FRUSTRATIVE NONREWARD AND THE BASAL GANGLIA:

FUNCTION OF NUCLEUS ACCUMBENS AND GLOBUS PALLIDUS EXTERNUS

DURING CONSUMMATORY REWARD DOWNSHIFT

By

Sara Guarino

Bachelor of Arts, 2016
St. Mary's University
San Antonio, Texas

Master of Science, 2018
Texas Christian University
Fort Worth, Texas

Keywords: Nucleus Accumbens, Globus Pallidus Externus, Consummatory Reward Downshift, Open Field, Chemogenetics

A Dissertation Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of requirements
for the degree of

Doctor of Philosophy in Experimental Psychology

Fort Worth, Texas
December 2021

FRUSTRATIVE NONREWARD AND THE BASAL GANGLIA: FUNCTION OF NUCLEUS ACCUMBENS AND GLOBUS PALLIDUS EXTERNUS DURING CONSUMMATORY REWARD DOWNSHIFT

By

Sara Guarino

Dissertation approved:

Dr. Mauricio R. Papini, Professor of Psychology

Dr. Kenneth J. Leising, Associate Professor of Psychology

Dr. Anna I. Pétursdóttir, Associate Professor of Psychology

Dr. Brenton G. Cooper, Associate Professor of Psychology

Dr. Heather M. Hill, Professor of Psychology

For The College of Science and Engineering

ACKNOWLEDGEMENTS

This dissertation research would not have been possible without the support of many people. I would like to thank my advisor, Dr. Mauricio R. Papini, for your mentorship, guidance, encouragement, and advise you provided throughout my time as your student. I would also like to thank Drs. Anna Pétursdóttir, Kenneth Leising, Brenton Cooper, and Heather Hill for contributing their expertise as members of my committee. Additionally, this research would not have been possible without the help and insight of graduate student Christopher Hagen; thank you for contributing to every aspect of this research, for introducing the perfusion procedure and the c-Fos assays to our lab. Many thanks to Quynh Nguyen for your dedication to this work and for your extensive help with behavioral data collection for this research during your time as undergraduate student in our lab. Special thanks to Lindy Bledsue for your constant support and tireless work in the vivarium. Extra special thanks to Heather Hill, not only for the great insight into my research work, but also for your constant support, encouragement, and friendship in each and every aspect of my life for the past 9 years. Many special thanks for too many reasons to be listed here go to Luca Ceresa, for your help with data collection for c-Fos assessment, but extra special thank you for sharing every mile walked in the snow to and from TCU during the winter storm. A very special thank you, Alessandra, for way too many reasons, but most importantly for being my best friend and yoga teacher. I could not have done it without your presence and "Yoga After Dark" during the COVID-19 pandemic. Thank you for sharing your passion for yoga that has been a life-changing experience and an inspiration for my future. To quote you, "Forward from here". Last, but not least, many thanks to my parents, Antonio and Elena, for their endless patience and support for the past 29 years of my life. Vi voglio un mondo di bene!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abbreviations

