largely unclear.
Many of the investigations of the function of this pathway have used extremely
course methods to manipulate the circuit. Lesions of the superior colliculus have been
performed using aspiration, which is problematic because it is nonselective and causes
massive damage to the superficial tectum and likely disrupts other behaviors in addition
to licking (i.e., perceptual signals). Muscimol inactivation is slightly better in its
specificity, but disrupts inputs from all afferents innervating the site of injection and is
therefore not specific to the nigrocollicular pathway. Furthermore, previous studies have
failed to measure the effects of collicular manipulations on voluntary licking. Rather,
they have utilized methods that induce involuntary licking, which is often considered
‘vacuous’ and void of goal-directedness. It is unknown whether vacuous licking relies on
the same neural systems as goal-directed, voluntary licking. Finally, electrophysiological
evidence of lick-related activity in this pathway is scant, leaving many questions about
the nature of nigrocollicular control of orofacial behavior unanswered. Because of this, it
remains unclear whether the SNR projections to the lateral regions of the superior
colliculus are critical for the regulation of goal-directed licking and if so, how the activity
of these neurons contributes to behavior. Fortunately, recent technological advances
have provided the tools necessary to directly address this question.
1.3 The Superior Colliculus

1.3.1 Anatomical organization of the superior colliculus

The superior colliculus is located in the midbrain tectum and is thought to be critical for many sensory-guided functions. The SC is a striated region made up of at least seven layers. These layers are further grouped into two basic functional units (though there is probably considerable heterogeneity within the groups): 1) the superficial layers and 2) the deep layers (Wurtz and Albano, 1980). The superficial layers are somatotopically organized and receive sensory input from the retina, head, limbs, and trunk (Drager and Hubel, 1976; Stein et al., 1976; Sparks and Nelson, 1987). Neurons in the superficial layers project to neurons within the deep layers, and there is a rough conservation of the somatotopic organization within the superficial projections to the deep layers. Deep SC neurons receive converging input from superficial SC as well as cortical and subcortical sources and are thought to integrate sensory and motor signals to direct behavior. Deep SC neurons project to a variety of structures, but notably for this discussion, they have reciprocal connections with the SNR and reticular formation (Comoli et al., 2003).

1.3.2 Functions of the superior colliculus

The SC has been extensively studied in the context of visually-guided behavior (i.e., eye and head movements). Generally, the superficial SC is thought to be involved in sensory processing, as these neurons receive input from primary (i.e., retina) and secondary (i.e., visual cortex) sensory regions. Neurons located here tend to respond to salient sensory stimuli and are tuned to detect specific aspects of these stimuli. Microstimulation of the superficial SC produces little effect on movement. However, as stimulating electrodes move down through the SC from superficial to deep layers, the
effect of stimulation on movement (most notably, eye movements) becomes greater (Wurtz and Albano, 1980). Neurons in the deep layers of the SC show tuning properties that are related to the direction of saccades in the dark, and stimulation of these neurons can elicit eye and head movement (Sparks and Nelson, 1987). The tuning properties of neurons recorded from the deep SC as well as eye movements generated by stimulation of this region tend to follow a roughly retinotopic map. For example, neurons within a given region of the visuo-motor SC may burst just before a saccade to a particular area of the visual field, and stimulation of this same region will produce a saccade to that same area.

Generally, it is thought that the SC regulates foveation, or adjusting behavior such that a perceptual signal falls upon the fovea of the eye. This explains why researchers have observed the saccade-related activity of these neurons during eye movements as well as the finding that microstimulation generates saccades. In agreement with this hypothesis, the discharge properties of SC neurons are dependent on eye position (Groh and Sparks, 1996), and the properties of saccades generated by electrical stimulation of the SC are determined in part by the eye position at the time of the stimulation (Groh, 2011). In rodents, which rely less on vision to guide behavior, the SC has also been shown to contribute to head and whisker centered orienting in response to visual, auditory, or tactile stimulation (Dean et al., 1989). These seemingly disparate functions may all be the result of a system that is designed to perform a sort of orienting of the organism. In the context of visually guided eye movements, this takes the form of foveation, as demonstrated by decades of work with primates (Wurtz and Albano, 1980). However, when other sensory modalities are isolated, the orienting may result in bringing a different perceptual signal to a desired point relative to the head,
ears, or whiskers. As will be discussed below, this framework may be important for understanding the contribution of the nigrotectal projections to orofacial behavior.

1.4 Disorders of the Basal Ganglia

Because the current understanding of basal ganglia function is still quite limited, important insights into the role that these circuits play in controlling behavior can be gained from human patients with basal ganglia pathologies.

1.4.1 Basal ganglia disorders and movement

Basal ganglia disorders often result in severe motor impairment. This motor impairment falls along a spectrum ranging from hypokinetic, in which patients cannot move as much as they would like to, to hyperkinetic, in which patients move too much (DeLong, 1990). The classic example of a hypokinetic disorder is Parkinson's disease (PD). In these patients, the dopaminergic neurons of the substantia nigra die, resulting in significantly reduced dopamine release in the dorsal striatum. Common behavioral consequences of the death of nigral dopamine neurons in PD are resting tremors, bradykinesia, and impaired posture.

Conversely, Huntington's disease (HD) is a hyperkinetic disorder. The chief symptom is chorea, or involuntary movements. The motor symptoms of HD are thought to be driven in part by death of striatal medium spiny neurons which is caused by buildup of Huntingtin protein and subsequent neurotoxicity. Numerous observations in humans and animal models have established that very large manipulations of the basal ganglia, such as those seen in PD and HD patients, are sufficient to generate profound
impairments in movement. However, since the pathologies that result in wholesale changes in motor control tend to be very wide-reaching it can be difficult to dissociate specific motivational symptoms from more motor-related symptoms.

1.4.2 Basal ganglia disorders and motivation

One outcome that is common in patients with focal lesions within the basal ganglia (often the caudate nucleus, which is the DMS homologue) is apathy, specifically athymia and abulia (Bhatia and Marsden, 1994; Levy and Dubois, 2006). Apathy is characterized by a general lack of motivation and a reduction in voluntary, goal-directed behavior. Apathetic patients often fail to initiate actions, including speaking and eating. This is the most common symptom of patients with damage that is restricted to the caudate nucleus (Caplan et al., 1990), and it is thought to be distinct from general motor deficits.

As further support for a role of the caudate in motivation, anorexic patients have severely reduced glucose metabolism (as measured by positron emission tomography) in the caudate (Herholz et al., 1987). Conversely, obese individuals show enhanced activation of the caudate when viewing high-calorie food stimuli compared with controls (Rothemund et al., 2007).

PD patients often show weight loss and reduced appetite. These patients are generally thought to have supersensitivity to dopamine receptor stimulation. When PD patients are treated with L-DOPA, it is common for them to begin compulsively over eating (Voon et al., 2009). Together, these results support the findings from animal studies that motivation for food can be dissociated from general motor deficits.
Furthermore, they suggest a specific role of the basal ganglia in controlling motivated behavior.

### 1.4.3 Basal ganglia disorders and orofacial control

Patients with pathologies of the basal ganglia frequently present with impaired orofacial control. As a byproduct of dopamine receptor supersensitivity, chronic treatment with dopaminergic drugs such as L-DOPA can cause hyperkinetic symptoms in PD patients. A common hyperkinetic outcome of chronic L-DOPA treatment is tardive dyskinesia, which is characterized by uncontrolled movement of the tongue and jaws (Gerlach et al., 1974).

Coordination between multiple consummatory motor systems is critical for survival. The activation of muscles of mastication must be coordinated with activation of muscles responsible for breathing and swallowing (Kleinfeld et al., 2014). PD patients frequently have deficits that suggest dysregulation of these systems. Specifically, PD patients often develop aspiration pneumonia, which is caused by inhaling food or saliva and can lead to death. These patients cannot optimally coordinate their chewing, breathing, and swallowing and have high prevalence of aspiration during feeding (Gross et al., 2008).

Speech deficits also occur as the result of basal ganglia pathologies. Proper control of the muscles of the tongue and jaws is necessary for articulation during speaking. PD patients often have disrupted speech—both on and off medication—because they have limited control of the tongue and jaws (Ackermann and Ziegler, 1991). Moreover, the inability to initiate speaking or slowness of speech are common outcomes of focal basal ganglia lesions and are particularly common when lesions are
restricted to the lentiform nucleus (composed of the putamen and globus pallidus) (Bhatia and Marsden, 1994).

Together, these clinical observations suggest that descending basal ganglia pathways contribute to oral behavior in many species, including humans. While much of the clinical data have emphasized the role of dopamine and its direct downstream targets in the striatum in regulating appetitive and consummatory behavior, the outputs of the basal ganglia are likely involved in these processes as well.

1.5 Dopamine transporter knockout mice as a model of hyperdopaminergia

A genetic model that has proven particularly useful in the study of dopamine function is the dopamine transporter (DAT) knockout mouse (DAT\(^{-/-}\)). These mice were produced through homologous recombination and have impaired dopamine uptake, which results in persistently elevated synaptic dopamine levels (Giros et al., 1996). This chronic hyperdopaminergic tone provides a convenient way to test dopamine's function in behavior. DAT\(^{-/-}\) mice exhibit a variety of behavioral deficits (Rodriguiz et al., 2004; Costa et al., 2007), and are often used as a mouse model of attention deficit hyperactivity disorder (ADHD) (Cook et al., 1995; Giros et al., 1996; Gill et al., 1997) or schizophrenia (Wong et al., 2012). These mice are generally hyperactive, supersensitive to novelty, and have impaired social investigation (Rodriguiz et al., 2004).

However, whether DAT\(^{-/-}\) mice have motivational deficits is less clear. DAT\(^{-/-}\) mice have enhanced preference for sucrose during free consumption (Costa et al., 2007). In a forced swim test, DAT\(^{-/-}\) mice spent no time immobile (Spielewoy et al., 2000), which is suggestive of enhanced motivation but could alternatively be caused by a potentiated locomotor response to the novel environment. Furthermore, it has been
argued that DAT\textsuperscript{-/-} mice are less sensitive to extinction following extensive training on a task in which they have to nose poke for food (Hironaka et al., 2004). Together, these studies suggest that DAT\textsuperscript{-/-} mice may have alterations of motivated behavior, but a thorough analysis of appetitive and consummatory behavior has not been performed.

1.6 Summary and Rationale

For decades, the basal ganglia have been thought to contribute to feeding behavior. Much attention has been given to striatal dopamine and its function in appetitive processes; however, much of the evidence supporting (or refuting) this hypothesis is clouded by ineffective behavioral measures and coarse manipulations of brain circuitry. By comparison, even less attention has been given to nuclei located downstream of the striatum, such as the substantia nigra pars reticulata. This nucleus is part of a descending pathway from the basal ganglia that is critical for voluntary appetitive and consummatory behavior. The signals that these pathways convey to downstream targets and the way in which they orchestrate feeding behavior is unknown. The following chapters will attempt to shed light on these problems.
2. Bidirectional modulation of substantia nigra activity by motivational state

2.1 Introduction

Motivational state is a key determinant of behavior. Food-seeking behavior, for example, persists until the animal is sated. A variety of satiety signals have been studied in the brain, especially in the hypothalamus (de Araujo et al., 2006). There is also an extensive literature on the neural basis of reward seeking behavior (Kelley et al., 2005), implicating the basal ganglia and ascending dopaminergic projections from the midbrain. But it is not clear whether, and how, satiety signals from homeostatic feeding control circuits can influence basal ganglia circuits mediating goal-directed behavior (Kelley et al., 2005).

One possibility is that satiety signals can directly alter the activity of midbrain dopamine (DA) neurons (Palmiter, 2007; Coizet et al., 2010). As a major neuromodulator, DA regulates synaptic transmission and plasticity in the basal ganglia (Surmeier et al., 2007; Zhou et al., 2009). It has been implicated in action selection (Graybiel, 2008; Kravitz et al., 2010), attention (Bromberg-Martin et al., 2010), learning (Morris et al., 2004; Everitt and Robbins, 2005; Cohen et al., 2012; Lee et al., 2012), and motivation (Satoh et al., 2003; Berridge, 2007; Bunzeck et al., 2009; Krashes et al., 2009; Matsumoto and Hikosaka, 2009). Phasic activity of DA neurons was reported to follow the presentation of a food reward or a stimulus that predicts the reward (Mirenowicz and Schultz, 1996; Flagel et al., 2011). Specific depletion of DA in the dorsal striatum, a target of the nigrostriatal DA pathway, can also disrupt the motivational control of feeding behavior (Palmiter, 2007).

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DA neurons of the SN pars compacta (SNC) are highly connected with the SN pars reticulata (SNR), a major output nucleus of the basal ganglia (Lee and Tepper, 2009). SNR outputs can inhibit DA neurons, while the release of DA can also alter the firing patterns of SNR GABA neurons (Zhou et al., 2009). The SNR contains GABAergic neurons that project to motor initiation regions in the brainstem and the thalamus. It appears to play a major role in selecting the appropriate behaviors based on the current motivational state of the animal (Yin et al., 2008; Fan et al., 2012). But the functional relationship between DA and GABA neurons in the substantia nigra is poorly understood.

To study the effects of motivational state on the SNR GABAergic output of the basal ganglia and the SNC dopaminergic input to the basal ganglia, we recorded from both regions simultaneously using multi-electrode arrays as hungry mice became sated on a fixed time reward schedule. In this task, a cued food reward is delivered once a minute, resulting in progressive and controlled satiation of the animal and reduced reward seeking behavior. We hypothesized that shifts in motivational states can also alter the output of the basal ganglia by dynamically shaping the activity of the substantia nigra neurons. We found populations of DA and GABA neurons whose activity was bidirectionally modulated by the motivational state of the animal.

2.2 Materials and Methods

2.2.1 Subjects

Male C57BL/6J mice (Jackson Laboratories) aged 2-4 months ($n = 7$) were used. During testing, food was restricted to maintain the mice at approximately 85% of free-feeding body weight. Water was available at all times in the home cages. All
experiments were conducted during the dark phase of the animal's light cycle, in accordance with the Duke University Institutional Animal Care and Use Committee guidelines.

2.2.2 Surgery

Mice were anesthetized with isoflurane (induction at 3%, maintained at 1%) and head fixed on a stereotax (Kopf). A craniotomy (~1 mm by 2 mm) above the site targeting the right SN was created. Dura was removed, and the microarray was slowly lowered into place, targeting the final coordinates (in mm relative to Bregma): AP -3.2, ML +1.2, DV -4.6. Microarrays (Innovative Neurophysiology) were 2x8 arrays of 7 mm-long, 50 µm-diameter tungsten recording electrodes (5 mm reference electrode) with 150 µm electrode spacing and 200 µm row spacing attached to an Omnetics connector. Microarrays were grounded to screws placed in the skull using a 0.008" silver ground wire and then fixed in place with dental acrylic. Mice were allowed to recover for at least one week following surgery before the start of recording.

2.2.3 Histology

Following the completion of all behavioral experiments, mice were deeply anesthetized with isoflurane and transcardially perfused with 0.9% saline followed by 10% buffered formalin solution. Brains were sliced into 80 µm coronal sections with a Vibratome 1000 Plus, stained with thionin, and examined with a light microscope to verify placement of the electrode tips within SN (Paxinos and Franklin, 2003) (Figure 1a).
2.2.4 Behavior

Behavioral testing took place in a Med Associates operant chamber (35 cm x 28 cm x 22 cm) designed for in vivo electrophysiology and housed inside light-resistant and sound-attenuating walls as described previously (Rossi et al., 2012; Rossi and Yin, 2012). The walls and floor grid were made of Plexiglas with a food cup in the center of one wall, into which 14 mg food pellets (Bio-Serv rodent purified diet) were dispensed. On the wall opposite the food cup was a 3 W, 24 V house light. Before testing each day, a 16-channel head stage (Blackrock Microsystems) was connected to the array, and a single cable extended from the head stage to a motorized commutator (Dragonfly R&D Inc) that allowed the mouse to rotate freely. At the start of fixed time 60s (FT60; n = 7 mice; mean 13.9 ± 3.15 s.e.m. sessions per mouse) testing, the house light was illuminated, and one pellet was delivered into the food cup every 60s for two hours. The pellet dispenser made a distinct and audible sound ~550 ms before the pellet was available to the mouse, serving as a cue predicting reward. After 120 pellets had been delivered, the house light was turned off, and the session ended. During testing, entries into the food cup were recorded using an infrared photo beam located just inside the entrance to the food cup. Each time the beam was broken, one entry was recorded using computers with the Med-PC-IV program (Med Associates). During extinction testing (2 mice, 4 experiments), the procedure was the same except the pellets were not dispensed into the food cup, but the dispenser still sounded. During pre-feeding sessions (4 mice, 9 experiments), mice were given free access to food pellets for a minimum of 1 hour or until they consumed at least 0.75g.
2.2.5 Data Collection and Analysis

Single unit activity was recorded using the Cerebus data acquisition system (Blackrock Microsystems) (Yu et al., 2012). Neurons were selected based on activity observed prior to the start of testing on each day. Data were filtered with analog and digital band-pass filters (analog high-pass 1\textsuperscript{st} order Butterworth filter at 0.3 Hz, analog low-pass 3\textsuperscript{rd} order Butterworth filter at 7.5 kHz). Single unit data were separated with a high-pass digital filter (4\textsuperscript{th} order Butterworth filter at 250 Hz) and sampled at 30kHz. Single units were processed using online sorting algorithms and then re-sorted offline (Offline Sorter, Plexon). Only single-unit activity with clear separation from background noise was used for analyses (Yin et al., 2009; Fan et al., 2011). Single units were analyzed using NeuroExplorer (Nex Technologies) and custom software (Matlab).

2.2.6 Cell Type Classification

Neurons were classified as GABAergic or dopaminergic based on spike duration. Spike duration was measured using the full width half-max value, or the width of the valley at half of the maximum depth (Figures 1d-e) (Fan et al., 2012). These sorting parameters were then tested using injections of the dopamine D2 receptor agonist, quinpirole. Quinpirole (1mg/kg; Sigma) was injected i.p. (4 experiments in 3 mice) while neural activity was being recorded. Neurons that were classified as dopaminergic based on the duration of their waveforms were confirmed to be dopaminergic when the rate of firing was reduced by quinpirole. Neurons classified as GABAergic did not reduce their firing rate following quinpirole injection (Figure 2).
Figure 1. Single unit recording in substantia nigra. (a) Representative photomicrograph of electrode tracks in the pars compacta (SNC; arrow; left). Schematic representation of the electrode placement (right; SNR, pars reticulata). Coordinates are relative to Bregma. Sample traces of DA (b) and GABA (c) neurons showing the narrower spike waveform of GABA neurons. Insets are inter-spike interval histograms. (d) Average waveforms (± s.e.m.) for all recorded DA and GABA neurons. We classified the neurons based on the waveforms of their action potentials. DA neurons, for example, are characterized by longer spike durations than GABA neurons. n is indicated by the numbers. (e) Full width half max (FWHM) values of classified DA and GABA neurons. (f) Schematic illustration of the FT60 task. One pellet was delivered into the food cup every 60 s (vertical black lines). An auditory cue preceded the pellet delivery by ~550 ms (red lines). Vertical scale bars represent 68 µV (b) and 23 µV (c), and horizontal scale bars represent 500 µm in a, and 200 µs in b and c (10 ms in insets).
2.2.7 Population JPSTH Analysis

Only neurons recorded simultaneously and on different electrodes were included in the analyses to avoid shadowing effects (Bar-Gad et al., 2001). To obtain population joint peri-stimulus time histograms (pJPSTH), we first calculated the raw JPSTH by plotting the peri-reward spikes of each pair of neurons (raw JPSTH; one on the vertical axis and one on the horizontal axis). The predictor was defined as the cross product of the PSTHs of each pair of neurons. Both the raw JPSTHs and predictors were calculated with sliding bins of 100 ms. The predictor matrix was then subtracted from the raw JPSTH of each pair of neurons bin by bin to control for correlations between spike trains due to co-variation in spike rates, generating the 'corrected' JPSTH (Aertsen et al., 1989; Joshua et al., 2009). Each bin of the corrected JPSTH was then normalized by the standard deviation of the trial to trial response (SD of predictor matrix) (Joshua et al., 2009). The pJPSTH was obtained by averaging the normalized JPSTHs. Diagonal mean and s.e.m. values were obtained from the corrected but non-normalized pJPSTHs using a width of 500 ms.