µl: microliter

AAV: adeno-associated virus

ACC: anterior cingulate cortex

ANC: anticipatory negative contrast

ANOVA: analysis of variance

AP: anterior/posterior

BG: basal ganglia

BLA: basolateral amygdala

CDP: chlordiazepoxide

CeA: central nucleus of the amygdala

cm: centimeter

CNO: clozapine N-oxide

CP: caudate-putamen

cRD: consummatory reward downshift

dB: decibel

DA: dopamine

DMS: dorsomedial striatum

DMSO: dimethyl sulfoxide

DREADDs: designer receptors exclusively activated by designer drugs

DPDPE: [d-pen2,d-pen5]encephalin

DV: dorsal/ventral

EGFP: enhanced green fluorescent protein

EPM: elevated plus maze

Exc: excitatory

GABA: gaba-amino butyric acid

GPe: globus pallidus externus

GPi: globus pallidus internus

GT: gustatory thalamus

IC: insular cortex

Inh: inhibitory

Ip: intraperitoneal

IRt: intermediate zone of reticular formation

iSNC: instrumental successive negative contrast

kg: kilogram

LHb: lateral habenula

mg: milligram

min: minute

ML: medial/lateral

MSN: medium spiny neuron

NAc: nucleus accumbens, core and shell

NTS nucleus of the tractus solitarius

OF: open field

PBN: parabrachial nucleus

PBS: phosphate buffer saline

pCREB: phosphorylated cAMP-response element binding *protein*

PCRt: parvocellular reticular formation

PFA: paraformaldehyde

PFC: prefrontal cortex

PVT: paraventricular nucleus of the thalamus

RD: reward downshift

s: second

SEM: standard error of the mean

SNc: substantia nigra pars compacta

SNr: substantia nigra pars reticulata

STN: subthalamic nucleus

Veh: vehicle

VLT: ventrolateral thalamic nucleus

VAT: ventroanterior thalamic nucleus

VVC: viral vector control

Frustrative Nonreward and the Basal Ganglia: Function of Nucleus Accumbens and Globus Pallidus Externus During Consummatory Reward Downshift

Incentive Relativity: Early Studies

Most animals interact with their environment to obtain resources crucial for their survival and reproductive success. These resources, including food, water, shelter, social partners, and maternal care, are incentives that have absolute and relative values. Thorndike's (1911) traditional view of learning illustrated the absolute value of rewards. This view was based on the strengthening and weakening of stimulus-response associations, according to which animals learn a novel behavior or modify an existing one when that behavior is followed by the presentation of a reward. Changes in behavior were the result of food strengthening stimulus-response associations. However, Thorndike's view assumed that no actual learning occurred about the food.

Tinklepaugh (1928) argued that animals form reward expectations and experimentally demonstrated the relative value of incentives. In his experiment with monkeys, Tinklepaugh used bananas as the high-value incentive and lettuce as the low-value incentive. During training sessions, monkeys watched the experimenter placing food rewards, either a piece of lettuce or a banana, under one of two cups, whereas the other cup remained empty. After positioning the food item under the cup, a board screen was lowered to hide the cups from the monkey's view. After a brief interval, the board screen was raised, and the monkey was allowed to make its choice and eat the reward. Although the banana was the preferred reward, monkeys would eat the lettuce too, suggesting that the lettuce also had incentive value. During occasional testing sessions, the monkey watched the experimenter place the banana under the cup, but then the

experimenter replaced the banana for a piece of lettuce while the cups were hidden from the monkey's view. When the monkey picked up the cup and found the lettuce in place of the expected banana, it searched around, shrieked at the experimenter, and moved away from the cups leaving the lettuce untouched. The rejection of the less preferred reward suggested that the monkey had developed expectations about the reward and had detected the discrepancy between the current and the expected reward. The change in behavior following the unexpected switch in rewards was due to the violation of an expectation and supported the view that incentives have relative value. These findings suggested that behavior directed at the low-value reward depended on experience with a previous incentive of higher value obtained under similar conditions. This finding argued against Thorndike's view of learning as a stimulus-response process, supporting the view that monkeys had developed a detailed expectancy of the reward.

Elliott (1928) reported similar results using rats trained in a complex maze. In his experiment, Elliott used a wet mixture of cereal as the high-value incentive, and sunflower seeds as the low-value, but acceptable, incentive. Rats were trained to learn the path to the goal box where the reward, either the high- or the low-value incentive depending on group assignment, was delivered. Rats learned the correct path to the goal box faster when rewarded with wet cereal than when rewarded with sunflower seeds. However, a shift from the high-value cereal to the low-value sunflower seeds increased the number of errors and the latency to reach the goal box for rats rewarded with cereal during training sessions (i.e., downshifted group) relative to rats rewarded with sunflower seeds throughout the experiment (i.e., unshifted group). These findings suggested that rats, like the monkeys in Tinklepaugh's experiment, had formed an expectation about a

specific reward and the violation of this expectation reduced the motivation to reach the goal, causing behavioral disruption.