2.2.8 Video Tracking

Videos were recorded at 30 frames per second and analyzed offline. Using custom software (Matlab), the mouse’s horizontal and vertical position within each frame were tracked. The position data were then smoothed using a moving average filter (boxcar average of 30ms). Mouse speed was calculated as the derivative of the position data. This was low-pass filtered at 50 pixels/frame, and missing points were interpolated to remove any artifacts.
2.2.9 Statistical Analysis

All recorded neurons were tested for significant modulation following the onset of the reward cue. Paired $t$-tests compared the firing rate during the 500 ms just preceding the reward cue to the 500 ms immediately following reward cue (phasic window) on each trial. Neurons whose firing rate following the reward cue was significantly greater than the firing rate during the 500 ms preceding the cue ($p < 0.05$) were classified as showing a phasic response. Modulation was determined using the number of spikes in the phasic window from each trial pooled into 10-trial blocks. A one-way ANOVA was performed on the twelve 10-trial blocks using the number of spikes in each trial as independent samples. Neurons were classified as modulated if there was a significant effect of block ($p < 0.05$). Significantly modulated neurons were then tested to assess directionality of the change by comparing the average values of the first and last blocks. 'Increasing' neurons were those modulated neurons in which the average response from the first block of 10 trials was lower than the response of the last block. 'Decreasing' neurons showed the opposite pattern.

2.3 Results

2.3.1 Classification of DA and GABA Neurons

Using 16-electrode arrays, we recorded from DA ($n = 264$) and GABA ($n = 761$) neurons in the SN from 7 male mice. GABA neurons can be distinguished from DA neurons on account of their narrower spike waveforms and higher tonic rate of firing (Figure 1, DA $4.2 \pm 0.29$ Hz, GABA $8.1 \pm 0.33$ Hz, mean ± s.e.m.). The classification of DA and GABA neurons was based on visual inspection of their waveforms, especially the spike duration. To confirm classification, we also used a pharmacological
manipulation. We injected mice with quinpirole, which activates D2-like DA autoreceptors on the presynaptic terminals of DA neurons and presumably reduces the firing rate of DA but not GABA neurons (Schultz, 1986; Jin and Costa, 2010) (Figure 2; 4 tests in 3 mice; 18 GABA, 13 DA neurons). In all tested cases, the firing rates of neurons that were classified as DA were reduced by the quinpirole injection ($t_{(12)} = 4.98$, $p < 0.001$), whereas neurons classified as GABA showed no significant reduction (GABA: $t_{(17)} = 1.68$, $p > 0.05$).

2.3.2 Behavior

Food deprived mice (maintained at ~85% of free feeding body weight) were trained under a fixed time schedule of reward. Under this schedule, a 14 mg food pellet (Bio Serv, rodent purified diet) was delivered into a food cup every 60s (FT60) for two hours (Figure 1f). The distinct sound of the pellet dispenser predicted the presence of the pellet in the cup. The mice learned to enter the food cup as soon as the sound was detected. The session length and inter-reward interval were determined by previous work, which showed that the mice could finish all the pellets (~1.68 g total) in the allotted time. After each session, we confirmed that each mouse was able to finish the pellets.
Figure 2. Effects of quinpirole on dopaminergic and GABAergic neurons. To confirm the classification of cell types, we performed additional pharmacological experiments. Quinpirole (1mg/kg), a D2 receptor agonist, was injected intraperitoneally during a recording session (red arrows). Example responses of DA (a-c) and GABA (d-f) neurons to quinpirole treatment are shown. Immediately following injection, the rate of dopaminergic firing decreased (b), whereas GABAergic neurons were unaffected (e). (c, f) Inter-spike interval histograms for the neurons in a and d. In a and d, vertical scale bars represent 76 µV and 137 µV, respectively. Horizontal scale bars represent 200µs. Scale bars in c and f represent 25 ms.
2.3.3 Phasic Activity and Synchrony Following Reward Cue

The delivery of the pellet elicited a phasic response in many neurons we recorded (Figure 3). Approximately 63% (128/202) of DA neurons and 45% (272/605) of GABA neurons recorded during FT60 sessions showed a significant increase in firing rate immediately following the reward cue (paired t-test, \( p < 0.05 \)). Some neurons showed significant inhibition immediately following the cue (10 DA, 35 GABA; \( p < 0.05 \)). Only those neurons showing significant excitation following the reward cue were included in further analyses.

Figure 3. Dopaminergic and GABAergic neurons showed a phasic response to reward cue. (a) Activity of neurons that increased following reward cue normalized to the maximum firing rate; sorted according to increasing cumulative maximum values. Each row represents one neuron. X axis is time from reward cue onset (s). \( n \) is indicated in parentheses. (b) Representative peri-event histograms and rasters for individual DA (top) and GABA (bottom) neurons showing phasic responses following the reward cue (10 ms bins).
Figure 4. Synchrony is enhanced immediately after the reward cue. Examples of synchrony between pairs of SN neurons following the reward cue. (a) Example of a corrected joint peri-stimulus time histograms (JPSTH) in the left column, corresponding predictor matrix (middle) and histogram of JPSTH diagonal (right; 500 ms width) for a pair of simultaneously recorded DA neurons (a), GABA neurons (b), and DA-GABA pairs (c); DA activity is plotted on the horizontal axis. Axes are time relative to the reward cue. The predictor matrix is subtracted bin-by-bin from the raw JPSTH, and then normalized by the standard deviation of the predictor matrix (100 ms x 100 ms bins).
We also found synchrony in simultaneously recorded nigral neurons following the reward cue (Figures 4, 5). We used joint peri-stimulus time histograms (JPSTH) to assess how synchrony between pairs of neurons changes following the cue (Joshua et al., 2009). We first calculated individual JPSTH for simultaneously recorded pairs of neurons that showed a phasic burst following the reward cue (Figure 4) (Bar-Gad et al., 2001). Each predictor matrix (Figure 4 middle column) was subtracted from the raw JPSTHs to account for synchrony that may occur due to co-variation in spike trains.

**Figure 5.** Population JPSTH analysis. (a) Normalized population JPSTH (pJPSTH) of all DA-DA pairs ($n = 185$ pairs). Synchrony was enhanced immediately after the onset of the cue. (b) Synchrony among GABA-GABA pairs ($n = 475$ pairs) was not affected by the reward cue. (c) Synchrony between DA and GABA neurons ($n = 394$ pairs) was enhanced following the reward cue. pJPSTH bins are 100 ms x 100 ms. Values are normalized by the standard deviation of predictor matrices. (d) The diagonal of each corrected pJPSTH is plotted (width = 500 ms, bins = 100 ms). DA-DA and DA-GABA pairs showed enhanced synchrony following the cue, whereas cue-elicited synchrony was not observed among GABA-GABA pairs. Only those neurons showing significant excitation following the reward cue were included in the analysis.
This yielded an estimate of cue-induced synchrony for pairs of neurons (Figure 4 right column). Individual JPSTHs were then pooled to produce a population JPSTH (pJPSTH; Figure 5). We found that simultaneously recorded pairs of DA neurons showed enhanced synchrony following the reward cue (Figures 4a, 5a). In contrast, although pairs of GABA neurons showed strong synchrony, this synchrony was not affected by the reward cue (Figures 4b, 5b). Synchrony between DA and GABA neurons was also enhanced after the cue (Figures 4c, 5c-d; two-way ANOVA, Cell type × Time, Interaction: $F_{(58,31530)} = 0.92, p = 0.65$; Cell type: $F_{(2,31530)} = 456.6, p < 0.0001$; Time: $F_{(29,31530)} = 3.14, p < 0.0001$). Taken together, these results demonstrate a coordinated GABAergic and dopaminergic phasic response to a reward cue.

### 2.3.4 Motivational Modulation of Neural Activity

To assess the role of this coordinated neuronal response to the reward cue and its relationship to food-seeking behavior, we examined changes in neural activity as the animals became sated during the session. In both DA and GABA neuron populations, we identified two distinct types of motivational modulation: neurons that increased phasic activity as the animals became sated ('increasing'; $n = 68$ GABA, $n = 19$ DA) and others that decreased phasic activity as animals became sated ('decreasing'; $n = 138$ GABA, $n = 74$ DA) (Figure 6).
Figure 6. Motivational modulation of phasic responses to reward cue. Motivational modulation of DA and GABA activity in SN. (a) Sample PSTH illustrating the motivational shift in phasic cue response of decreasing (a) and increasing (b) DA neurons separated into four 30 min blocks (bin size = 30 ms). GABA neurons also showed similar decreasing (c) and increasing (d) motivational modulation.
2.3.4.1 Stability of single unit recording during each session

The observed effects could be attributed to lack of stability in the recording. To explicitly rule out this possibility, we examined the waveform characteristics of each modulated neuron during the first and second hours of the recording session (Figure 7). Among the modulated neurons, neither the FWHM (Figure 7a; paired $t$-test; DA: $t_{(116)} = 0.06, p > 0.05$; GABA: $t_{(255)} = 1.76, p > 0.05$) nor the peak of the inter-spike interval histogram (Figure 7b; DA: $t_{(116)} = 0.48, p > 0.05$; GABA: $t_{(255)} = 0.40, p > 0.05$) changed during the recording session. There was no evidence to suggest that changes in recording stability contributed to the modulation observed.

2.3.4.2 The effects of satiety, pre-feeding, and extinction

During the session, all mice became sated and progressively reduced their food cup entries (Figures 8a,c; One-way ANOVA, main effect of time on rate of entry; $F_{(5,72)} = 29.25, p < 0.0001$). Overall the phasic response of both DA and GABA neurons was reduced as mice became sated (Figure 9a).
Figure 7. Modulation is not due to changes in neuron isolation. To test whether changes in neuronal isolation could account for the modulation observed in single unit activity, waveform characteristics were calculated for all modulated neurons for each half of the recording session. Neither FWHM (a) nor the peak of the inter-spike interval (ISI; b) changed during the two-hour recording sessions (paired t-tests, \( p > 0.05 \)). (c) Sample traces from the first and second half of the session for modulated neurons. The range of ISI values shown is 0-30 ms.
Figure 8. Food-seeking behavior gradually decreases with satiety. (a) Following reward delivery, mice entered the food cup to collect the pellet. Representative data from one mouse are shown. (b) The rapid increase in food cup entries after the reward cue was eliminated during extinction and following pre-feeding. Traces are from the same mouse. FT60 data is the average from all four blocks in a. (c) Rate of entries decreased as mice became sated during FT60 sessions. It was also reduced during extinction and following pre-feeding (average of all mice on all testing days; 20 min bins). (d) The latency to enter the food cup following reward delivery increased during extinction and following pre-feeding treatment. (e) Latency to enter the food cup for all mice on all testing days was pooled to yield population averages. (f) The proportion of DA and GABA neurons that exhibited significant inhibitions following the reward cue was unaffected by pre-feeding and extinction. (g) The number of excitatory responses varied as a function of motivational state for both DA and GABA neurons. * represents significant difference at alpha of 0.05.
If the increasing and decreasing neurons (Figure 6) are indeed modulated by motivation, then we would not expect a similar pattern when the motivational state does not change significantly during the session. Pre-feeding the animals prevents significant changes in the motivational state by inducing satiety at the beginning of the recording session. Pre-feeding reduced the rate of food cup entries (Figures 8b,c) and increased the latency to enter the food cup following reward delivery (Figures 8d,e; one-way ANOVA, $F_{(2,354)} = 91.22, p < 0.0001$). Pre-feeding also dramatically altered neural activity in the SN. The proportion of neurons that were inhibited (DA: 6/39; GABA: 2/72) following the reward cue was not affected (Figure 8f; $\chi^2$ FT60 vs pre-feed; DA $\chi = 1.903$, $p > 0.05$, GABA $\chi = 1.47$, $p > 0.05$). The proportion of DA neurons excited by the reward cue was reduced by pre-feeding (Figure 8g; 15/39; $\chi = 5.23$, $p < 0.05$), but the proportion of excited GABA neurons (29/72) was unaffected by pre-feeding ($\chi = 0.99$, $p > 0.05$).

While pre-feeding prevents changes in motivational state by inducing satiety in the mice immediately before the testing session, changes in motivational state can also be prevented using extinction. We recorded neural activity while the cue was still presented but no pellet was delivered into the food cup. The mice therefore remained hungry during the extinction session. Like pre-feeding, extinction had a dramatic effect on the behavior and neural activity. Extinction quickly reduced the rate of food cup entries (Figure 8c) and increased the latency to enter the food cup following reward delivery (Figure 8d,e). Extinction also reduced the magnitude of the phasic response (Figure 9c) and the proportion of DA (6/23) and GABA (15/84) neurons that were excited following the reward cue (Figure 8g; $\chi^2$ FT60 vs pre-feed; DA $\chi = 14.16$, $p < 0.001$, etcetera.
GABA $\chi = 8.70, p < 0.01$), without affecting the proportion of inhibited neurons (Figure 8f; DA (0/23): $\chi = 2.51, p > 0.05$; GABA (6/84): $\chi = 0.21, p > 0.05$).

To determine whether changes in the magnitude of the phasic response of DA and GABA neurons reflected changes in motivational state, we compared the activity in the first half with activity in the second half of the recording session (Figure 9). Because the phasic response was significantly reduced during pre-feeding and extinction sessions (Figures 8g, 10), we also included neurons that did not exhibit significant phasic excitation in the analyses to determine if there was motivational modulation of neuronal activity in the absence of a statistically significant phasic peak. During pre-feeding sessions, increasing DA neurons showed no modulation of activity, but decreasing neurons still showed evidence of modulation (Figure 11b).

Following pre-feeding, motivational modulation was much reduced (Figure 9). During extinction (Figure 9c), the phasic response in both DA and GABA neurons was almost completely abolished. Modulation of the neural activity was absent (Figures 11c, 12c). Thus, changes in the phasic response of both DA and GABA neurons may reflect changes in the motivational state of the animal. When animals are pre-fed to induce satiety before testing, modulation is reduced, but not entirely eliminated. This may be caused by the fact that mice are not completely sated at the start of the recording session. They typically consume some but not all of the pellets that are delivered, which may account for the presence of some modulation during pre-feeding sessions. Although the mice ate all the pellets during FT60 sessions, it is possible that the lack of overall activity towards the end of the session, rather than motivational state per se, may be responsible for the observed effects. That is, the mice were no longer active toward the end of the session, as they became sated.
Figure 9. Average response of all excited neurons, including those that do not show motivational modulation. To compare the basal activity and mean responses between the classes we examined the responses of all neurons that were excited following the cue. The activity (mean ± s.e.m.; 20 ms bins) of all neurons with excitatory responses to the reward cue, regardless of motivational modulation, is plotted for each half of FT60 (a), pre-feeding (b) and extinction (c) sessions. Overall, there was a reduction in the magnitude of the phasic response as mice became sated. During extinction (c) the phasic response is nearly abolished for both DA and GABA neurons. The response to the cue (d) and to reward receipt (e) are dissociable. The population responses are shown for DA (n = 128) and GABA (n = 272) neurons recorded during FT60 sessions. (f-g) Baseline firing rates do not vary between FT60, pre-feed, or extinction sessions for DA or GABA neurons presented in a-c (one-way ANOVAs, F < 1.0, p > 0.05).
Figure 10. Motivational modulation is absent following pre-feeding and during extinction. Example of the cue response of a DA neuron in FT60 (a), pre-feed (b), and extinction (c) sessions. Each line represents the average response from a 30-trial block. 10 ms bins, 100 ms Gaussian smooth. Corresponding waveforms are shown below.
Figure 11. Summary of phasic response in DA neurons. To compare the magnitude of the satiety effect to the magnitude of the mean response, we plotted the responses of all DA neurons showing a cue-elicited phasic burst. (a) The phasic response to the cue of increasing DA neurons \( (n = 19) \) was higher for the second half of FT60 sessions than it was for the first half. Decreasing DA neurons \( (n = 74) \) showed the opposite pattern. The phasic response of unmodulated neurons \( (n = 35) \) did not change over time. Modulation of the phasic response was reduced by pre-feeding (b; \( n = 5 \) increasing, 15 decreasing, 19 unmodulated) and eliminated during extinction (c; \( n = 2 \) increasing, 2 decreasing, 18 unmodulated). The phasic response of increasing neurons was nearly abolished following pre-feeding and during extinction, and the phasic response of decreasing neurons was abolished during extinction.
Figure 12. Summary of phasic response in GABA neurons. GABA neurons show the same pattern of modulation as DA neurons. (a) The phasic response to the reward cue of increasing GABA neurons \((n = 68)\) was higher for the second half of FT60 sessions than it was for the first half. Decreasing GABA neurons \((n = 138)\) showed the opposite pattern. The phasic response of unmodulated neurons \((n = 66)\) did not change. Modulation of the phasic response was reduced by pre-feeding \((b; n = 15 \text{ increasing}, 24 \text{ decreasing}, 33 \text{ unmodulated})\) and eliminated during extinction \((c; n = 7 \text{ increasing}, 4 \text{ decreasing}, 73 \text{ unmodulated})\). The phasic response of increasing neurons was nearly abolished following pre-feeding and during extinction, and the phasic response of decreasing neurons was abolished during extinction.
To examine this possibility, we used automated video tracking of mice to quantify their behavior during the recording session. Mice spent almost all of their time near the food cup (Figure 13a), and their movement velocity did not change significantly during the session, though they entered the food cup less frequently with satiety (Figure 13b).