These two experiments assessed the behavioral changes following manipulations of the qualitative value of rewards. However, manipulations of the quantitative value of rewards can produce similar behavioral changes. Crespi (1942) tested violation of expectations in situations involving the manipulation of the quantitative value of rewards using solid food in the runway task. During training sessions, rats learned to cross the runway for large or small amounts of pellets. A large-to-small reward downshift for rats that received large amounts of pellets during training resulted in increased latency to cross the runway relative to unshifted rats that received small amounts of pellets throughout the experiment. Additionally, a small-to-large reward upshift for rats that received small amounts of pellets during training resulted in decreased latency to cross the runway relative to their training session latency. Crespi (1942) defined these changes in behavior following reward shifts as "depression" and "elation" effects and described them as reflecting emotional responses evoked by the violation of expectations.

Zeaman (1949) reported similar reward downshift and reward upshift effects in an experiment involving manipulation of amounts of cheese in the runway task, a situation analogous to that of Crespi's experiment. Zeaman (1949) coined "negative contrast" or "positive contrast" to define the behavioral changes observed following reward downshift or reward upshift. Specifically, "negative contrast" described a worse-than-expected reward manipulation, whereas "positive contrast" described a better-than-expected reward manipulation. In both cases, the "contrast effect" referred to the difference in

behavior between the experimental group (downshifted or upshifted) and the respective unshifted control group (always small reward or always large reward, respectively).

In the experiments described thus far, the value of the reward is shifted after several training sessions. Bower (1961) introduced a contrast procedure in which rewards of different values were presented to animals in different contexts, but within the same session. In Bower's experiment, rats gained access to a large reward when they crossed a black alley and to a small reward when they crossed a white alley. Latencies of these rats were compared to latencies of rats that received either only the large reward or only the small reward. Rats that could gain access to both the large reward and the small reward had slower speeds when they crossed the white, small-reward alley (i.e., simultaneous negative contrast) and higher speeds when they crossed the black, large-reward alley (i.e., simultaneous positive contrast) than their respective unshifted controls. These became known as simultaneous contrast effects.

Spear and Hill (1965) designed an experiment involving solid food pellet consumption in the T-maze situation that incorporated these two types of contrast paradigms, and introduced the distinction between successive and simultaneous contrast effects. During the simultaneous-contrast phase (preshift) of this experiment, a low-value reward (one regular food pellet) that was held constant across groups, and a high-value reward that varied in its qualitative (regular or sucrose pellets) or quantitative (1, 2, or 10 pellets) value was presented simultaneously. Regardless of the qualitative value of pellets, animals displayed lower running speed to the low-value reward than to the high-value reward; also, the higher the magnitude of the high-value reward, the lower the

running speed to the low-value reward; however, animals did not differ in running speed to the high-value reward.

After several sessions under these conditions, during the following successive-negative contrast phase (postshift), the high-value reward was shifted to 2 pellets across groups, so that some animals received a worse-than-expected reward (10-to-2 pellets), some animals received a better-than-expected reward (1-to-2 pellets) relative to the simultaneous-contrast phase, and control animals received the same reward (2-to-2 pellets). The low-value reward remained unchanged throughout the two experimental phases. Animals displayed lower running speed to the low-value reward in the postshift when they received a larger high-value reward during the preshift phase. However, regardless of the magnitude of the high-value reward, running speed to the low-value reward did not change. Although this procedure produced simultaneous-contrast effects during the preshift phase, there was no evidence of successive-contrast effects during the postshift phase. The exposure to the simultaneous-contrast phase may have decreased the likelihood of observing the successive-contrast effect.

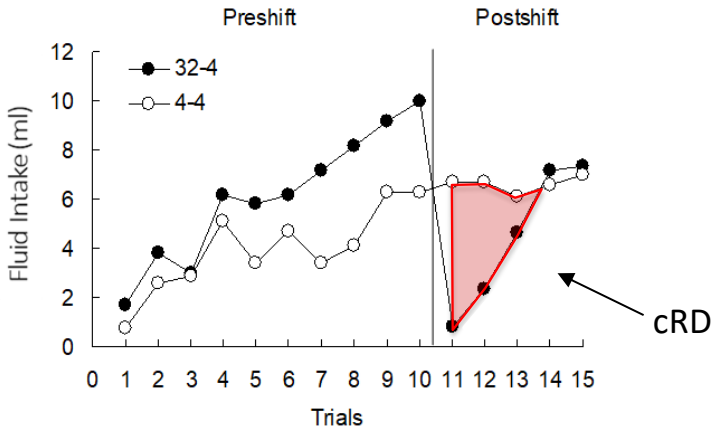
Consummatory Reward Downshift (cRD)