Figure 13. Mice are active throughout the recording session. To ensure that motivational modulation was not caused by mice falling asleep or losing interest in the pellets, we used video tracking to record the position of mice during recording sessions. Mice spent most of their time near the food cup (a). Speed was calculated (pixels/frame) throughout the session. Mice remained active for the entire two-hour session (b).
Figure 14. Cue-elicited synchrony of neural activity is modulated by motivational state. pJPSTH diagonals (mean ± s.e.m.; 100 ms x 100 ms bins; 500 ms diagonal width) are shown for DA-DA (n = 185 FT60; n = 81 pre-feed; n = 45 extinction), GABA-GABA (n = 475 FT60; n = 346 pre-feed; n = 389 extinction), and DA-GABA (n = 394 FT60; n = 144 pre-feed; n = 138 extinction) pairs recorded during FT60 (a), pre-feeding (b), and extinction (c). The diagonals of the corrected and normalized pJPSTHs are in the top row, while the diagonals of the predictor matrices are in the bottom row. The predictor was defined as the cross-product of the JPSTH for each pair of neurons. Pre-feeding reduced cue-elicited synchrony for DA-DA and DA-GABA pairs while increasing overall synchrony between GABA-GABA pairs (b). Extinction reduced synchrony between DA-GABA and DA-DA pairs without affecting GABA-GABA pairs (c).
2.3.5 Motivational modulation of synchrony

We next examined how cue-evoked synchrony varied as a function of motivational state. We found cue-evoked synchrony between DA-DA and DA-GABA pairs during FT60 sessions (Figure 14a), but both pre-feeding (Figure 14b; two-way ANOVA [Cell Type x Time], Interaction: $F_{(58,17040)} = 0.36, p = 1.00$; Cell Type: $F_{(2,17040)} = 1908, p < 0.0001$; Time: $F_{(29,17040)} = 0.36, p = 1.00$) and extinction (Figure 14c; two-way ANOVA [Cell Type x Time], Interaction: $F_{(58,17070)} = 0.09, p = 1.00$; Cell Type: $F_{(2,17070)} = 346.1, p < 0.0001$; Time: $F_{(29,17070)} = 0.11, p = 1.00$) reduced cue-induced synchrony between DA-DA and DA-GABA pairs. Analyses include all neurons shown in Figures 11-12. Interestingly, while GABA-GABA synchrony showed no change relative to the reward cue, overall synchrony was elevated following pre-feeding treatment. These results suggest that cue-evoked synchrony, in addition to the magnitude of the phasic response to the reward cue, depends on an animal's motivational state.

2.4 Discussion

We recorded from DA and GABA neurons in the substantia nigra as mice became progressively sated over the course of a 2-hr session, using a FT60s reinforcement schedule in which a food pellet was delivered once a minute. We found that phasic activity of both DA and GABA neurons in response to the reward cue (sound of the pellet dispenser) was bidirectionally modulated by motivational state (Figure 6). For both cell types, we identified two distinct populations of neurons based on how motivational state changes their activity: those that increased their phasic response as
the animal became sated, or "increasing neurons", and those that decreased their phasic response, or "decreasing neurons."

The phasic response to the reward cue was largely eliminated when we prevented any changes in motivational state using extinction (Figures 10-12). Extinction prevents satiety, but the lack of reward delivery may result in rapid learning that eliminated the cue-evoked phasic activity as the cue becomes an invalid predictor of reward. Therefore, it is difficult to determine from the extinction tests alone whether the observed modulation is caused by satiety. It is necessary to prevent a change in motivational state without changing the reward contingency. We therefore examined the effects of pre-feeding before the recording session, which should also prevent significant changes in motivational state by rapidly sating the mice. If the animal was already sated before the session, then there should not be significant changes in motivational state within the 2-hr session. We found that pre-feeding reduced the modulation of the cue-evoked response in decreasing DA and GABA neurons, and it also abolished any modulation of the phasic response of increasing neurons (Figures 11-12). Together the data from the extinction and pre-feeding experiments suggest that the changes in DA and GABA activity in the SN may reflect changes in motivational state.

Even though extinction and pre-feeding tests are intended to prevent changes in motivational state (Rossi and Yin, 2011b), they also affect the behavior of the animals. Therefore, we cannot rule out the possibility that the observed modulation during FT60 sessions, the reduced modulation following pre-feeding, and the abolished phasic response during extinction are dependent on the behavior of the mice. Extinction and pre-feeding dramatically changed the mouse's behavior (Figure 8). The reduced food-seeking behavior coincides with the reduction of the phasic response of both DA and GABA neurons to the reward cue. Since the rate of food cup entries was reduced by
both pre-feeding and extinction, it is likely that the related changes in the phasic DA and GABA signals are indeed related to some component of behavior. One possibility is that following pre-feeding or during extinction, mice lost interest in the food pellets, no longer attending to the pellet-predicting cue. The specific relationship between behavior (i.e. locomotion, response vigor, attending to the cue) and neural output remains to be explored.

Interestingly, although the phasic response was abolished by extinction, it was not completely eliminated by pre-feeding. In contrast to the phasic response of decreasing neurons, the phasic response of increasing neurons was much more sensitive to pre-feeding (Figures 11b, 12b). While the phasic response of decreasing DA and GABA neurons was still present following pre-feeding, the phasic response of increasing neurons was completely eliminated. The functional implications of this disparity are unclear, but one possibility is that the output of these two populations of neurons (increasing and decreasing) reflects distinct contributions to behavior. This possibility remains to be tested.

Although the phasic response to the reward cue in DA neurons is similar to what has been reported previously (Schultz, 1998), the observation of very similar phasic activity in GABA neurons in the SNR is novel. Indeed, the spiking of GABA neurons is often synchronized with that of DA neurons following the reward-predicting cue, which can be seen as cue-elicited synchronization among DA neurons and GABA neurons (Figure 5). Thus DA neurons are not the only neurons that show this particular type of cue-elicited phasic activity, and whatever signal this phasic response represents cannot only be conveyed by DA, since GABA neurons in the SNR have a different pattern of anatomical connectivity. As these are putative projection neurons, the observed phasic activity should directly affect downstream structures in the thalamus and brainstem.
It is possible that the synchrony between DA and GABA neurons is driven by common inputs, e.g. from the striatum (Redgrave and Gurney, 2006; Lee and Tepper, 2009; Yin et al., 2009; Coizet et al., 2010). It is also possible that synchrony is a result of electrical coupling. DA neurons, for example, are known to express gap junctions (Vandecasteele et al., 2005). In addition, the dendritic release of DA can also depolarize GABA neurons by activating transient receptor potential (TRP) channels (in particular TRPC) (Zhou et al., 2009), which is another potential mechanism for the observed effects. How satiety signals (hormonal or neural) can influence synaptic transmission and electrical coupling in the substantia nigra remains poorly understood, but our results suggest that they have a potent effect on the output of the basal ganglia as well as dopaminergic signaling.

In addition to the surprising similarity in the event-related firing patterns of DA and GABA neurons, we also observed some significant differences between these cell types. GABA neurons have much higher tonic firing rates than DA neurons, though their phasic response is more similar in magnitude. The baseline firing rate of GABA neurons was also more strongly modulated by motivational state (Figure 12a), possibly because the tonic basal ganglia output more directly reflects the overall activity level of the animal.

Our results confirm and extend previous reports of reward-elicited synchrony in firing among midbrain dopamine neurons (Joshua et al., 2009). The observed synchrony cannot simply be explained by rate modulation: DA and GABA neurons show similar rate modulations following the reward cue, but DA-DA pairs exhibit significant changes in cue-elicited correlations whereas GABA-GABA pairs fail to show enhanced post-cue synchrony. Pre-feeding did not increase the firing rates of GABA neurons
(Figure 12b), but synchrony between GABA-GABA pairs was enhanced (Figure 14b). The observed synchrony can therefore be independent of changes in firing rate.

Our finding that there are two distinct populations of DA neurons is in agreement with recent work (Schultz, 1998; Matsumoto and Hikosaka, 2009; Henny et al., 2012). Hikosaka and colleagues, for example, have shown that the activity of SNC DA neurons can be divided into two major classes: some neurons respond to predictors of reward but others respond to predictors of aversive outcomes (Matsumoto and Hikosaka, 2009). An intriguing question is whether the DA neurons that are responsive to aversive outcomes are the same as those that increase phasic firing as animals become sated.

A prominent hypothesis is that phasic DA activity encodes a reward prediction error (RPE), based on electrophysiological studies in monkeys (Schultz, 1998). According to this hypothesis, phasic activity of DA neurons reflects the reward prediction error and serves as a teaching signal that regulates plasticity in regions receiving dopamine, e.g. striatum (Schultz, 1998; Niv and Schoenbaum, 2008; Glimcher, 2011). Although we replicated previous finding of phasic DA activity in response to the reward cue, our results cannot be explained by the RPE hypothesis.

The phasic DA activity we recorded does not reflect a reward prediction error. In our experiments, the prediction error is always the same because the reward cue always predicts the reward (except for the extinction sessions). Yet, DA activity changes systematically according to shifts in motivational state, with some increasing while others decreasing their response. In other words, there was no significant change in the predictability of the reward, only changes in motivational state.

One possible defense of the RPE hypothesis is that the "effective reinforcement" signal does not necessarily remain the same even if the physical reward remains the same, since the food can become less rewarding and presumably less effective as a
reinforcement signal (Doll et al., 2012). But even a modification of the RPE hypothesis to incorporate reduced subjective reward value cannot explain the present findings. The motivational modulation we observed is bidirectional: with satiety, some DA neurons increase their phasic response while others decrease their phasic response. This observation is incompatible with any version of the RPE hypothesis.

Interestingly, we found that DA neurons are not the only neurons showing cue-evoked phasic activity, as GABA neurons show a similar profile in relation to the reward predicting cue. Our recent work also found a similar pattern with an operant temporal differentiation task (Fan et al., 2012). These GABA neurons are a major source of outputs from the basal ganglia, targeting many brain regions. The phasic response of these neurons is expected to directly contribute to the behavioral output, though in the present study we did not measure the behavioral output during the cue presentation. It would be crucial in future experiments to examine the contribution of such a signal—both from GABA and DA neurons—to behavior, though better and more precise behavioral measures will be required. Previous work on the role of DA has not carefully examined the behavior of the animal, often using very limited measures under highly artificial conditions. Further progress will depend on establishing the link between reward-related behavior, which is clearly modulated by motivational state (Leblond et al., 2011), and the signals generated by the DA neurons and their target regions.

In accord with a growing body of work (Cagniard et al., 2006b; Jin and Costa, 2010; Shiner et al., 2012), DA neurons appear to be more important for the regulation of performance than for learning driven by prediction errors. The activity of both DA and GABA neurons reflects the motivational state of the animal and reward-seeking behavior. Given the widespread targets of SNC DA neurons and SNR GABA neurons, similar motivational modulation should also be found in the diverse brain regions.
receiving their projections, such as the cortex, striatum, brainstem, and thalamus. The obvious question is how nigral DA and GABA neurons receive information on the motivational state of the animal. Based on known anatomical connectivity, these signals can come from brainstem parabrachial nucleus and the hypothalamus (Blevins et al., 2004; Kelley et al., 2005). Hormones implicated in regulating the homeostatic system can also directly affect dopamine neurons; e.g., leptin and insulin can inhibit dopamine neurons, whereas ghrelin can excite them (Palmiter, 2007). More detailed analysis of the anatomical projections and synaptic transmission between these regions is needed to elucidate these functional interactions. While many studies of reward processing have chosen to focus on the dopamine neurons located in the ventral tegmental area (VTA), relatively few have addressed the role of dopamine neurons of the substantia nigra in reward processing (Fan et al., 2012; Rossi et al., 2013b). Metabolic signals are known to interact with dopamine circuits in both the VTA and SN (Palmiter, 2007; Narayanan et al., 2010). As such it is possible that similar motivational modulation of neural activity could be observed in the VTA as well.
3. Striatal region-specific viral rescue restores normal reward seeking, but not instrumental learning and hyperactivity, in dopamine transporter knockout mice

3.1 Introduction

Dopamine (DA) has been implicated in motor control, motivation, attention, and learning (Berridge, 2007; Salamone et al., 2009; Howe et al., 2013). A genetic model that has proved particularly useful in the study of DA function is the dopamine transporter (DAT) knockout mouse (DAT−/−), which shows impaired dopamine uptake and increased synaptic DA level (Giros et al., 1996). DAT−/− mice exhibit a variety of behavioral deficits (Rodriguez et al., 2004; Costa et al., 2007), and are often used as a mouse model of attention deficit hyperactivity disorder (ADHD) (Cook et al., 1995; Giros et al., 1996; Gill et al., 1997).

Because DAT−/− mice lack DAT in the whole brain, how specific behavioral deficits are produced remains unclear. DA neurons from the midbrain project to the entire forebrain, especially the striatum. Due to considerable functional heterogeneity within the striatum, the respective contribution of dopaminergic signaling in specific striatal regions remains unclear (Corbit et al., 2001; Thorn et al., 2010; Yin, 2010; Rossi and Yin, 2011a). Therefore, we used a genetic rescue approach by restoring DAT levels in DAT−/− mice and assessing the consequent impact on behavior.

Because striatal DA is known to regulate motivation for food and feeding behavior (Szczytpka et al., 1999a; Szczytpka et al., 2001; Salamone and Correa, 2012), we tested the effect of increased DA on Pavlovian reward approach behavior (food cup entry to collect food pellets) and instrumental learning (lever pressing for food pellets) on DAT−/− mice. We then used lentiviral-mediated gene delivery to restore DAT in three different striatal regions (dorsomedial striatum (DMS), dorsolateral striatum (DLS), or...
nucleus accumbens (NAC)) in DAT\textsuperscript{+/−} mice, to identify the role of DAT in specific striatal regions.

3.2 Materials and Methods

3.2.1 Subjects

Subjects were C57BL/6J DAT-KO (DAT\textsuperscript{+/−}; \(n = 35\)) mice and their littermates including heterozygous (DAT\textsuperscript{+/−}; \(n = 13\)) and wild-type (DAT\textsuperscript{+/+}; \(n = 34\)) aged 3-10 months (Giros et al., 1996), maintained at 85-90\% of their ad libitum body weight. Water was available at all times in the home cages.

3.2.2 Surgery

Following FT60 training, mice were injected with 3xHA-DAT-TdT targeting either DMS (\(n = 7\)), DLS (\(n = 8\)), or NAC (\(n = 7\)). Viral injections were performed as previously described (Rossi et al., 2013b) targeting the following coordinates (in mm relative to Bregma): DMS (two injection sites per hemisphere): 0 AP, ±1.5 ML, -2.7 DV and +1.1 AP, ±1.3 ML, -2.7 DV; DLS (two injection sites per hemisphere): +0.2 AP, ±2.3 ML, -3.3 DV and +1.1 AP, ±2.2 ML, -2.7 DV; NAC (one injection site per hemisphere): +1.4 AP, ±0.75 ML, -3.9 DV. DV coordinates were measured from brain surface. All injections were 0.5 µl over 5 min. Mice were allowed to recover for one week before behavioral testing began.
3.2.3 Behavior

3.2.3.1 Food seeking

Behavioral testing took place in Med Associates operant chambers as described previously (Rossi and Yin, 2012). A food cup was located in the center of one wall opposite a house light. Entries into the food cup, a type of Pavlovian approach behavior, were detected by a photobeam. During testing, one 14 mg food pellet (Bio Serv) was delivered into the food cup every 60 seconds (FT60) for 60 min. Food seeking was assessed by monitoring the rate of food cup entries. Extinction lasted 60 min during which the light was on, yet no pellets were delivered. 'Sated' sessions were conducted after 24 hours of unrestricted access to home chow.

3.2.3.2 Instrumental learning (lever pressing for food pellets)

Following FT60 testing, mice were trained to press a lever for food pellets for 7 consecutive days. Each session ended after 100 lever presses or 60 min.

3.2.3.3 Locomotor activity

Mice were placed in a circular chamber, and the position of each mouse was tracked at 30 frames/s using a camcorder from above. The custom Matlab software used for data analysis was described previously (Rossi et al., 2013a). Speed was calculated frame-by-frame based on changes in position. Average speed during the first three minutes after mice were placed into the test chamber was used as a measure of locomotor activity for a subset of mice (DAT\(^{+/+}\), \(n = 15\); DAT\(^{+/-}\), \(n = 7\); No rescue, \(n = 5\); DMS, \(n = 3\); DLS, \(n = 5\); NAC, \(n = 6\)).
3.2.4 Cloning of lentiviral vector and lentivirus production

We removed the stop codon from a mammalian expression vector (pcDNA3.1, Invitrogen) containing human DAT cDNA tagged at the N-terminus with three hemagglutinin (3xHA) epitopes (3xHA-DAT) and added 2A peptide sequence and tdTomato coding sequences (Shaner et al., 2004) to generate 3xHA-DAT-TdT. Then we subcloned 3xHA-DAT-TdT into a modified FUGW (Lois et al., 2002) lentiviral vector. Lentiviruses were produced as previously described (Dittgen et al., 2004). Human embryonic kidney 293FT cells (Invitrogen) were transfected using lipofectamine 2000 (Invitrogen) with the expression of two helper, Δ8.9 and vesicular stomatitis virus G-protein, plasmids at 10, 7.5, and 5.5 μg of DNA per 10-cm plate (Naldini et al., 1996). After 72 h, the supernatant of four plates were pooled, spun at 1,000x g for 5 min, filtered at a 0.45 μm pore size, spun at 25,000x g for 4 h, and the pellet was resuspended in 20 μl of PBS.

3.2.5 Western blot

We transfected EK293T cells (2x10^6 per well, 6-well plate) with either 3xHA-DAT or 3xHA-DAT-TdT construct (2 μg per well) using Lipofectamine 2000 (Life Technologies). Whole cell lysates were prepared in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% IGEPAL CA-630, 0.5% Sodium deoxycholate, 0.1% SDS), supplemented with 1% protease inhibitor cocktail (P8340, Sigma). Protein (40 μg) from whole cell lysates was separated on SDS gels and transferred to PVDF membranes. Membranes were incubated in TBST (137 mM NaCl, 20 mM Tris, 0.05% Tween 20), supplemented with 5% non-fat milk for 1hr and probed with mouse monoclonal antibodies against HA (1:2000, Covance). After primary antibody incubation, membranes were washed 3x with TBST for 10 min and incubated with
peroxidase conjugated goat anti-mouse IgG (1:2000, Thermo Scientific) for 1 h. Immunoreactive bands were visualized with chemiluminescent substrate (SuperSignal West Femto Chemiluminescent Substrate, Thermo Scientific).

### 3.2.6 Immunostaining

Dissociated primary cortical neuronal cultures were prepared from E18 rat pups (Kaech and Banker, 2006). 1.5x10⁵ cells per well in a 12-well dish were plated and neurons were transfected after 8 days in vitro with either 3xHA-DAT or 3xHA-DAT-TdT construct (1 μg per well) using Lipofectamine 2000 (Life Technologies). Neurons were fixed 24 h post-transfection for 30 min with 4% PFA in PBS with 5% sucrose. Fixed neurons were permeabilised with 0.1% Triton-X in PBS for 10 min, blocked with 5% normal donkey serum in PBS (blocking buffer) for 1 h, and incubated overnight in mouse monoclonal anti-HA antibody (1:500, Covance). Fixed neurons were washed in PBS (3x) for 10 min, and incubated with Alexa 488 goat anti-mouse IgG (1:500, Life Technologies) for 2 h. Nuclei were labeled with DAPI (1:5000, Life Technologies).

### 3.2.7 Histology

Mice were perfused with 4% PFA. Brains were post-fixed for 24 h then sliced at 60 μm and stained with DAPI. TdTTomato fluorescence was visualized with an Axio Zoom.v16 (Zeiss) microscope.
3.3 Results

3.3.1 Enhanced reward seeking in DAT⁻/⁻ mice

Consistent with previous reports, DAT⁻/⁻ mice weighed less (Figure 15a; ANOVA: $F_{(2,73)}=212.10, p<0.0001$, post hoc tests $ps <0.05$) and were hyperactive relative to both DAT⁺/⁺ and DAT⁺/⁻ mice, showing significantly higher speed in their locomotor activity tested in an open field chamber, whether they were hungry or sated (Figure 15b-c; two-way repeated measures ANOVA [Genotype x Hunger]: effect of Genotype, $F_{(2,43)}=39.94$, $p<0.0001$; effect of Hunger, $F_{(1,43)}=13.26$, $p<0.001$; no Interaction, $F_{(2,43)}=1.74$, $p<0.05$).