The experiments described thus far assessed the behavior of animals involving instrumental searching for the incentive, and therefore defined instrumental reward downshift contrast (iRD). However, Vogel et al. (1968) developed a procedure producing similar effects, but involving direct consumption of the incentive, and therefore defined consummatory reward downshift (cRD). A typical cRD design involves a comparison of consummatory behavior between a group exposed to a 32-to-4% sucrose downshift and an unshifted control group that received access to 4% sucrose for all experimental

sessions (Flaherty, 1996). During each preshift session 32-to-2% sucrose groups are given access to a highly preferred, 32% sucrose solution, and are allowed to consume this solution for 5 min. Then, the sucrose solution is downshifted to a less preferred, but acceptable, 4% sucrose solution. Rats receiving this unexpected downshift usually display suppressed consummatory behavior on the first downshift experience relative to the unshifted control group that received 4% sucrose throughout the sessions. Over the following downshift sessions, downshifted animals begin to gradually recover consummatory behavior to the level of the unshifted control group. Reward downshift involves the detection of the negative discrepancy between obtained and expected rewards, rejection of the downshifted reward resulting in consummatory suppression during early downshifted sessions, and recovery to baseline levels of consumption of the downshifted reward over the following downshifted sessions. The difference in behavior between the downshifted and unshifted conditions during postshift sessions defines the cRD effect (Figure 1).

Figure 1

Results of a Typical cRD Experiment Involving 32-to-4% Sucrose Downshift



Note. Data from Papini, 2006.

Furthermore, the manipulation of the reward discrepancy between the preshift and postshift sucrose concentration modulates the strength of the cRD effect, which depend on the ratio of preshift-to-postshift sucrose concentration (Papini & Pellegrini, 2006; Pellegrini & Papini, 2007). Following this understanding, all experiments included in this dissertation utilized different reward disparities for the downshifted groups, including 32-to-2% and 8-to-2% sucrose, rather than the more typical 32-to-4% sucrose downshift, to facilitate the observation of effects resulting from neural manipulations (Experiments 2-3) that floor effects could obscure. A potential problem with using a 32-to-2% sucrose downshift is that the low lick frequency in the unshifted control group always receiving access to 2% sucrose could obscure the development of the cRD effect. However, the absence of a significant cRD effect does not necessarily imply the absence of emotional activation, as revealed by some manipulations. For example, mild consummatory suppression after low sucrose disparities can be enhanced by opioid receptor antagonist treatment (Pellegrini et al., 2005) and by peripheral pain (Ortega, Daniel, et al., 2011). None of these factors affected the behavior of unshifted controls.

Effects of Psychoactive Drugs on cRD Effect

Pharmacological approaches have been used to explore the role of drugs in the modulation of the aversive emotional response induced by unexpected reward downshifts, which has been described in terms of frustrative nonreward (Amsel, 1958, 1992). Critical for the proposed research, selective pharmacological manipulation to target early and late downshift sessions proposed a session-specific effect of two classes of psychoactive drugs when administered on the first vs. second reward downshift session.

Anxiolytics. Benzodiazepine anxiolytics, such as chlordiazepoxide (CDP), activate GABAergic neurons by specifically binding to the $GABA_A$ receptor site of the γ-amino butyric acid (GABA) receptor (Genn, Tucci et al., 2004). CDP is an allosteric neuromodulator of $GABA_A$ receptors that does not directly activate GABA receptors but enhances the action of GABA when the endogenous ligand has already activated the receptor. Alcohol has anxiolytic effects similar to those produced by CDP and also modulates GABAergic function (Tan et al., 2011).