Dopamine level has been found to regulate motivation for food (Cagniard et al., 2006b; Costa et al., 2007; Cacciapaglia et al., 2011; Rossi et al., 2013a). We presented hungry mice with one food pellet every 60 seconds in a fixed-time food reward task (FT60). There was no difference in body weight between genotypes when normalized by their baseline weight, suggesting similar levels of food-deprivation (Figure 15e; ANOVA: $F_{(2,73)}=2.02$, $p=0.14$). Intriguingly, DAT⁻/⁻ mice ($n = 34$) exhibited elevated food cup entries relative to both DAT⁺/⁺ ($n = 29$) and DAT⁺/⁻ ($n = 13$) controls (Figure 15d left).
Figure 15. DAT-/- mice are more motivated for food than controls. (a) DAT-/- mice weigh less than DAT+/+ mice. (b) DAT-/- mice were more active than DAT+/+ and DAT+/-- controls in an open field test. Average speed during 3min exposure. (c) Examples of open field tracking. (d) DAT-/- mice show elevated rates of food cup entries during the inter-reward interval on FT60 tests. When sated, DAT-/- mice (n=16) no longer entered the food cup more than DAT+/+ (n=15) and DAT+/-- (n=5) mice. In extinction, when food was no longer delivered, all mice also stopped entering the food cup. Thus, enhanced rates of food cup entries cannot be attributed to general hyperactivity. (e) When normalized to baseline, all groups show similar levels of deprivation. (f) The rate of DAT-/- food cup entries was elevated only when mice were hungry and pellets were being delivered, confirming that entries are food-directed. Values are mean and s.e.m. * p<0.05.
To test whether DAT⁻/⁻ mice entered the food cup more than DAT⁺/+ mice due to increased motivation for food or simply due to general hyperactivity, we tested the mice following 24 h of unrestricted access to food (Figure 15d middle) and during extinction, in which no pellets were delivered (Figure 15d right). Under both conditions, the rate of food cup entries was reduced in all animals, and the group difference in food cup entries was completely abolished (Figure 15f; two-way ANOVA: main effect of Genotype, $F_{(2,112)}$=7.22, $p<0.01$; main effect of Condition, $F_{(2,112)}$=33.66, $p<0.0001$; Interaction, $F_{(4,112)}$=7.66, $p<0.0001$). Post hoc analyses revealed that DAT⁻/⁻ mice entered the food cup more than DAT⁺/+ and DAT⁺/- mice during FT60 only when they were hungry, but not when sated or during extinction ($p<0.001$), indicating that increased food cup entries in DAT⁻/⁻ mice was due to increased motivation for food.

Figure 16. DAT⁻/⁻ mice show impaired instrumental learning. (a) DAT⁻/⁻ mice are severely impaired at learning to lever press for food pellets. (b) Inter-response intervals for the first eight rewards during initial acquisition are shown. After three presses, DAT⁻/⁻ mice show significantly longer inter-response intervals than DAT⁺/+ and DAT⁺/- mice. Values are mean ± s.e.m.
DAT\(^{-/}\) mice also showed profound impairment at learning to press a lever for food relative to both DAT\(^{+/+}\) and DAT\(^{+/}\) mice (Figure 16a; two-way ANOVA [Genotype x Session]: main effect of Genotype, \(F_{(2,282)}=11.44, p<0.0001\); main effect of Session, \(F_{(6,282)}=14.61, p<0.0001\); Interaction, \(F_{(12,282)}=4.00, p<0.0001\)). Post hoc analysis showed that DAT\(^{-/}\) mice pressed at a lower rate compared to both DAT\(^{+/+}\) and DAT\(^{+/}\) mice on sessions 3-7 (\(p<0.01\)). To assess the rate of learning during initial instrumental acquisition, we calculated the inter-response interval (IRT) between rewards (Figure 16b) for the initial lever presses. DAT\(^{-/}\) mice failed to reduce their IRT during the first eight presses compared to both DAT\(^{+/+}\) and DAT\(^{+/}\) mice (two-way ANOVA [Genotype x Press Number]: main effect of Genotype, \(F_{(2,287)}=12.61, p=0.001\); no main effect of Press Number, \(F_{(7,287)}=1.19, p>0.05\); no Interaction, \(F_{(7,287)}=1.82, p>0.05\)). The latency to the first press was no different between DAT\(^{-/}\) and DAT\(^{+/+}\) mice (planned comparison: \(t_{(41)}=0.03, p>0.05\)), but the IRT between presses 4-8 was significantly longer for DAT\(^{-/}\) mice (\(p<0.05\)). These results suggest that DAT\(^{-/}\) mice fail to acquire the instrumental contingency, despite performing the initial exploratory presses.

3.3.2 Striatal region-specific viral rescue

The main target of dopaminergic projections is the striatum, the input nucleus of the basal ganglia. Yet the striatum is a large and functionally heterogeneous region (Yin et al., 2008), and it is not clear which particular striatal region regulates motivation for food in DAT\(^{-/}\) mice. To test whether selective restoration of DAT function in striatal subregions could restore food seeking in DAT\(^{-/}\) mice, we injected 3xHA-DAT-TdT into DMS, DLS, or NAC (Figure 17, 18a-f).
Figure 17. Generation and validation of a lentiviral HA-tagged DAT expression construct. (a) Design of a lentiviral construct. FUGW-3xHA-DAT-P2A-Tdtomato-WPRE (3xHA-DAT-TdT). hUBC, human ubiquitin c promoter; HA, hemagglutinin; hDAT, human dopamine transporter; P2A, porcine teschovirus-1 2A; TdT, a red fluorescent protein; WPRE, Woodchuck hepatitis B virus post-transcriptional regulatory element. The P2A peptide sequence enables simultaneous expression of hDAT and TdT. (b) Western blot analysis from HEK293T cells, transfected with either 3xHA-DAT or 3xHA-DAT-TdT. ~80kDa bands (arrowheads) indicate a mature form of DAT. ~55kDa (asterisks) indicate an immature form of DAT. (c) Immunostaining of neurons transfected with 3xHA-DAT-TdT (DIV8). Only neurons expressing TdT are positive for HA staining, indicating DAT expression. Scale bar, 20 µm.
Figure 18. Lentiviral expression of DAT in striatum of in DAT-/- mice. (A-C) Representative images of injection sites from DMS (A), DLS (B), and NAC (C). hDAT, red; DAPI, blue. Scale bars, 200 µm. (D-F) The largest (gray) and smallest (black) infection sites are shown across the anterior-posterior axis of each region. (G) DAT protein is absent in DAT-/- mice but restored following viral rescue.
We replicated the finding that DAT\textsuperscript{−/−} mice are more motivated for food rewards than DAT\textsuperscript{+/−} and DAT\textsuperscript{+/+} mice (Figure 19a). Lentiviral infection of DAT into DMS reduced excessive food cup entries in DAT\textsuperscript{−/−} mice (Figure 19b; planned comparison, No rescue vs. DMS rescue: two-way ANOVA: no effect of Rescue and no Interaction, $p$s>0.05, main effect of Time, $F_{(5,65)} = 3.44, p < 0.01$). By contrast, restoration of DAT in the DLS reduced the excessive food seeking to control levels (No rescue vs. DLS rescue: main effect of Rescue, $F_{(1,70)} = 8.06, p < 0.05$; no effect of Time, $p > 0.05$; Interaction, $F_{(5,70)} = 6.12, p < 0.0001$). Post hoc analysis showed that DLS rescue mice entered less than DAT\textsuperscript{−/−} controls between 20s and 60s from reward delivery ($t$-test, $p < 0.05$). Likewise, restoration of DAT in the NAC also reduced food seeking (No rescue vs. NAC rescue: no main effect of Rescue or Time, $p > 0.05$, but there was a significant Interaction, $F_{(5,65)} = 4.29, p < 0.01$). Post hoc analysis revealed a significant group difference at 30 s following reward delivery ($p < 0.05$).

Figure 19. DAT rescue in DLS reduces excessive food seeking in DAT\textsuperscript{−/−} mice. (a) DAT\textsuperscript{−/−} mice show elevated food seeking during the inter-reward interval. (b) DAT rescue in DLS ($n = 8$) and NAC ($n = 7$), reduces food cup entries compared to controls ($n = 8$). DAT restoration in DMS ($n = 7$) did not rescue food seeking (* $p < 0.05$).
The reduction in food cup entries among DLS and NAC mice was not due to differences in satiety, as the extent of food deprivation (% body weight) was similar among all groups (ANOVA: $F(3,29) = 0.35, p > 0.05$).

We then tested whether striatal DAT restoration was sufficient to restore DAT$^{-/-}$ locomotion to control levels. DAT$^{-/-}$ speed was not reduced by viral rescue of DAT (Figure 20a; ANOVA of DAT$^{-/-}$ groups, $F(3,18) = 0.73, p > 0.05$). DAT restoration did not alter the amount of weight gained during behavioral testing (Figure 20b; Kruskal-Wallis ANOVA: $K = 4.51, p > 0.05$).

Figure 20. Striatal rescue of DAT does not restore hyperactivity or instrumental responding in DAT$^{-/-}$ mice. (a) In an open field test, speed remained significantly elevated above DAT$^{+/+}$ and DAT$^{+/}$ controls for all DAT$^{-/-}$ groups. (b) DAT rescue does not cause mice to gain more weight than controls. (c) All DAT$^{-/-}$ mice were significantly impaired at learning to press a lever for food. Viral rescue did not restore instrumental learning. (d) Food cup entries during instrumental training were comparable between groups, indicating comparable Pavlovian approach responses ($p > 0.05$).
Because Pavlovian approach behavior and instrumental lever pressing are known to be dissociable (Dickinson et al., 2000; Yin et al., 2008), we tested whether DAT\(^{-/}\) mice showed altered instrumental learning (Figure 20c). DAT\(^{-/}\) mice were severely impaired at learning to press a lever for food compared to both DAT\(^{+/}\) and DAT\(^{+/}\) mice (two-way ANOVA [Genotype x Session]: main effect of Genotype, \(F_{(5,408)} = 15.94, p < 0.0001\); main effect of Session, \(F_{(6,408)} = 9.53, p < 0.0001\); Interaction, \(F_{(30,408)} = 4.18, p < 0.0001\)). All DAT\(^{-/}\) groups were impaired relative to DAT\(^{+/}\) and DAT\(^{+/\}}\) mice on every session \((p < 0.05)\). Viral restoration of DAT did not rescue instrumental learning. The deficit is not due to competing behaviors such as excessive food seeking in DAT\(^{-/}\) mice because food cup entries are similar (Figure 20d; final session, one-way ANOVA, \(F_{(5,71)} = 2.27, p > 0.05\)).

3.4 Discussion

In agreement with previous work (Pecina et al., 2003), we found that DAT\(^{-/}\) mice showed enhanced motivation for food rewards, as measured by their much higher rate of food cup entries when hungry. Yet when they were sated, their food seeking behavior was not higher than controls, even though they still exhibited hyperactivity as measured in an open field (Figure 15f). Thus we ruled out the possibility that enhanced food seeking is a result of a more general hyperactivity. This finding dissociates general hyperactivity and food seeking behavior in DAT\(^{-/}\) mice, suggesting that these are mediated by distinct neural mechanisms (Roitman et al., 2004).

Paradoxically, despite their enhanced reward seeking, DAT\(^{-/}\) mice were dramatically impaired in instrumental learning—learning to press a lever to earn food reward. Despite extensive training, they were unable to learn the instrumental
contingency (Figure 16). These results establish a clear dissociation between Pavlovian approach behavior, measured by food cup entries, and instrumental lever pressing.

3.4.1 Lentiviral DAT rescue in specific striatal regions

Our observations suggest that hyperdopaminergia results in two dissociable behavioral changes: 1) general hyperactivity, as measured by locomotion in an open field, and 2) a selective increase in food-directed behaviors that is abolished by satiety. As the DAT knockout is global, it is unclear which brain regions might mediate the observed effects. As the striatum is the major target of dopaminergic projections in the brain, we designed a lentiviral vector to reintroduce DAT in the striatum. Because different striatal regions have been shown to have distinct roles in behavior (Yin et al., 2008), we selectively injected the lentiviral vector containing DAT in three different striatal regions (DLS, DMS, and NAC) which have distinct patterns of anatomical connectivity (McGeorge and Faull, 1989).

When DAT was selectively restored in the DLS or NAC (Figure 19), the excessive food seeking in DAT\(^{-/-}\) mice was reduced to control levels. This finding suggests that increased DA signaling in the DLS and NAC is responsible for the high rate of reward seeking observed in DAT\(^{-/-}\) mice. Previous work using the opposite strategy of genetically depleting DA has also implicated striatal DA in feeding behavior (Darvas and Palmiter, 2010): feeding and motivation for food were absent in DA deficient mice, but restored following viral restoration of DA signaling in the DLS.

These results suggest that the level of DA in the DLS and NAC is critical for controlling the vigor of Pavlovian reward approach behavior, in agreement with our recent finding of motivational modulation of DA neuron activity (Rossi et al., 2013a). The DMS group, which showed no significant effect, serves as a control for viral infection.
Restoration of dopamine signaling in the DLS, but not DMS or NAC, was shown by previous work to rescue aphagia and restore food motivation in dopamine deficient mice (Szczyepka et al., 1999a; Szczyepka et al., 2001). That is, when DLS DA is too low, animals are aphagic, whereas excessive DA signaling in the DAT−/− mice results in excessive reward-seeking. Previous work has also implicated accumbens DA in motivation for food rewards (Salamone and Correa, 2012). Thus, synaptic dopamine in the DLS and NAC may serve as gain control in food seeking behavior, though the relative contributions of striatal outputs from these two regions remain unclear.

One possible explanation for why DAT−/− mice show excessive food seeking is that they are more sensitive to depletion of energy reserves because of their smaller size. Our results, however, do not support this explanation. After viral restoration of DAT in DLS and NAC, food seeking was reduced, yet weight was not altered (Figure 20b). If energy reserves were driving food seeking, then the reduction in food seeking as a result of viral rescue should also be accompanied by a change in weight.

3.4.2 DA and instrumental learning

Since instrumental learning is among the most important forms of learning, its virtual abolishment in DAT−/− mice is a striking phenotype, which may resemble learning deficits observed in ADHD. Our results therefore have strong implications for the understanding of learning deficits associated with hyperdopaminergic disorders like ADHD.

DAT−/− mice are unable to reliably learn associations between instrumental actions and their outcomes. The food reward fails to reinforce lever pressing, even though the knockout animals were capable of pressing the lever initially (Figure 16b). Although viral rescue produced normal food seeking behavior, it did not affect general
hyperactivity or instrumental learning (Figure 20). Only excessive food seeking behavior was rescued by restoring DAT in the DLS and NAC. Not only do DAT$^{-/}$ mice show a striking deficit in instrumental learning, this deficit was not rescued by introducing DAT back in any of the three striatal regions. Because the viral rescue restored normal Pavlovian approach behavior to food rewards, but failed to alter general hyperactivity, our finding suggests that the latter may be responsible for the dramatic deficits in instrumental learning.

It should be noted, however, that the lack of effects on instrumental learning and hyperactivity could simply be due to the lack of sufficient viral infection, though the rescue was dramatically successful in reducing excessive food cup entries on the FT60 task. Without direct measures of striatal dopamine in these regions, it is difficult to ascertain the extent to which dopaminergic signaling in DAT$^{+/}$ mice was altered by the viral rescue. Future studies using direct measures of dopamine in specific striatal regions in freely moving mice will be needed to answer this question (Howe et al., 2013).

Food cup entries or nose pokes are a part of normal behavioral repertoire of rodents, whereas an arbitrary action like pressing a lever requires learning the instrumental contingency between action and outcome (Dickinson, 1994; Yin et al., 2008). Such learning requires a trial and error learning process and the generation of behavioral variability before the correct action is generated and selected (Staddon, 1983b; Derusso et al., 2010).

Previous work also found enhanced motivation for food rewards in DAT knockdown mice, but normal instrumental learning (Cagniard et al., 2006a; Yin et al., 2006; Beeler et al., 2012). This discrepancy is of particular interest, because DAT knockdown mice only show a moderate ($\sim 70\%$) increase in DA signaling, whereas the increase in DAT$^{-/}$ mice is $\sim 500\%$ (Jones et al., 1998). Interestingly, heterozygous
(DAT+/−) mice, which have moderately increased DA tone comparable to that in the knockdown mice, showed no instrumental learning deficits (Figure 16) (Jones et al., 1998). Conversely, DA deficient mice are also unable to learn to press a lever for food (Robinson et al., 2007). Together these results suggest that there is an optimal level of synaptic dopamine necessary for instrumental learning. One possibility is that initial acquisition of lever pressing requires behavioral variability, as the animal explores different options, via trial and error, before it presses the lever for the first time. Excessive DA signaling may impair the behavioral variability needed to chance upon the correct action. This possibility is supported by the finding that DAT+/− mice are not only hyperactive, but also show highly stereotyped and repetitive behaviors (Wong et al., 2012; Fox et al., 2013). Hyperactivity and stereotypy can impair successful exploration of the environment, which are critical for instrumental learning, in accord with the learning deficits in ADHD.
4. Dopaminergic modulation of pattern generation and behavioral variability in licking

4.1 Introduction

The previous chapters have examined the neural substrates that contribute to food seeking behavior in freely-behaving mice. The results strongly implicate the basal ganglia in regulating appetitive processes. However, it is often difficult to discern the precise relationship between neural activity and behavior. When freely moving mice approach a food cup in response to a auditory cue (Chapter 2), it is unclear exactly what aspect(s) of behavior the burst in dopamine and GABA activity relates to, since the behavior occurring within each trial is unique. To address this ambiguity, the next two chapters will focus on consummatory behavior, which is much more stereotyped than appetitive behavior and consequently better understood.

Dopamine, a major neurotransmitter that mainly modulates activity in the basal ganglia, has been implicated in a variety of motivated behaviors (Zhou and Palmiter, 1995; Berridge, 2007; Rossi et al., 2013a). Extensive dopaminergic projections target the striatum, the input nucleus of the basal ganglia, where dopamine is released and influences the responsiveness of striatal neurons to glutamatergic cortical inputs (Gerfen and Surmeier, 2011).

Previous studies have suggested a critical role of dopamine in appetitive and consummatory behaviors for food and water (Szczypta et al., 2001; Salamone and Correa, 2012). For example, systemic administration of dopamine receptor agonists potentiates orofacial movements and induces aberrant licking and chewing (Olpe, 1978; Zarrindast et al., 1992). Moreover, in dopamine transporter (DAT) knockout (DAT−/−) mice, which show prolonged increases in dopamine signaling, the overall rate of licking
for sucrose rewards was greatly increased (Costa et al., 2007). Nevertheless, exactly how dopamine can modulate consummatory behaviors remains unclear.

In this study, we used an established DAT−/− mice, an established animal model of hyperdopaminergia, to study the role of dopamine in modulating licking behavior. Licking is a relatively stereotyped behavior in rodents, with a rate of 5-9 Hz (Marowitz and Halpern, 1973; Halpern, 1975; Murakami, 1977; Weijnen, 1998). Despite the stereotypical pattern, however, studies have shown that the microstructure can be modulated by a variety of factors, such as perceptual feedback, satiety, palatability, and so on (Cone, 1974; Cone et al., 1975a; Mamedov and Bures, 1984).

We quantified the microstructure of licking in DAT−/− mice and their wild type (DAT+/+) littermates as they voluntarily licked sucrose solution. We found that, compared to DAT+/+ mice, DAT−/− mice showed pronounced differences in their pattern of licking. In addition, they also showed more rapid onset of behavioral variability following the imposition of an extinction contingency and more rapid extinction of licking behavior.

4.2 Materials and Methods

4.2.1 Subjects

Dopamine transporter knockout \((n = 11)\) and wild-type \((n = 8)\) littermates (8-12 weeks old) were used for experiments as previously described (Giros et al., 1996). During testing, mice were maintained on 23 hour water deprivation schedule. All experiments were conducted during the light phase of the animal's light cycle.
4.2.2 Behavior

4.2.2.1 Measuring Licking

Sucrose (10% w/v) was available from a standard water bottle in one wall of an operant chamber (35 cm x 28 cm x 22 cm). The spout was recessed ~3 mm within a plastic tube to prevent the mice from contacting the lick sensor with their paws. Licks were recorded using a contact lickometer (Slotnick, 2009). When mice touched the metal spout with their tongues, a circuit was completed, which triggered a voltage change lasting the duration of the contact. Voltage was sampled at 2000 Hz using the Cerebus acquisition system (Blackrock Microsystems).

Testing took place in daily 30-min sessions. Thirsty mice were first acclimated to the testing chamber for 30 min and allowed to freely consume sucrose solution. The following day, mice were tested in the same chamber, and licks were recorded (sucrose condition). The next day, the extinction test was conducted in which the same set up was used, except the spout contained no sucrose solution. On the following day, reinstatement, sucrose solution was once again present in the spout.

4.2.2.2 Measuring Locomotion

Locomotion tests were conducted after completion of the licking tests with a subset of the same mice used in the previous licking experiments ($n = 8$ DAT$^{+/+}$; $n = 9$ DAT$^{-/-}$). Mice were placed in a circular chamber for three minutes and video was taken from directly above. Position was tracked frame-by-frame offline using custom software (Matlab) as described previously (Rossi et al., 2013a).
4.2.3 Data Analysis

From the analog voltage signal, we generated timestamps corresponding the onset and termination of each lick (0.5 ms resolution). From this we calculated the duration (time from onset to termination), duty cycle (the ratio of the duration of the contact with the lickometer to the period for each lick cycle), and inter-lick interval (ILI; time from lick offset to the next lick onset) as well as the number and rate of licks. Bout analysis was used to determine the properties of bouts of licking. Based on previous analysis of rodent licking (Spector et al., 1998a), we defined the start of a bout as 3 or more licks occurring at >3 Hz and being preceded by at least 1 second in which no licks were recorded. The end of the bout was defined as the last lick that was followed by at least 1 second in which no licks were recorded. Power spectral density analysis was used to assess the component frequencies of the lick trains (Neuroexplorer, Nex Technologies). The lick rate was calculated as the inverse of the median inter-lick interval (ILI) of all licks occurring within a bout.
4.3 Results

4.3.1 DAT\(^{-/-}\) mice are hyperactive and show altered lick patterning relative to DAT\(^{+/+}\) controls when freely licking for sucrose solution

In agreement with previous data (Chapter 3 and (Giros et al., 1996)), DAT\(^{-/-}\) mice were hyperactive compared to DAT\(^{+/+}\) mice during an open field test (Figure 21a,b). Overall, DAT\(^{-/-}\) mice traveled much farther than DAT\(^{+/+}\) littermates (Figure 21b; \(t_{15} = 10.33, p < 0.0001\)). This is in agreement with previous studies showing hyperlocomotion in hyperdopaminergic mice (Giros et al., 1996).

Figure 21. Hyperactivity in DAT\(^{-/-}\) mice. (a) Locomotion was tracked during 3 min in an open field chamber. DAT\(^{+/+}\) mice showed no decline in locomotion during the test, while DAT\(^{+/+}\) mice did (left). (b) Overall, DAT\(^{-/-}\) mice covered more distance than DAT\(^{+/+}\) (\(p < 0.0001\)).
Figure 22. Recording of voluntary licking in DAT⁺ mice. (a) Schematic of apparatus. Mice stood on a metal ground plate and licked a spout that converted licks to a voltage signal. (b) Example traces from lickometer showing a sample bout of licking. Lick timestamps are shown as lines. Scale bar is 1 s. (c) Traces corresponding to the shaded regions from panel b showing individual licks as upward deflections for DAT⁺/⁺ and DAT⁻/⁻ mice. Note that the lick pattern is different in DAT⁻/⁻ mice.
To assess whether chronically elevated dopamine alters consummatory motor output, we designed a contact lickometer that detected individual licks (Figure 22a). We found that DAT\(^{-}\) mice had altered lick patterning compared to DAT\(^{+}\) mice when voluntarily licking for sucrose solution (Figure 22b,c). Overall, the lick cycle of DAT\(^{-}\) mice (within bout rate: 7.66 ± 0.13 Hz) appeared slower relative to DAT\(^{+}\) mice (within bout rate: 8.28 ± 0.20 Hz). DAT\(^{-}\) mice showed longer lick durations than DAT\(^{+}\) mice (Figure 23a; Mann-Whitney \(U = 10.00, p = 0.0057\)) and shorter ILIs (Figure 23b; \(U = 17.00, p = 0.028\)). The duty cycle (the ratio of the duration of the contact with the lickometer to the period for each lick cycle) was increased in DAT\(^{-}\) mice relative to DAT\(^{+}\) mice (Figure 23c; \(U = 19.00, p = 0.043\)).

**Figure 23.** DAT\(^{-}\) mice show altered lick patterning when licking for sucrose. (a) Distribution of lick durations. DAT\(^{-}\) mice show longer durations than DAT\(^{+}\). (b) Distribution of ILIs. DAT\(^{-}\) mice show shorter ILIs. (c) Distribution of duty cycles. DAT\(^{-}\) mice show higher duty cycles than DAT\(^{+}\) mice. (d) DAT\(^{-}\) mice show elevated power spectral density at lower frequencies. * \(p < 0.05\). Values are mean and s.e.m.
To further examine the pattern of licking, we used power spectral density analysis to extract the component frequencies of bouts of licking (Figure 23d). DAT\textsuperscript{-/-} mice showed higher power at lower lick frequencies compared to DAT\textsuperscript{+/+} mice (two-way ANOVA [Genotype x Frequency]: main effect of Genotype, $F_{(1,850)} = 19.82, p = 0.0004$; main effect of Frequency, $F_{(50,850)} = 25.68, p < 0.0001$; interaction between Genotype and Frequency, $F_{(50,850)} = 1.65, p = 0.0036$).

Figure 24. DAT\textsuperscript{-/-} mice show increased rate of licking and number of licks in a bout. (a) Rate of licking throughout the session. (b) Number of bouts. (c) DAT\textsuperscript{-/-} mice lick more in each bout. * $p < 0.05$. 
The overall lick rate was increased for DAT$^{-/-}$ mice (Figure 24a; two-way ANOVA [Genotype x Time]: no main effect of Genotype, $F_{(1,85)} = 3.32$, $p = 0.086$; main effect of Time, $F_{(5,85)} = 13.37$, $p < 0.0001$; interaction between Genotype and Time, $F_{(5,85)} = 2.57$, $p = 0.032$). The interaction was driven by more licks for DAT$^{-/-}$ mice during the first 5 min of the session (Bonferroni post test, $p < 0.01$). DAT$^{-/-}$ mice took marginally fewer bouts than DAT$^{+/+}$ mice (Figure 24b; $t_{(17)} = 1.92$, $p = 0.07$) but had more licks per bout than DAT$^{+/+}$ mice (Figure 24c; unpaired t-test with Welch's correction for unequal variance: $t_{(12)} = 3.10$, $p = 0.0092$).

4.3.2 In extinction, in which no solution is present, DAT$^{-/-}$ mice no longer show altered lick patterning relative to DAT$^{+/+}$ controls

To test whether the differences in the patterning of DAT$^{-/-}$ licking were influenced by feedback related to the presence of sucrose in the spout, we conducted licking tests in extinction, when no solution was present. When mice licked the dry spout, the rate of licking was slower than when they licked for sucrose (DAT$^{+/+}$: 6.47 ± 0.21 Hz; DAT$^{-/-}$: 6.32 ± 0.23 Hz). In addition, the observed differences in the pattern of licking disappeared (Figures 25 and 26). During extinction, DAT$^{-/-}$ mice no longer had increased lick durations (Figure 25b; $U = 38.50$, $p = 0.68$), shorter ILIs (Figure 25c; $U = 35.5$, $p = 0.51$), or higher duty cycles (Figure 25d; $U = 33.00$, $p = 0.39$). Neither group showed enhanced power within the 7-9 Hz range (Figure 25e).

4.3.3 DAT$^{-/-}$ mice are more sensitive than DAT$^{+/+}$ controls to extinction

DAT$^{-/-}$ mice were more sensitive to the imposition of the extinction contingency than DAT$^{+/+}$ mice (Figure 26). Both groups reduced the rate of licking during extinction (Figure 26a; two way ANOVA [Genotype x Time]: no main effect of Genotype, $p > 0.05$;
main effect of Time, $F_{(5,85)} = 7.12, p < 0.0001$; no Interaction, $p > 0.05$). When the lick rate was normalized by the average rate of licking during the preceding sucrose session, DAT$^{-/-}$ mice extinguished licking more quickly than DAT$^{+/+}$ mice (Figure 26b; two way ANOVA [Genotype x Time]: main effect of Genotype, $F_{(1,85)} = 9.23, p = 0.0074$; main effect of Time, $F_{(5,85)} = 6.79, p < 0.0001$; no interaction between Genotype and Time, $F_{(5,85)} = 0.61, p = 0.69$).

DAT$^{-/-}$ mice took fewer bouts than DAT$^{+/+}$ mice (Figure 26c; $t_{(17)} = 3.50, p = 0.0028$). The number of licks per bout was greatly reduced for both groups compared to the sucrose condition, yet DAT$^{-/-}$ mice still tended to lick more per bout (Figure 26d; t-test with Welch’s correction for unequal variance: $t_{(13)} = 3.97, p = 0.0016$).
Figure 25. Lick patterning during extinction. (a) Representative lickometer traces for DAT\textsuperscript{+/+} (top) and DAT\textsuperscript{−/−} (bottom) mice. When the data from the entire extinction session were compared, neither lick duration (b), ILI (c), nor duty cycle (d) were different between DAT\textsuperscript{+/+} and DAT\textsuperscript{−/−} mice when licking in extinction. (e) Power spectral density of licking.
Figure 26. DAT\(^{-/-}\) mice are more sensitive than WT mice to imposition of the extinction contingency. (a) Rate of licking throughout the 30-min extinction session. (b) Rate of licking throughout the session normalized by the average rate of licking for sucrose. DAT\(^{-/-}\) mice reduce their lick rate more quickly than WT mice. DAT\(^{-/-}\) mice show fewer bouts of licking (c) and more licks per bout (d). (e) Representative examples of lick duration throughout the session from WT (top) and DAT\(^{-/-}\) (bottom) mice. (f) Median duration of licks. Sucrose indicates data from the last rewarded session. (g) Inter quartile range of lick duration. * \(p < 0.05\).
To understand the time course of behavioral adaptation to altered feedback during extinction, we analyzed how lick duration changed over time (Figures 26e-g). DAT\textsuperscript{-/-} mice adapted more quickly to the dry spout than DAT\textsuperscript{+/+} mice. Because all mice licked different amounts, we divided the licks of each mouse into three equally sized bins and to compare lick duration and variability throughout the extinction session. The duration of licks was stable throughout the session, and there was no group difference (Figure 26f; two way ANOVA [Genotype x Time]: no main effect of Genotype, $F_{(1,34)} = 0.75, p > 0.05$; no main effect of Time, $F_{(2,34)} = 2.98, p = 0.06$; no interaction, $F_{(2,34)} = 1.21, p > 0.05$). In response to the dry spout, mice increased the variability of lick durations. DAT\textsuperscript{-/-} mice, however, did so more quickly than DAT\textsuperscript{+/+} mice as measured by the inter quartile range (Figure 26h; no main effect of Genotype, $F_{(1,34)} = 1.06, p > 0.05$; main effect of Time, $F_{(2,34)} = 3.53, p = 0.04$; interaction between Genotype and Time, $F_{(2,34)} = 4.27, p = 0.02$).

**4.3.4 Altered lick patterning in DAT\textsuperscript{-/-} mice is restored when sucrose is reinstated**

Following extinction, licking for sucrose solution was reinstated (Figure 27). Licking during reinstatement was highly similar to pre-extinction licking (Figure 27a; DAT\textsuperscript{+/+}: 8.36 ± 0.21 Hz; DAT\textsuperscript{-/-}: 7.60 ± 0.20 Hz). DAT\textsuperscript{-/-} mice had longer lick durations (Figure 27b; $U = 7.00, p = 0.0026$), shorter ILIs (Figure 27c; $U = 12.50, p = 0.01$), and higher duty cycles (Figure 27d; $U = 12.00, p = 0.0093$) than DAT\textsuperscript{+/+} mice. Enhanced power was also observed in the 7-9 Hz frequency range (Figure 27e).

We compared the effects of extinction early in the session (first 50 licks) with the last 50 licks (Figures 27f,g). Lick duration was altered by extinction and returned to pre-
Figure 27. Altered lick patterning in DAT⁻/⁻ mice during reinstatement. (a) Representative lickometer traces for DAT⁺/⁺ (top) and DAT⁻/⁻ (bottom) mice. Lick duration (b), ILI (c), and duty cycle (d) are all different between DAT⁺/⁺ and DAT⁻/⁻ mice when licking for sucrose solution is reinstated. (e) Power spectral density of licking returns to pre-extinction levels. (f-g) DAT⁻/⁻ mice adapt licking behavior to altered feedback during extinction more quickly than DAT⁺/⁺ mice. The first 50 licks during extinction was considered 'early' and the last 50 licks was considered 'late.' Duration of licks (f) and inter-quartile range of lick durations (g) returns to pre-extinction levels during reinstatement. * p < 0.05.
extinction levels during reinstatement (Figure 27f; main effect of Genotype, $F_{(1,51)} = 9.03, p = 0.008$; main effect of Time, $F_{(3,51)} = 4.74, p = 0.005$; no interaction between Genotype and Time, $F_{(3,51)} = 2.20, p = 0.10$). The inter quartile range of the lick durations was also altered by extinction and returned to pre-extinction levels during reinstatement, but DAT$^{+/+}$ adapted more slowly (Figure 7g; no main effect of Genotype, $F_{(1,51)} = 0.19, p = 0.67$; main effect of Time, $F_{(3,51)} = 4.41, p = 0.008$; interaction between Genotype and Time, $F_{(3,51)} = 3.29, p = 0.03$).

The global rate of licking was similar between groups (Figure 28a; two way ANOVA [Genotype x Time]: no main effect of Genotype, $F_{(1,85)} = 0.85, p = 0.37$; main effect of Time, $F_{(5,85)} = 36.56, p < 0.0001$; no interaction between Genotype and Time, $F_{(5,85)} = 1.23, p = 0.30$). Both groups took a similar number of bouts (Figure 28b; unpaired t-test with Welch’s correction for unequal variance: $t_{(14)} = 0.69, p > 0.05$). DAT$^{-/-}$ mice had more licks per bout during reinstatement (Figure 28c; unpaired t-test with Welch’s correction for unequal variance: $t_{(14)} = 2.33, p = 0.035$).

Figure 28. Summary of licking during reinstatement. (a) Rate of licking throughout the session is similar between groups. (b) The number of bouts is similar between groups. (c) DAT$^{-/-}$ mice lick more in each bout, * $p < 0.05$. 
4.4 Discussion

DAT\(^{-/-}\) is a well-established mouse model of hyperdopaminergia (Giros et al., 1996; Spielewoy et al., 2000). In agreement with previous work, we found general hyperactivity in these mice. Our novel finding is a pronounced change in the pattern and timing of licking in these mice. DAT\(^{-/-}\) showed increased individual lick duration, reduced inter-lick interval, and more licks per bout. In addition, when the feedback was altered during extinction, we found a dramatic difference in the consequent change in licking behavior: whereas controls showed a gradual increase in behavioral variability over time, the DAT\(^{-/-}\) were much more sensitive to the change in the feedback function, showing an immediate and transient burst of variability following the detection of the extinction contingency (Figure 26g). This more rapid generation of behavioral variability is also coupled with greater extinction (Figure 26b).

4.4.1 Generation of licking pattern

In recent years, licking behavior has increasingly become a focus of neuroscience research because it is highly reliable and stereotyped, permitting convenient characterization of the microstructure of behavior (Gutierrez et al., 2010; Johnson et al., 2010; Komiyama et al., 2010; Ostlund et al., 2013; Guo et al., 2014).

Although the pattern generator underlying licking is well characterized, the top down influences, including the role of the basal ganglia, remain largely unknown. Some cortical regions are known to show lick related activity (Gutierrez et al., 2006; Komiyama et al., 2010). Neurons from these regions may drive licking via striatal projections. The neurons within the lateral striatum exhibit oscillatory activity that corresponds to licking behavior (Mittler et al., 1994). Injection of amphetamine in the ventrolateral striatum induces oral stereotypy and voracious licking (Kelley et al., 1988), and unilateral
depletion of nigrostriatal dopamine in rats results in impaired licking in which the lick rate slows and tongue force is reduced (Skitek et al., 1999).

Output from the basal ganglia is thought to regulate orofacial movements via projections to the motor regions of the superior colliculus (Taha et al., 1982; Gunne et al., 1988). The superior colliculus, in turn, sends projections to rhythmically active motor and premotor neurons in the reticular formation that control the tongue and jaws (Morimoto et al., 1966; Wiesenfeld et al., 1977; Sparks and Hartwich-Young, 1989; Brozek et al., 1996; Travers et al., 1997b).

Licking is generally considered to be a highly stereotyped behavior generated by a central pattern generator. However, as shown in the present study, the pattern is certainly influenced by feedback from the liquid during licking. DAT\(-/-\) showed higher overall rate of licking for sucrose. This result is not surprising given previous work (Costa et al., 2007; Perona et al., 2008). But our analysis of the licking microstructure revealed for the first time a significant change in the patterning of licking. These changes are characterized by a dramatic increase in the persistence of a bout of licking (more licks per bout), as well as an increase in the individual lick contact duration. Their high rate of licking is primarily due to an increase in the number of licks per bout, i.e. more persistent licking once the bout is initiated. On the other hand, during each bout, their licking is actually slower, characterized by longer duration of contact with the spout and shorter inter-lick intervals. This observation can be explained by the hypothesis that DAT\(-/-\) mice attempt to maximize sucrose intake. In accord with this interpretation, the overall proportion of contact time during a bout (duty cycle) is increased in the DAT\(-/-\) group.
4.4.2 Extinction

Here we showed that in control mice, the lick duration increased under extinction, when sucrose reward was no longer delivered. DAT$^{+/+}$ mice gradually increased variability of lick duration during extinction, as shown in Figure 6g.

Extinction, as a procedure, represents a drastic change in the feedback function, and has long been shown to result in new learning. As shown by the rate of licking, all mice reduced their overall rate of licking over time, but the DAT$^{-/-}$ mice showed a more rapid reduction (Figure 6b). Moreover, they immediately increased behavioral variability, whereas the controls showed a more gradual change.

An increase in behavioral variability is present in initial instrumental learning (Derusso et al., 2010; Costa, 2011; Yin, 2014b). But such variability could be a general feature of learning, including the behavioral adaptations following exposure to the extinction contingency. The mice learned to stop licking, but initially they exhibited “exploration” by varying the pattern and timing of licking. Despite the high stereotyped licking pattern, both the inter-lick-interval and the lick duration could be varied, and all mice increased such behavioral variability following extinction.

4.4.3 The function of dopamine

Our results are in accord with previous work showing a critical role of dopamine in the performance of orofacial movements. For example, studies have shown reduced lick frequency in rats with unilateral striatal dopamine depletion (Skitek et al., 1999) and increased frequency of orofacial movements following systemic or intrastratal administration of dopamine agonists (Fray et al., 1980; Redgrave et al., 1980; Arnt et al., 1987; Delfs and Kelley, 1990). Yet our results suggest that increasing dopamine
transmission produces two distinct types of effects—one related to performance and the other related to learning.

The net effect of dopamine is to modulate the gain of the reward seeking system, altering performance to maximize the input by prolonging the contact duration (duty cycle). This suggests that dopamine is operating at a hierarchically higher level, that has access to net sucrose intake. This level, presumably corresponding to the basal ganglia circuits, can simultaneously modulate all three effects (ILI, contact duration, and bout persistence) toward maximizing sucrose intake. As a result, the overall yield per bout of licking is increased.

The second effect is related to learning, in this case to behavioral adaptation to the imposition of the extinction contingency. As has long been established, extinction can generate new types of learning (Bouton, 2004). Here dopamine appears to modulate the level of behavioral variability in licking pattern generation (Costa, 2011). This effect is in agreement with work on dopaminergic modulation of song variability in song birds (Leblois et al., 2010; Leblois and Perkel, 2012). The prolonged dopamine signaling in DAT−/− mice may therefore increase variability depending on the behavioral context, whether it is modulation of song production based on social context or the modulation of licking variability following the imposition of the extinction contingency. Because the generation of such behavioral variability is critical during the exploratory phase of learning, dopamine can also play a key role in learning.
5. Nigro-tectal regulation of consummatory behavior

5.1 Introduction

Consummatory behaviors such as eating and drinking are critical for survival and energy homeostasis (Nakamura et al., 1990a; Fowler and Mortell, 1992a). Licking and chewing consist of patterned orofacial movements that require precise coordination of the muscles of the tongue and jaws. In rodents and many other species, drinking is accomplished by stereotyped repetitive licking movements with a relatively constant frequency (Spector et al., 1998a). The neural substrates of consummatory behavior have been well characterized at the level of lower motor neurons, and the organization of the brainstem nuclei involved in mastication and licking is well characterized (Travers et al., 1997a). But it remains unclear how the brainstem nuclei responsible for central pattern generation are controlled by descending commands from the brain.

A major source of descending signals from the brain is the basal ganglia, a large group of subcortical nuclei implicated in voluntary behavior. By common consensus, there are multiple parallel basal ganglia networks with functional specialization (Alexander and Crutcher, 1990; Yin et al., 2008), and the outputs of such networks project to many areas, including thalamus, tectum, and reticular formation (Beckstead, 1983; Deniau and Chevalier, 1992). Classic lesion and inactivation studies have suggested that the nigro-tectal projections are involved in orofacial behaviors (Redgrave et al., 1980; Taha et al., 1982; Wang and Redgrave, 1997). The basal ganglia output from the substantia nigra pars reticulata (SNR) can influence activity of central pattern generators in the medullary reticular formation via their projections to the tectum.
Cats and rats that have been decerebrated early in life, and thus lack intact basal ganglia, fail to show appropriate voluntary licking for milk and water even though they are able to orient their heads and move toward auditory, olfactory, and tactile stimuli. These animals only show reflexive licking and chewing when food or milk is placed into contact with the lips (Bignall and Schramm, 1974; Grill, 1980). Interestingly, voluntary licking behavior is relatively normal in cats that only lack the cerebral cortex (Bjursten et al., 1976), suggesting that the presence of voluntary licking, which is based on motivational cues, is driven at least in part by the basal ganglia, though recent studies have shown that descending motor cortical projections can influence licking in adult mice (Li et al., 2015).

In primates, cats, and rodents, the major basal ganglia output, the SNR, is implicated in appropriately executing and patterning orofacial movements (Arnt and Scheel-Kruger, 1980; Taha et al., 1982; Gunne et al., 1988). When the GABA receptor agonist, muscimol, is injected into the SNR in cats and rats, voracious licking is observed (Wolfarth et al., 1981; Taha et al., 1982; Cools et al., 1983). In addition, the projections from the SNR to the lateral part of the superior colliculus (SC) have been implicated in regulating orofacial movements (Redgrave et al., 1980; Taha et al., 1982; Wang and Redgrave, 1997).

However, how basal ganglia output influences the relevant downstream targets to generate voluntary drinking behavior is still poorly understood. It is unclear what type of signals might be sent by SNR neurons or how they relate to the initiation, termination, and patterning of drinking behavior. To address these questions, we recorded single unit activity from the substantia nigra and superior colliculus. We found that both regions contained neurons whose firing rates were time locked to individual licks during self-initiated drinking. However the lick-related signals in the SNR and SC tend to be
antiphasic in nature, consistent with the presence of inhibitory nigrotectal projections. To selectively activate the nigrotectal projections, we used optogenetics to stimulate the GABAergic SNR afferents in the deep layers of the SC. Photo-stimulation of the nigrotectal projections transiently inhibited the activity of the lick-related tectal neurons and suppressed self-initiated drinking in a frequency-dependent manner.

5.2 Materials and Methods

5.2.1 Subjects

For nigrotectal stimulation experiments Vgat-ires-Cre mice (Vong et al., 2011) aged 3-9 months at the start of experiments were used (n = 13). For SN recordings, male c57bl/6 mice (n = 6; 4-6 months) were used. For SC recordings, c57bl/6 (n = 4; 4-6 months, two male) were used. Mice were maintained on a 12:12 light cycle and were given ad libitum food in their home cages. Except where indicated, mice were maintained on a 22.5 hour water deprivation schedule. During testing, water was restricted. Mice were allowed to freely consume sucrose solution during licking tests and were given access to water for 1 hour following all recording sessions.

5.2.2 Surgery

5.2.2.1 Virus injection

For all surgeries, mice were anesthetized with isoflurane (induction at 3%, maintained at 1%) and headfixed within a stereotaxic frame (Kopf). For optogenetics experiments, craniotomies were made bilaterally above the lateral SN and the lateral SC. Using a microsyringe, 0.5 µl of AAV-EF1α-DIO-ChR2(H134R)-eYFP (ChR2; n = 7, 4 male) or the control vector AAV-EF1α-DIO-eYFP (n = 6; 4 male) was injected over 10
minutes into the SN targeting the coordinates (in mm relative to Bregma): AP -3.2, ML ± 1.6, DV -4.3 from brain surface. Custom made optic fibers were then implanted targeting the lateral superior colliculus (AP -3.5, ML ± 1.3, DV -2.0). Fibers were secured in place with dental acrylic and skull screws. Mice were allowed to recover for at least two weeks before testing began.

5.2.2.2 Electrode implantation

Mice were anesthetized and headfixed. A craniotomy was made above the site targeting the SN (4 left, 2 right) and dura was removed. Electrode arrays (16-channel, 4x4 tungsten microwires, 35 µm diameter, 150 µm electrode spacing, 200 µm row spacing, 6 mm length; Innovative Neurophysiology) were lowered into place within the SN. A silver ground wire was secured to the skull screws and the array was secured in place with dental acrylic. Mice were implanted in the SN or the SC targeting the above coordinates. Procedures were the same for SC implants except the electrodes were custom made 4 mm long tungsten microwire arrays.

5.2.2.3 Optrode implantation

Vgat-Cre mice were injected with ChR2 as described above. In one hemisphere, an optic fiber was implanted targeting the lateral SC. An optrode was implanted targeting the lateral SC in the opposite hemisphere (n = 3). The optrode was a custom made 4 mm long, 4x4 tungsten microwire array with an optic fiber attached to the lateral side and angled so the cone of the emitted light encompassed the electrode tips. Optrodes were constructed as previously described (Sparta et al., 2012).
5.2.3 Tracing of Nigro-protectal Projections

Wild type mice (p60) were infected by 150nl of EF-1a promoter-driven Flex-AAV-GFP within the SNc (AP: 3.64; ML: 1.6; DV: 4.0). To visualize specific neurons in substantia nigra area that are directly connected with superior colliculus, 150 nl of rabies virus glycoprotein-coated Lenti-FuGB2-Cre (synapsin promoter) (Kato et al., 2011; Nelson et al., 2013) was injected into the superior colliculus (AP: 3.28; ML: 1.0; DV: 2.2). Two weeks after infection, brains were removed, postfixed overnight at 4°C, and then cryo-protected with 30% sucrose in TBS. Brains were cut into 50 µm coronal sections by cryostat (Leica CM 3000). Sections were treated with blocking solution (TBS containing 5% normal goat serum and 0.2% Triton X-100) for 2 hr and incubated overnight at 4°C with rabbit anti-tyrosine hydroxylase polyclonal antibody (1:1,000; Calbiochem) or guinea pig anti-VGAT polyclonal antibody (1:250; Synaptic Systems). After washing three times for 10 min per wash with TBST (TBS containing 0.2% Triton X-100), sections were incubated with Alexa Fluor® 555 IgG (1:500; Molecular Probes) for 1 hr at room temperature. Sections were counterstained with a 4',6-diamidino-2-phenylindole solution (DAPI; Sigma-Aldrich). After washing four times, the sections were coverslipped with FluorSave (CalBioChem) aqueous mounting medium. For axonal fiber tracing, images were taken by tile scan imaging using LSM 710 confocal microscope (Zeiss) with a 10× objective under control of Zen software (Zeiss). To verify the cell type (GFP-positive neurons) in substantia nigra, Images were acquired by z-series (0.5 µm intervals) using a 63× oil-immersion objective.

5.2.4 Consummatory behavior and optogenetic stimulation

Behavioral testing took place in a Med Associates operant chamber (35 cm x 28 cm x 22 cm) designed for in vivo electrophysiology and housed inside a light and sound
attenuating box. The walls and floor grid were made of Plexiglas, and a standard stainless steel drinking spout was located in the center of one wall. The spout was connected to a contact lickometer which recorded the time and duration of licks at 2000 samples/s. Freely-behaving mice were allowed to voluntarily lick for 20% sucrose solution.

For recording experiments, mice were connected to a Blackrock Microsystems recording system prior to testing via a cable extending from the mouse's head to a motorized rotating commutator (Dragonfly), which allowed unimpeded movement.

For optogenetic stimulation experiments, mice were connected to a 473 nm DPSS laser (Shanghai Laser & Optics Century) via fiber optic cables and placed inside the testing chamber (Rossi et al., 2013b). A rotating beam-splitting (50:50) optical commutator (Doric) prevented the mice from becoming tangled in the cables. Optical stimulation was triggered by contact with the lickometer. Stimulation was either pulsed (10 mW; 3-100 Hz, 5 ms square pulse width, 1 s duration) or constant (10 mW; 1 or 2 s duration). There was a 5 s inter trial interval imposed after the initiation of stimulation. Stimulation parameters were consistent within a session, but the order of stimulation was randomized between mice.

For optrode experiments, the experimental set up was the same as the previous experiments, except the mice were connected to both the laser and the recording system. Stimulation occurred in a blocked design in which mice had 1-4 blocks of trials (5-30 trials per block). Each session began with baseline trials in which no stimulation was presented and had at least two blocks (pseudo-randomized). For days in which two different frequencies were tested, the order of stimulation was randomized between mice. To test the efficacy of stimulation when mice were not licking, pulse trains were delivered in blocks of 12 trials (10 s inter trial interval) during free behavior. The
stimulation frequency was held constant within a block but varied between blocks and the order of stimulation was unique for each mouse.

**5.2.5 Motion tracking**

Mice were connected to the laser as described above and placed on an elevated platform (14 x 22.9 cm rectangle, elevated 33.7 cm), which was exposed on three sides to allow for visibility during motion capture. The three dimensional location of a 6.35 mm diameter spherical reflective marker placed on the head was recorded at 100 frames per second using eight infrared motion tracking cameras (Motion Analysis) placed around the platform. The location of the reflective marker was captured in millimeters relative to an x, y, z coordinate origin.

Stimulation trials were conducted in blocks of 10-12, each block consisting of a single stimulation parameter, with the order of these blocks randomized between mice. Stimulation occurred during free behavior, when the mouse was displaying a neutral posture, with a minimum inter-trial interval of 10 s. Stimulation was a 1 s pulse train, with the frequency varying between conditions (3, 10, 50, 100, or laser continuously on), and with a pulse width of 5 ms. Laser, motion capture, and lever press data sets were aligned using TTL timestamps in the Cerebus data acquisition system (Blackrock Microsystems). Head speed in three-dimensional space was calculated from the raw x, y, z position data generated using the Cortex software (Motion Analysis) during motion capture.

**5.2.6 Histology**

Following completion of experiments, mice were deeply anesthetized with isoflurane and perfused with 4% PFA. Brains were post fixed in PFA for ~24 hours and
then transferred to sucrose. For electrophysiology experiments, brains were sliced at 100 µm and stained with thionin. For optogenetics experiments, brains were sliced at 60 µm with a Vibratome 1000 Plus. Slices were incubated with primary chicken anti-GFP (1:1000, AbCam) and Vgat primary rabbit anti-Vgat (1:1000, Millipore) with 10% goat serum and 0.25% Triton X-100 overnight at 4 °C. Secondary antibodies (Alexa Fluor 594 goat anti-rabbit and Alexa Fluor 488 Goat anti-Chicken) were used to visualize Vgat and GFP, respectively (1:250, Molecular Probes). Images were acquired with a Zeiss Axio Zoom.V16.

5.2.6.1 Fluorescence quantification

To quantify ChR2-eYFP fluorescence intensity, a single image of a coronal brain section was acquired for each mouse (Zeiss Axio Zoom.V16). Identically sized regions of interest were drawn within the SNR, lateral SC, and medial SC of each hemisphere. The average pixel intensity for each region of interest was taken. Intensity values were normalized to the background fluorescence (equally-sized sample from hippocampus of the same hemisphere).

5.2.7 Single unit recording and cell classification

Single-unit activity was recorded using the Cerebus data acquisition system (Blackrock Microsystems) as described previously (Fan et al., 2012; Rossi et al., 2013a; Barter et al., 2014). For SN recordings, neurons were classified as either putative GABA or putative DA based on the mean firing rate and waveform shape (Fan et al., 2012; Ungless and Grace, 2012; Rossi et al., 2013a; Barter et al., 2014).
5.2.8 Analysis

Bout analysis was used to determine lick bouts. Based on previous analysis of rodent licking (Spector et al., 1998a), we defined the start of a bout as 3 or more licks occurring at >3 Hz and being preceded by at least 1 second in which no licks were recorded. The end of the bout was defined as the last lick that was followed by at least 1 second in which no licks were recorded.

To examine the relationship between licking and single unit spike rate, the lick rate and spike rate within a bout of licks were sampled in 10 ms bins and Pearson correlation was then performed on the binned rates. To determine the phase relationship between the lick cycle and the neural oscillations, the maximum and minimum firing rates within the lick cycle were calculated. The time of minimum and maximum firing rates were transformed to degrees such that 0 degrees represents the time of tongue contact with the spout and 180 degrees represents the time in which the tongue is fully retracted.

For optrode experiments, a neuron was determined to be light responsive if its firing rate was reduced during the stimulation epoch relative to the firing rate during same amount of time immediately preceding stimulation (paired t-test, \( p < 0.05 \)).

The probability that licking would pause following stimulation was calculated for optogenetics experiments as the probability that no licks would occur within 500 ms of the termination of stimulation. This was calculated for the first 10 trials (early) and the last 10 trials (late) of each session.
5.3 Results

5.3.1 SN GABA neurons, but not DA neurons, reflect lick microstructure

Neuronal activity was recorded from DA and GABA neurons within the substantia nigra (Figures 29a-b) as freely-behaving mice voluntarily licked for sucrose solution using the same lickometer described above (Chapter 4; Figure 22). DA and GABA neurons were classified based on their mean firing rate and the width of the waveform (Figures 29c-d) as previously described (Fan et al., 2012; Rossi et al., 2013a; Barter et al., 2014). In agreement with previous studies using rats, voluntary licking in rodents is highly stereotyped, consisting of bouts of rhythmic protrusions and retractions of the tongue at a relatively fixed rate (6-9 Hz). We found that mice licked in frequent bouts, and within bouts, the lick rate was stable (7.7 ± 0.06 Hz) (Fowler and Mortell, 1992b; Spector et al., 1998b; Weijnen, 1998).

During bouts of licking, the mouse's tongue exhibits rhythmic, stereotyped cyclic protrusions and retractions (Weijnen, 1998) (Figure 30a). This rhythmic tongue movement is reflected in neuronal activity of GABA neurons in the SNR but not DA neurons (Figures 30b-f). Many GABA neurons showed oscillatory activity that peaked at the time of tongue protrusion (when the tongue contacts the lickometer) and was tightly coupled with the pattern of licking (Figures 30d-e). The firing rate of GABA neurons during a bout of licking was positively correlated with the lick rate (Figure 30e, \( r = 0.94, p < 0.0001 \)).
Figure 29. Extracellular recording in the substantia nigra during consummatory behavior. (a) Schematic of electrode array placement within the SN. (b) Photomicrographs of electrode placements for two mice. Electrode positions are indicated by arrowheads. (c) Example waveforms of isolated dopamine and GABA neurons. Median waveforms are shown as colored lines. (d) Cells were classified as dopaminergic or GABAergic based on the width of their waveforms. Inset is population average (mean and s.e.m.) of classified neurons.
Figure 30. SN GABA neurons reflect the microstructure of licking. (a) Probability histogram of licks occurring within a bout of licking. (b) Example GABA neuron that shows oscillatory activity tightly coupled with the lick pattern. (c) Example of dopamine neuron that does not reflect the lick pattern. (d) Lick response and GABAergic neuronal response are overlayed. Data correspond to histograms in a and b. (e) Firing rate of the example GABA neuron is positively correlated with lick rate ($p < 0.0001$). (f) Population response of all recorded DA and GABA neurons.
While dopamine neurons do not oscillate with the lick cycle, a subset (25%, 4/20) burst following the initiation of a drinking bout (Figure 31a-b). These same neurons show a modest pause in activity following the completion of a bout (Figure 31c-d).

Figure 31. Dopamine neurons signal the boundaries of a drinking bout. (a) Example of a neuron that bursts after the initiation of a bout of licking (paired t-test of activity during the 500 ms before and after the first lick in a bout, $p < 0.05$). (b) Population response (4/20 neurons) of neurons that have a phasic burst of activity after the start of a bout. (c) The same neuron from (a) pauses after the end of a bout. (d) Neurons that signal the start of a bout pause once the bout is completed.
5.3.2 Neurons in the lateral SC reflect lick microstructure

The projections from SNR to the lateral regions of the superior colliculus have been implicated in controlling orofacial behavior (Redgrave et al., 1980; Taha et al., 1982; Yasui et al., 1994; Wang and Redgrave, 1997). We recorded single unit activity from the lateral SC during voluntary licking for sucrose solution (Figure 32). Licking behavior was similar to SN recording mice (7.6 ± 0.05 Hz) (Figure 33). Contact duration was similar between SN and SC mice (Figure 33a-b, Mann-Whitney $U = 65.00, p = 0.53$). The period of the lick cycle was also similar between groups (Figure 33c-d, $U = 72.50, p = 0.38$). Similar to the SNR, neurons in the SC showed oscillatory activity that was tightly coupled with the lick cycle (Figure 34a-e). However, in contrast to SNR neurons, which are generally in phase with the lick cycle, SC neurons tend to be out of phase with the lick cycle. The activity peaks during the retraction phase of the lick cycle, resulting in a strong negative correlation between lick rate and spike rate (Figure 34d, $r = -0.89, p < 0.0001$).

Figure 32. Extracellular recording from the superior colliculus. (a) Schematic representation of the recording location ($n = 4$ mice). (b) An example of an isolated single unit. Median waveform is in green. (c) Cluster analysis of SC waveforms. Inset is the average (± s.e.m.) of all waveforms ($n = 74$ neurons).
5.3.3 SN GABA neurons oppose the activity of SC neurons during consummatory licking

SNR and SC neurons exhibit opposing tuning properties during licking (Figure 35a-f). Many SNR and SC neurons fire oscillatory bursts of action potentials that are coupled with the lick cycle. However, these oscillations are antiphase to one another. Many SNR neurons (61%, 53/87) have their peak firing rate at the time of tongue contact, whereas fewer have oscillations that are 180 degrees out of phase with the lick cycle, exhibiting their minimum firing rate at the time of spout contact (23%, 20/87). In contrast, few SC neurons (5%, 4/74) show oscillations that are in phase with licking and peak at the time of spout contact, while a larger proportion (22%, 16/74) are antiphase to the lick cycle and have their minimum response at the time of contact (Figure 35e; $\chi^2 = 13.31, p < 0.0001$).
Figure 34. SC neurons reflect licking microstructure. (a) Probability histogram of licks occurring within a bout of licking. (b) Example SC neuron that shows oscillatory activity tightly coupled with the lick pattern. (c) Lick response and neuronal response are overlayed. Data correspond to histograms in a and b. (d) Firing rate of the example neuron is negatively correlated with lick rate ($p < 0.0001$). (f) Population response of all recorded SC neurons and the lick rate (values are mean and s.e.m).

The antiphase nature of the SNR and SC neural signatures of licking is also evident in the correlation between the lick rate and the neural firing rate (Figure 35f). The activity of many of the recorded SNR neurons was positively correlated ($p < 0.05$) with the lick rate (49%, 43/87). Fewer SNR neurons were negatively correlated with the lick rate (14%, 12/87). The SC, in contrast, shows the opposite pattern. A greater proportion of neurons were negatively correlated with lick rate (45%, 33/74) than were positively correlated (19%, 14/74; $\chi^2 = 24.08, p < 0.0001$).
Figure 35. Opposing neural responses of SNR and SC during licking. (a, b) Population heatmaps showing all recorded neurons in SNR (a) and SC (b) aligned to the start of a lick within a bout. Vertical line represents lick contact. (c) Distribution of the peak response of neurons. (d) Average (± s.e.m.) response during the lick cycle of SC and SNR neurons. (e) The proportion of neurons that have their maximum or minimum response rate at the 0 phase of the lick cycle ($\chi^2 = 13.31, p < 0.0001$). (f) Proportion of neurons whose firing rate is positively or negatively correlated ($p < 0.05$) with the lick rate during a bout of licking ($\chi^2 = 24.08, p < 0.0001$).
Figure 36. SNR GABA neurons project to lateral SC. (a) Tracing approach. Lenti-FuGB2-Cre was injected into lateral SC yielding retrogradely-transported Cre. AAV-FLEX-GFP was injected in SNR, resulting in selective expression of GFP in nigrotectal neurons. (b) Confocal image of GFP labeled neurons. (c) No overlap is observed between TH and GFP expressing neurons. (d) GFP colocalizes with Vgat expressing neurons. (e) Percent of GFP labeled neurons that colocalize with TH or Vgat.
5.3.4 SN GABA neurons project to lateral SC

To confirm the existence of SNR projections to the lateral SC, a retrogradely transported virus encoding Cre recombinase (Lenti-FuGB2-Cre) was injected into the SC of a wild-type mouse. A virus encoding Cre-dependent GFP reporter (AAV-FLEX-GFP) was injected into the SNR (Figure 36a). Nigrotemporal projections were observed (Figure 36b). Because GABAergic and dopaminergic neurons are found in close proximity to one another within the SN, we wanted to determine the cell type specificity of the nigrotemporal projections. GFP-expressing nigrotemporal neurons colocalized with vesicular GABA transporter (Vgat) but not with tyrosine hydroxylase (TH) (Figures 36c-e).

5.3.5 Optogenetic activation of nigrotemporal projections disrupts consummatory behavior

To test whether selective perturbation of these neurons during drinking can alter behavioral output, we expressed Cre-dependent ChR2 bilaterally in the SNR of Vgat-Cre mice (n = 7) and targeted the axon terminals within the lateral SC with optic fibers (Vgat::ChR2$^{SNR\rightarrow SC}$; Figure 37). ChR2 was restricted to non-dopaminergic, Vgat-expressing neurons in the SNR (Figure 37b-c). ChR2-expressing terminals were found in the SC and were particularly dense within the lateral region. Fluorescence intensity was quantified within the SNR and the medial and lateral SC regions of each hemisphere. Fluorescence intensity was similar between the SN and lateral SC, but was lower in the medial SC compared with both the SN and lateral SC (Figure 37e, ANOVA: $F = 22.24, p < 0.0001$, Tukey's multiple comparison test: $p < 0.05$ for medial SC vs. SN and lateral SC).
Figure 37. ChR2 expression in GABAergic SNR→Sc neurons. (a) Example of ChR2-eYFP expression within the SNR. Axons are found within the lateral SC. (b) eYFP expression overlaps with Vgat. (c) There is no overlap between TH (red) and eYFP-expressing neurons. White arrows, Th+ neurons; orange arrows, eYFP+ neurons. (d) Fiber placements (circles) and virus injection sites (triangles) are shown for ChR2 (blue symbols) and eYFP (green symbols) mice used in optogenetics experiments. Coronal sections are from the Allen Mouse Brain Atlas (Lein et al., 2007). (e) eYFP fluorescence intensity was quantified in the SN, lateral SC, and medial SC. The medial SC had less fluorescence than the SN and lateral SC (***p < 0.0001).
Figure 38. Vgat::ChR2$^{SNR→SC}$ activation disrupts consummatory behavior. (a) Schematic representation of optogenetic stimulation strategy. Cre-dependent ChR2 was bilaterally injected into the SNR of Vgat-cre mice. Optic fibers targeted nigroretinal axon terminals in the lateral SC. (b) Depiction of lick-triggered optogenetic stimulation. A computer program triggered the laser after the start of a licking bout. (c–e) Peri-stimulation lick plots from one mouse. (c) When no stimulation is present, the lick rate remains elevated following the initiation of a lick bout. When 1 s (d) or 2 s (e) constant illumination (~10
mW) is delivered during a lick bout, licking is temporarily interrupted and rebounds following the termination of stimulation. The inset in (d) is a single trial example of when licking was completely abolished during stimulation. (f) Group \((n = 7)\) average (± s.e.m.) peri-stimulus lick rate when constant illumination is used. (g) Peri-stimulation lick rate when pulsed stimulation (1 s trains; 5 ms square pulses; ~10 mW at 3-100 Hz) is used. (h) Average (± s.e.m.) lick rate during stimulation. The lick rate decreases as a function of stimulation frequency (one-way ANOVA, \(p < 0.0001\)).

Figure 39. Vgat::ChR2SNR→Sc activation does not change the global rate of licking but alters the lick pattern. (a) The rate of licking was not affected by stimulation. (b) The number of bouts was increased constant illumination. (c) The number of licks per bout was decreased by constant illumination. (d) Probability that mice pause licking as a result of stimulation. * \(p < 0.05\). Values are mean and s.e.m.
During behavioral tests, laser stimulation was triggered when mice licked for sucrose solution (Figure 38a-b). Constant illumination of the nigrosectal projections reduced the lick rate for the duration of the stimulation (Figure 38c-f). Vgat::ChR2^{SNR→SC} stimulation reduced the lick rate in a frequency-dependent manner (Figure 38g-h; one-way repeated measures ANOVA, $F_{(6,36)} = 19.55$, $p < 0.0001$, Dunnett's multiple comparison test: $p < 0.01$ for No Stim vs 10 Hz, 50 Hz, 100 Hz, 1 s on, and 2 s on). High frequency stimulation as well as constant illumination were most effective at disrupting licking.

Vgat::ChR2^{SNR→SC} stimulation did not affect the overall lick rate (Figure 39a; $F_{(6,36)} = 1.50$, $p = 0.21$). The pattern of licking, however, was altered. Vgat::ChR2^{SNR→SC} stimulation increased the number of licking bouts (Figure 39b; $F_{(6,36)} = 5.91$, $p = 0.0002$) and reduced the number of licks per bouts (Figure 39c; $F_{(6,36)} = 2.45$, $p = 0.04$). This is consistent with stimulation transiently interrupting the motor output associated with consummatory behavior. Motivational state impacted the efficacy of stimulation. When mice were more motivated (early in the session), constant illumination was less effective at eliciting a pause in licking behavior than when mice were less motivated for sucrose late in the session (Figure 39d; two-way repeated measures ANOVA: main effect of Motivation $F_{(1,42)} = 19.23$, $p < 0.0001$; main effect of Frequency $F_{(6,42)} = 8.18$, $p < 0.0001$; interaction between Motivation and Frequency $F_{(6,42)} = 3.97$, $p < 0.003$). Constant illumination was more effective at suppressing licking when mice were less motivated ($p < 0.05$).
To test whether the nonspecific effects of laser illumination or viral infection contributed to the reduced licking rate during Vgat::ChR2^{SNR→SC} stimulation, Vgat-Cre mice (n = 6) were injected with the AAV-FKEX-eYFP control vector (Vgat::eYFP^{SNR→SC}). Under similar experimental conditions, laser stimulation in the absence of ChR2 had no effect on the lick rate (Figure 40; \(F_{(6,30)} = 1.59, p = 0.18\)).

Figure 40. Control mice are unaffected by laser stimulation. (a-c) Peri-stimulation lick rasters for no stimulation (a), 1 s constant illumination (b) or 2 s constant illumination (c). Vgat::eYFP^{SNR→SC} Stimulation had no effect on the rate of licking. (d) Group average peri-stimulation lick rate. (g) The rate of licking was unaffected by light stimulation in the absence of ChR2. Values are mean and s.e.m.
Since the superior colliculus has been implicated in head movement and orienting behavior (Redgrave et al., 1992; Wang and Redgrave, 1997), we wondered whether the Vgat::ChR2\textsuperscript{SNR→SC} stimulation used to disrupt orofacial behavior caused aberrant head movements. To test whether nigro-tectal stimulation induced head movement, we stimulated Vgat::ChR2\textsuperscript{SNR→SC} and Vgat::eYFP\textsuperscript{SNR→SC} mice while we tracked the mouse’s head using a high-resolution tracking system. There was no effect of stimulation on head speed (Figure 41; two-way repeated measures ANOVA: no main effect of Group, $F_{(1,36)} = 2.66, p = 0.13$; no main effect of Frequency, $F_{(4,36)} = 1.56, p = 0.20$, no interaction between Group and Frequency $F_{(4,36)} = 0.71, p = 0.59$).

Figure 41. Vgat::ChR2\textsuperscript{SNR→Sc} activation does not cause head movements. The speed of the mouse’s head was tracked at 100 Hz using high-resolution motion tracking. There was no effect of stimulation frequency, no difference between ChR2 and eYFP mice, and no interaction (two-way ANOVA, $p > 0.05$).
5.3.6 Vgat::ChR2$^\text{SNR→SC}$ activation inhibits SC neurons to disrupt licking

To understand the effect of Vgat::ChR2$^\text{SNR→SC}$ stimulation on downstream neurons, we performed extracellular recordings from neurons in the superior colliculus while mice ($n = 3$) licked for sucrose (Figures 42-44). As in the previous experiment, Vgat::ChR2$^\text{SNR→SC}$ activation reduced the lick rate (Figure 42b, paired t-test: lick rate during stimulation trials vs. lick rate during within-session no stimulation baseline trials: $t = 4.52, p < 0.05$). Consistent with the hypothesis that GABAergic SNR neurons inhibit downstream targets, the activity of SC neurons (65%, 11/17) was transiently suppressed during stimulation (Figure 42c; paired t-test of spike rate during stimulation vs. rate immediately before stimulation, $p < 0.05$). The spike rate of these neurons was reduced during the stimulation epoch and rebounded immediately after stimulation ceased (Figure 42d-e, planned comparison paired t-tests pre vs. stim: $t = 50.62, p < 0.0001$ and stim vs. post: $t = 10.78, p < 0.0001$). When the activity of SC neurons is optogenetically suppressed ($t$-test using the activity from all trials within the session: $t_{(34)} = 3.85, p = 0.001$), the overall lick rate was also suppressed (Figure 42f-g; t-test with Welch's correction for unequal variance: $t_{(31)} = 6.27, p < 0.0001$).

SC neurons responded to stimulation with a frequency-dependent suppression of firing during free behavior in which the mice were not licking (Figure 43a; repeated measures ANOVA of firing rate in 18 neurons recorded from 3 mice: $F_{(6,125)} = 9.14, p < 0.0001$; 50 Hz, 100 Hz, 1s ON and 2s ON conditions were reduced relative to baseline rates, $p < 0.001$). When stimulation occurred during licking, we observed a similar frequency-dependent reduction in the lick rate as in the previous Vgat::ChR2$^\text{SNR→SC}$ experiments (Figure 43b; repeated measures ANOVA: $F_{(6,20)} = 6.23, p = 0.0035$; 50 Hz, 100 Hz, 1s ON and 2s ON conditions were reduced relative to baseline rates in which no stimulation occurred, $p < 0.05$). The reduction in lick rate coincided with suppression of
the firing rates of SC neurons that was similar to the suppression observed during stimulation that was not contingent on licking (Figure 43c; ANOVA: $F_{(6,134)} = 7.37$, $p < 0.0001$; 50 Hz, 100 Hz, 1s ON and 2s ON conditions were reduced relative to baseline rates in which no stimulation occurred, $p < 0.01$).

Since SC neurons show oscillations in activity that are coupled with the lick cycle (Figure 34), we wondered what effect Vgat::ChR2$^{SNR\rightarrow SC}$ stimulation had on these oscillations. Similar to the previous recordings, SC neurons recorded in optrode experiments showed oscillatory activity that was out of phase with the lick cycle (41%, 7/17 neurons). During trials in which stimulation occurred, yet licking was not entirely eliminated, the lick pattern was disrupted. In agreement with the disrupted consummatory behavior, the oscillatory activity of SC neurons was also disrupted (Figure 44), suggesting that the oscillatory waves of SNR and SC activity contribute to the patterning of orofacial behavior.
Figure 42. (on previous page) Vgat::ChR2<sup>SNR→SC</sup> activation inhibits SC neurons during consummatory behavior. (a) Schematic of optrode recording. (b) Activation of nigrothalamic projections during licking reduced the lick rate ($p < 0.05$). 65% (11/17 neurons from 3 mice) of neurons were inhibited during laser stimulation ($p < 0.05$). (c) Average response of all inhibited neurons. (d) Inhibition is transient. Spike rates rebound immediately after stimulation ($p < 0.0001$). (e) Population heatmap showing activity of all inhibited neurons during stimulation. (f) Example neuron recorded during licking. This neuron is inhibited by stimulation (right panel, $p < 0.01$). Laser stimulation does not affect the waveform shape. Sample waveforms of spikes recorded during the baseline and stimulation epochs are shown above the rasters. Median waveform is in red. (g) Licking behavior during the trials shown in (e). When the laser is on, the lick rate is reduced (right panel, $p < 0.01$). Values are mean and s.e.m.

Figure 43. Frequency-dependent reduction in SC spike rate and corresponding reduction in consummatory behavior. (a) During free behavior, the population response of SC neurons is reduced by high-frequency stimulation. (b-c) During consummatory licking, high-frequency Vgat::ChR2<sup>SNR→SC</sup> stimulation reduces the lick rate (b) and the firing rate of SC neurons (c). * $p < 0.05$ compared to BL values. Values are mean and s.e.m.
Figure 44. Vgat::ChR2 $^{SNR+SC}$ activation disrupts SC oscillatory activity. (a) Example neuron that shows oscillatory activity that is coupled with the lick cycle during the baseline period. When stimulation occurs during licking, consequently interrupting the pattern of consummatory behavior, the oscillatory activity is suppressed. (b) Population response (7 neurons from 3 mice) of neurons that paused at the time of spout contact during the baseline period. During stimulation trials, the oscillatory activity was suppressed. Values are mean and s.e.m.
5.4 Discussion

These experiments demonstrate that in freely moving mice, neurons in both the substantia nigra and the superior colliculus exhibit oscillatory activity that closely reflects the pattern of consummatory behavior (Figures 30 and 34). The putative GABAergic output neurons of the SNR tend to fire in phase with the lick cycle. That is, they show bursts of action potentials when the tongue extends, peaking at the time of spout contact. Neurons in the lateral part of the superior colliculus, which receive inhibitory input from the SNR, show an opposing pattern of activity during licking. These neurons tend to peak when the tongue is retracted and pause during spout contact. Channelrhodopsin-2 mediated activation of nigrotectal projections suppresses lick-related activity of SC neurons and consequently disrupts consummatory behavior. Together, these results suggest that the nigrotectal pathway is a critical mediator of consummatory drinking behavior.

5.4.1 Dopamine and consummatory licking

Interestingly, dopamine neurons of the SN, which have been implicated in regulating orofacial behaviors such as licking and chewing, did not show activity reflecting the lick microstructure. Dopamine activity may be more important for initiating and terminating bouts of licking as well as appetitive approach behavior (Chapters 2-4). This is supported by the finding that some dopamine neurons burst after the start of a drinking bout and pause following the completion of a bout (Figure 31). In accordance with the anatomical layout of the basal ganglia, dopamine may represent a functionally higher-order neural signal relative to the output neurons of the SNR. One possibility is that dopamine contributes to the macrostructure of a bout of licking, signaling the bout
boundaries (i.e., the transitions associated with starting and ending a bout) rather than the microstructure (i.e., tongue position).

### 5.4.2 Nigrotectal regulation of consummatory behavior

The putative GABAergic output neurons of the SNR and downstream SC neurons exhibit opposing patterns of activity during licking (Figure 35). Optogenetic activation of nigrotectal neurons during licking inhibits SC neurons (Figure 42). This is consistent with previous electrophysiological studies demonstrating the inhibitory effects of nigral neurons on post-synaptic collicular neurons (Chevalier et al., 1981; Hikosaka and Wurtz, 1983b). These studies have often considered the function of nigrotectal projections in visually guided eye movements (Hikosaka and Wurtz, 1983b; Sakamoto and Hikosaka, 1989), although the occulomotor related neurons may represent only a minority of nigral neurons (DeLong et al., 1983). In rodents, which do not rely heavily on vision, the nigrotectal projections are likely also related to orofacial behavior, whisking, or orienting.

#### 5.4.2.1 Nigrotectal projections regulate consummatory behaviors independently of orienting response

Vgat::ChR2<sub>SNR→SC</sub> stimulation did not produce overt head movements (Figure 41). Although the nigrotectal pathway has been implicated in orienting behavior in rodents and primates (Taha et al., 1982), we did not observe any stimulation induced head turning. In addition, licking during stimulation was often not completely abolished, but rather was slower and irregularly patterned (Figures 38 and 42). Thus, the mice were not completely breaking contact with the spout as would be expected if stimulation produced rearing or head turning. We hypothesize that this is due to the placement of
optic fibers within the deep and lateral regions of the superior colliculus, which may represent a region with specific orofacial motor functions. The superior colliculus is known to be topographically organized. In rodents, the SC is somatotopically organized such that the most lateral region receives input from the mouth and rostral face (Drager and Hubel, 1976).

The present data suggest a role for nigrotectal projections in selectively regulating orofacial behavior. One possibility is that the lick-related nigral activity represents a signal specifying the tongue position for downstream behavioral control systems. This is supported by previous work demonstrating that the firing rates of SNR GABA neurons correspond to the change in position of a mouse's head in space (Barter et al., 2015). When mice are at rest, the SNR neurons maintain tonic firing rates. Distinct populations of neurons monotonically increase firing rates as mice move their heads in a particular direction (e.g., to the mouse's left) and the same neurons decrease proportionally as the mouse moves its head in the opposite direction (e.g., to the right). Because of this, these neurons are thought to specify the position that a mouse moves its head. The same principle may underlie changes in tongue or mouth position during licking. However, the technology to precisely and continuously track the tongue position that would be needed to directly test this hypothesis is unavailable.

Lick related SC neurons are downstream of nigrotectal projection neurons. However, the exact relationship between the activity of lick-related SNR neurons and the post-synaptic neurons in the SC during licking remains to be tested. The optrode recording data demonstrate that Vgat::ChR2SNR→SC activation potently inhibits neurons in the lateral SC and that this inhibition is highly correlated with the magnitude of inhibition of consummatory behavior (Figure 43). The stimulation has the potential to activate all nigrotectal projection neurons, which are likely to be heterogeneous in function.
Presently there is no biochemical marker that distinguishes lick-related nigrotectal projections, but the anatomical layout may confer some orofacial specificity. This is supported by the fact that Vgat::ChR2<sup>SNR→SC</sup> stimulation did not induce overt head movements.

### 5.4.3 Relationship between SC and oral premotor neurons

In rodents, neurons in the lateral part of the superior colliculus located near the site of termination of the nigrotectal axons send projections to the medullary reticular formation (Yasui et al., 1994), which is the site of oral premotor neurons (Travers et al., 1997b, 2000; Stanek et al., 2014). The oral premotor neurons in the reticular formation make connections with hypoglossal motor neurons, which control the muscles of the tongue. The projections from the lateral SNR to the lateral SC are thus anatomically positioned to contribute to the generation of oral behaviors. Neurons within the reticular formation and the hypoglossal nucleus exhibit oscillatory activity—similar to that observed in the present study—that is tightly coupled with the lick cycle (Wiesenfeld et al., 1977; Nakamura et al., 1980; Travers et al., 2000). However, the activity of reticular neurons that are rhythmically active during licking is distributed throughout the lick cycle (Travers et al., 2000). Thus, it remains to be tested how the distinct antiphase oscillatory patterns observed in the SNR and SC relate to specific activity patterns of oral motor and premotor neurons.

### 5.4.4 Vgat::ChR2<sup>SNR→SC</sup> stimulation and motivation

One interpretation of the result that mice reduce their rate of licking during optogenetic stimulation of the nigrotectal pathway is that the stimulation reduces
motivation to consume the sucrose solution. While the present data cannot explicitly rule out this possibility, the time course of the behavioral response makes it unlikely to be the case. Motivational effects on neural activity tend to act slowly, gradually changing appetitive behavior (Rossi et al., 2013a). Since the reduction in lick rate occurred transiently within the stimulation epoch, and repeated stimulations did not alter the overall lick rate (Figure 39a), but rather interrupted an ongoing bout of licking (Figure 39b-c), we hypothesize that the effects are restricted to the orofacial motor output. While Vgat::ChR2SNR→SC stimulation did not seem to directly affect the motivation to lick, the animal's motivational state did influence the efficacy of stimulation (Figure 39d). Early in the session, when mice are highly motivated, constant illumination is less effective at causing a pause (>500 ms) in licking than late in the session, when they are less motivated.
6. General Discussion

6.1 Summary

6.1.1 Summary of chapter 2

We performed chronic extracellular recordings of electrophysiologically identified dopamine and GABA neurons of the substantia nigra as mice consumed food pellets that were preceded by an auditory cue. Food seeking behavior declined as mice gradually became sated. This gradual satiation was accompanied by gradual changes in the magnitude of the phasic neural response to the pellet-predicting cue. Within both GABA and dopamine neuron populations, subsets of neurons increased or decreased their phasic response as the mice became sated. When motivational state was held constant by pre-feeding, which rapidly induced satiety before testing, or by withholding the pellets thereby preventing satiety, the bidirectional modulation of the phasic responses of both populations was eliminated.

6.1.2 Summary of chapter 3

Hyperdopaminergic DAT knockout mice showed elevated food seeking behavior relative to heterozygotic and wild-type controls. This phenotype was prevented by pre-feeding and extinction, suggesting that the elevated food seeking was dissociable from general hyperactivity observed in these mice. Viral restoration of DAT within the dorsolateral striatum or the nucleus accumbens, but not the dorsomedial striatum, rescued food-seeking behavior but failed to rescue hyperactivity or impaired instrumental learning.
6.1.3 Summary of Chapter 4

Hyperdopaminergic DAT knockout mice exhibited altered consummatory behavior when voluntarily drinking sucrose solution. This was characterized by a persistence of licking within a bout, resulting in fewer bouts that contained more licks and a greater duty cycle of lick duration relative to wilt-type controls. DAT knockout mice were more sensitive to extinction than were wilt-type mice. Knockout mice adjusted their behavior, as measured by an increased variability in lick duration, much more quickly than controls, supporting the hypothesis that dopamine facilitates behavioral transitions.

6.1.4 Summary of chapter 5

Electrophysiological recordings from dopamine and GABA neurons in the substantia nigra during voluntary drinking revealed that GABA neurons, but not dopamine neurons, exhibited oscillatory activity that was highly correlated with the microstructure of licking behavior. SNR GABA neuron activity tended to be in phase with licking. Neuronal oscillations peaked near the time that the tongue was fully extended. Dopamine neurons, in contrast, did not show activity related to the lick microstructure, but rather signaled the start and end of bouts. Neurons recorded from the lateral region of the superior colliculus also showed oscillatory activity that was highly correlated with licking; however, these neurons tended to be antiphase to the SNR neurons. They peaked when the tongue was retracted and paused during extension. Optogenetic activation of the nigrotectal pathway during ongoing consummatory behavior, which inhibited SC neurons, interrupted licking behavior and reduced the lick-related oscillations of the SC neurons.
Together, the results of these experiments extend the current understanding of how the basal ganglia and an immediate downstream target, the superior colliculus, contribute to motivated behavior.

6.2 The function of dopamine in motivated behavior

A logical question that is raised by the results of Chapter 2 is what aspects of behavior does the changing cue response represent? One possibility is that the phasic dopamine burst signals transitions between repertoires of behavior (i.e., rest vs. food seeking). This would be in agreement with recent work suggesting that dopamine neurons in the SNC regulate transitions between action sequences (Jin and Costa, 2010). The magnitude of the phasic cue response is highly correlated with the magnitude of food seeking (rate of food cup entries) and latency to enter the food cup after the cue. It has previously been proposed that that tonic dopamine levels can invigorate responding (Niv, 2007). In the present study, however, tonic dopamine levels did not change with motivational state (Figure 11). Furthermore, phasic dopamine bursts have been argued to represent reward value (Morris et al., 2004) or a prediction error signal (Schultz et al., 1997). But the presence of two opposing neural populations, one that increases the phasic response and one that decreases the phasic response with satiety, presents a challenge for this type of interpretation. Since the perceived value of the pellet is only changing in one direction (i.e., value is decreasing as mice become sated), and the cue is a perfect predictor of the pellet, the presence of opposing, bidirectionally modulated signals cannot be explained by current theories. But if the phasic dopamine response is not representing a prediction error, then what is its function?
Recent reports have confirmed the presence of dopamine and GABA populations within the SN that have opposing response properties during free movement (Fan et al., 2012; Barter et al., 2014; Barter et al., 2015). Thus, I hypothesize that the changing phasic responses to the cue are related to some movement parameter (e.g., posture, head position). Indeed, subsequent projects addressing this very question have given support to these possibilities (Barter et al., 2014; Barter et al., 2015). When movement kinematics are carefully monitored, neural response properties are found to correlate quite strongly with behavior. Although the FT 60 task was insufficient to carefully track the kinematics of the mice, it is likely that the phasic responses were related to the mouse’s movements and that the changing response magnitudes reflect discrete changes in the properties of movement.

As discussed above, it is hypothesized that nigrostriatal dopamine may serve as a gain control on descending corticostriatal signals. The present data support this possibility. The phasic dopamine response of decreasing neurons may act as a gain function for descending commands that are intended to initiate movement toward the food cup. As the gain (i.e., dopamine signal) is reduced with satiety, we observe a corresponding reduction in the rate of food cup entries. This possibility is also supported by the DAT knockout data (Chapters 3 and 4). If the dopamine signal is a gain control on a descending command that corresponds to transitions or velocity of movement, then one would predict that hyperdopaminergic DAT knockout mice would show potentiated transitions during free behavior. This is exactly what is observed. DAT knockout mice have an extreme response to novelty and are hyperactive. Moreover, the DAT knockout mice change their consummatory behavior much more quickly than wild type mice when sucrose solution is unexpectedly removed from the drinking spout (Figure 26). The rapidity with which the hyperdopaminergic DAT knockout mice adjust their behavior in
response to extinction supports the hypothesis that dopamine regulates behavioral transitions.

Interestingly, DAT knockout mice show elevated food seeking behavior that is dissociable from general hyperactivity. This suggests that dopamine (or a subset of the dopamine neuron population) may have a more specific role in controlling food-directed behavior in addition to its more general role in movement. It has previously been argued that burst of action potentials from SNC dopamine neurons signal initiation and termination of action sequences (Jin and Costa, 2010). Similarly, we found that a small (~20%) subset of dopamine neurons recorded during consummatory licking signaled the boundaries of a bout of licking. These neurons exhibited a burst of action potentials immediately after a bout began and a moderate pause after the bout ended. One possibility is that the phasic dopamine burst that signals the start of a bout of licking is responsible for initiating and maintaining the lick bout (i.e., an action sequence). Based on this hypothesis, one might predict that DAT knockout mice, which have prolonged synaptic dopamine following phasic release (Giros et al., 1996), might show a corresponding persistence of the consummatory action sequence once it is initiated by a phasic dopamine burst. This possibility is supported by the fact that DAT knockout mice persist in licking once a bout is initiated more than control mice (i.e., more licks per bout).

Together, these data support the hypothesis that dopamine may be related to velocity (and by extension, transition) control. However, more work is required to parse the specific contributions of unique populations of dopamine neurons. For example, do the lick-related dopamine neurons project to the ventrolateral striatum, an area that has been explicitly implicated in controlling consummatory behavior?
6.3 *The function of SNR in motivated behavior*

While nigral dopamine has been studied extensively in the context of motivation and reward, the GABAergic output neurons of the SNR have received comparably little attention. We found that SNR GABA neurons—like the dopamine neurons of the SNC—fire phasic bursts of action potentials in response to the reward predicting cue (Figure 3). Like dopamine neurons, the magnitude of this phasic burst was bidirectionally modulated by the animal's motivational state. But unlike dopamine neurons, the tonic firing rates of modulated GABA neurons were also modulated by motivational state.

It has recently been proposed that GABAergic output neurons of the SNR signal the position of a mouse's head (Barter et al., 2015). The motivational modulation observed in GABA neurons may reflect changes in food-directed behavior that accompany satiety. In light of recent data, the most parsimonious explanation for these results is that the modulation of GABA neurons reflects changes in the mouse's head position relative to the food cup. When motivation is high, the mouse consistently moves toward the food cup in response to the cue in order to collect the pellet. When motivation is low, this movement changes, which is characterized by longer latencies to enter the food cup and reduced overall rates of entry (Figure 8). Thus the altered food-directed behavior is reflected in the firing rates of GABA neurons.

The downstream targets for the modulated neurons are unknown, but one efferent connection of particular relevance is the nigrotectal projection. The activity of SNR output neurons to the SC may represent a reference signal that allows downstream systems to adjust behavior in a continuous manner. This process has been studied in detail in the superior colliculus. Stimulation of particular regions of the visual SC produces foveation: bringing a perception into the fovea of the eye. This is
accomplished by moving the eyes until the desired object is perceived on the fovea. Other modalities may rely on similar principles. To address this, I used consummatory licking as a model behavior. Nigral control of licking, for example, may represent a sort of foveation for the tongue. In this way, the reference signal from the SNR to the SC may specify the position of the tongue relative to the mouth.

Though the present data support this possibility, additional experiments will be necessary to determine the exact relationship between tongue position and neural activity. Since position control is a continuous process, there is likely a continuous relationship between the neural systems that regulate the behavior and the behavior itself. Importantly, to test this, a continuous measure of tongue movements is required. The present lickometer design only gives a proxy for tongue position. We know when the tongue contacts the spout, and based on the established sinusoidal nature of the oscillations of the tongue during licking, we can deduce its position with some confidence. However, to establish the exact relationship between tongue position and the firing rates of lick-related SNR or SC neurons, we need a continuous position measure.

One way to accomplish this would be to use high-speed video tracking of the mouse’s tongue during licking. Combined with optrode manipulations in which we can perturb the circuit while measuring neural activity, we could begin to understand the system as a whole. That is, if the neural activity of a lick-related SNR neuron is increased by 50% of its baseline firing rate, what happens to the tongue’s position? The present data suggest that an increase in the firing rate of SNR neurons could produce a fixed tongue position, thereby interrupting the lick cycle altogether. However, nigrotectal activation reduced the lick rate on some trials, and on others abolished licking altogether. It is also difficult to know whether the inconsistent behavioral effects
observed during optogenetic stimulation of nigrotectal fibers is caused by inconsistent neuronal activation or some other unknown factor. One possibility is that the effectiveness of stimulation depends on the exact position or velocity of the tongue at the onset of stimulation. For example, it may be the case that when the tongue is fully retracted in the mouth, nigrotectal stimulation is more effective at abolishing licking, but if the stimulation begins when the tongue is transitioning between protrusion and retraction, the lick cycle is slowed rather than abolished because the ongoing oscillations are sufficient to override the effects of exogenous stimulation. Having a continuous measure of the tongue position will help to address this issue.

6.4 Implications

Understanding behavior is an essential goal of neuroscience research. Motivated feeding behavior, specifically, provides a particularly convenient window into the systems that control basic survival processes and allows researchers to systematically test the functional organization of the system. Feeding behavior is ubiquitous, and animals rely on both higher-order descending signals as well as lower level pattern generators to control intake of sustenance. The basal ganglia are especially important for controlling both appetitive and consummatory aspects of feeding. In human patients as well as in laboratory animals, disruption of basal ganglia pathways at multiple levels can cause profound deficits in both appetitive and consummatory behavior. However, the mechanisms underlying these deficits are not always clear.

The present data capitalize on recent technological advancements to selectively manipulate defined neuronal populations and extend our understanding of the neural
substrates of motivated behavior. These experiments implicate the nigrostriatal dopamine system in controlling general appetitive processes, such as the persistence of food seeking, while the downstream basal ganglia output is necessary for controlling consummatory motor commands. Specifically, a subset of the nigrotectal projections are important for controlling orofacial behavior. These data fit with the hypothesis that the basal ganglia serve as a transition control system for voluntary behavior and suggest that there are functionally distinct pathways within the basal ganglia that may contribute specifically to feeding behavior. Notably, the projections from the dorsolateral striatum to the lateral substantia nigra pars reticulata and those from the substantia nigra to the lateral superior colliculus are critical for feeding behavior. Hyperdopaminergia produces excessive food seeking, which can be rescued by viral restoration of the dopamine transporter within the dorsolateral striatum. Downstream from that, perturbation of the GABAergic nigrotectal pathway disrupts consummatory behavior. With these findings, future investigations can further hone in on the cellular mechanisms that underlie these processes and shed light on how this circuit may go awry in pathological patients.

6.5 Limitations and Future Directions

The present experiments can help to inform disorders of the basal ganglia as well as serve as a model circuit for understanding voluntary behavior. While these experiments have provided new insights into the regulation of appetitive and consummatory behaviors by the basal ganglia, it is important to remember that these pathways probably contribute to other aspects of behavior as well. For example, nigrostriatal dopamine is involved in a wide variety of behaviors, not just appetitive and consummatory behavior. One possibility that is supported by my experiments is that
nigrostriatal dopamine may act as a gain signal on a transition control system. This hypothesis can be directly tested by future experiments. Furthermore, the nigrosectal projections are likely to contribute to behaviors that extend beyond orofacial control. This pathway has been previously implicated in eye and head movements that underlie orienting and whisking. Here I demonstrate its importance in orofacial behavior.

Understanding the function of this pathway in the context of a precisely-defined, natural behavioral repertoire has the potential to inform our understanding of basic behavioral control. The anatomical and functional data suggest that systems for posture, orienting, and licking (and maybe others) may use similar processes to control behavior. But exactly how this is accomplished by the brain is still unclear.

A shortcoming of the present data is that we often have no molecular or anatomical markers to identify functionally distinct neural populations. As a result, many questions remain which may guide future investigations of basal ganglia regulation of feeding behavior. For example, what is the function of the 'increasing' and 'decreasing' phasic response and do these neurons have unique efferent or afferent connections? Do these functionally distinct populations have unique molecular biomarkers that can facilitate selective manipulation? How does SNR activity change in hyperdopaminergic mice, and are these changes in neuronal activity related to the observed appetitive and consummatory phenotypes? The persistence of licking during a bout that is observed in dopamine transporter knockout mice suggests that a transient dopamine signal—which would presumably be prolonged in these mice—may determine the duration of the bout. Thus, would selective manipulations of the dopamine neurons that signal the boundaries of a lick bout influence the persistence of licking? Are lick-related SNR neurons restricted to the nigrosectal pathway and what proportion of nigrosectal neurons are lick-related? Are there anatomical differences (i.e., afferent and efferent connections)
between SNR GABA neurons that are in phase with the lick cycle and those that are out of phase? Are the same SC neurons that are inhibited by nigrotemporal stimulation those that project to oral premotor neurons in the reticular formation? Future experiments will be needed to address these questions.
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Biography

Mark Allen Rossi was born in Detroit, Michigan, USA on February 10, 1987. He attended the University of Michigan-Dearborn in Dearborn, Michigan, USA and received his Bachelor of Arts degree in Psychology with a concentration in Biology in December 2009. He attended Duke University In Durham, NC, USA and received his Doctor of Philosophy in Psychology and Neuroscience in May 2015. At Duke University he published the following articles:

1) Prefrontal cortical mechanisms underlying delayed alternation in mice (*Journal of Neurophysiology*, 2012)

2) Operant self-stimulation of dopamine neurons in the substantia nigra (*PLoS ONE*, 2013)

3) Bidirectional modulation of substantia nigra activity by motivational state (*PLoS ONE*, 2013)

4) A wirelessly controlled implantable LED system for deep brain optogenetic stimulation (*Frontiers in Integrative Neuroscience*, 2015)