Forced administration of alcohol (Kamenetzky et al., 2008) or CDP prior to exposure to a downshifted sucrose solution attenuates the cRD effect (Becker & Flaherty, 1983; Rowan & Flaherty, 1991), suggesting that the cRD effect is mediated by a negative emotional reaction to the downshifted solution. Relative to unshifted controls, downshifted groups that received anxiolytic administration produced higher responding (i.e., less consummatory suppression) to the downshifted solution. However, these effects tend to be selective for the second downshift session (Flaherty, 1996; Flaherty et al., 1990; Ortega et al., 2014). Forced administration of these anxiolytics prior to the second downshift session attenuated contrast effects and produced faster recovery from the reward downshift. Forced administration of CDP or alcohol prior to the first downshift session did not affect cRD performance (Becker & Flaherty, 1982, 1983). However, when the length of downshift sessions was extended from the typical 5 min to 20 min, CDP reduced the contrast effect during the second 5 min of the 20-min downshift session (Flaherty et al., 1986). These results are consistent with the hypothesis that experience with the downshifted solution is necessary for CDP to reduce the negative emotion

induced by reward downshift and that the GABAergic system is active during late cRD downshift sessions. (Flaherty et al., 1990).

To further explore session specificity of CDP, Flaherty (1996) proposed the involvement of $GABA_A$ receptors in an endogenous recovery process that occurs over the sessions following the immediate effect of reward downshift. To test this hypothesis, a group of rats received an injection of GABA agonist muscimol to activate the receptor prior to the first downshift session, followed by systemic CDP injection. The combination of muscimol and CDP injection eliminated the effects of reward downshift, whereas CDP injection alone did not affect the cRD effect (Flaherty, 1996). These findings confirmed GABAergic activity is necessary for CDP to modulate the negative emotion produced by reward downshift, and supported the importance of this system for the recovery from reward downshift. These findings also suggest that GABA receptors are not active during the first downshift session when this session is 5 min long.

Opioids. Research also provides evidence of a modulating function for opioids in the cRD effect. Opioids bind to a variety of receptors located throughout the vertebrate central nervous system (Papini & Ortega, 2011). The nonselective opioid-receptor agonist morphine, which has a strong analgesic effect, reduced consummatory suppression in 32-to-4% sucrose groups when doses of 4 and 8 mg/kg were administered intraperitoneally before the first and second downshift sessions, but did not disrupt performance of the 4-to-4% sucrose control group (Rowan & Flaherty, 1987). Further, low doses (0.25, 0.5, and 1.0 mg/kg) of the opioid antagonist naloxone did not alter the cRD effect (Rowan & Flaherty, 1987), but higher doses (2 mg/kg) resulted in an enhanced suppression of consummatory behavior in the cRD task (Pellegrini et al., 2005). Finally, administration

of naloxone in combination with morphine eliminated the contrast-reducing effects of morphine (Rowan & Flaherty, 1987). The effects of selective opioid-receptor agonists, including kappa (κ) opioid receptor U50, 488H and the delta (δ) opioid receptor DPDPE ([D-Pen^2, D-Pen^5] enkephalin) have also been explored in the context of cRD (Papini, 2009). U50, 488H did not affect consummatory behavior when injected on the first downshift session, but reduced consummatory behavior when injected on the second downshift session in a dose-dependent manner (Wood et al., 2008). In contrast, DPDPE selectively attenuated consummatory suppression when injected on the first downshift session, but did not disrupt consummatory behavior when injected on the second downshift session. None of these agonists affected performance of unshifted controls (Wood et al., 2005). The selective effects of opioids on cRD suggest that opioid receptors are involved in reward loss rather than only in the modulation of consummatory behavior. Specifically, the pattern of results discussed above indicates that while δ-opioid receptors may be implicated in the onset of the cRD effect occurring on the first downshift experience, κ-opioid receptors may be implicated more in the recovery phase during the following downshift experiences.

Taken together, these findings support the view of reward downshift as a dynamic process, involving sequential processes of detection, evaluation, and conflict over the postshift phase, and suggest the possibility that different mechanisms selectively acting on each postshift stage may be involved. Psychopharmacological manipulation allows for the exploration of the systems involved in coping with violation of expectation and emotional learning. The transient nature of the cRD effect and the well-established behavioral conditions leading to consummatory suppression (e.g., Flaherty, 1996; Torres

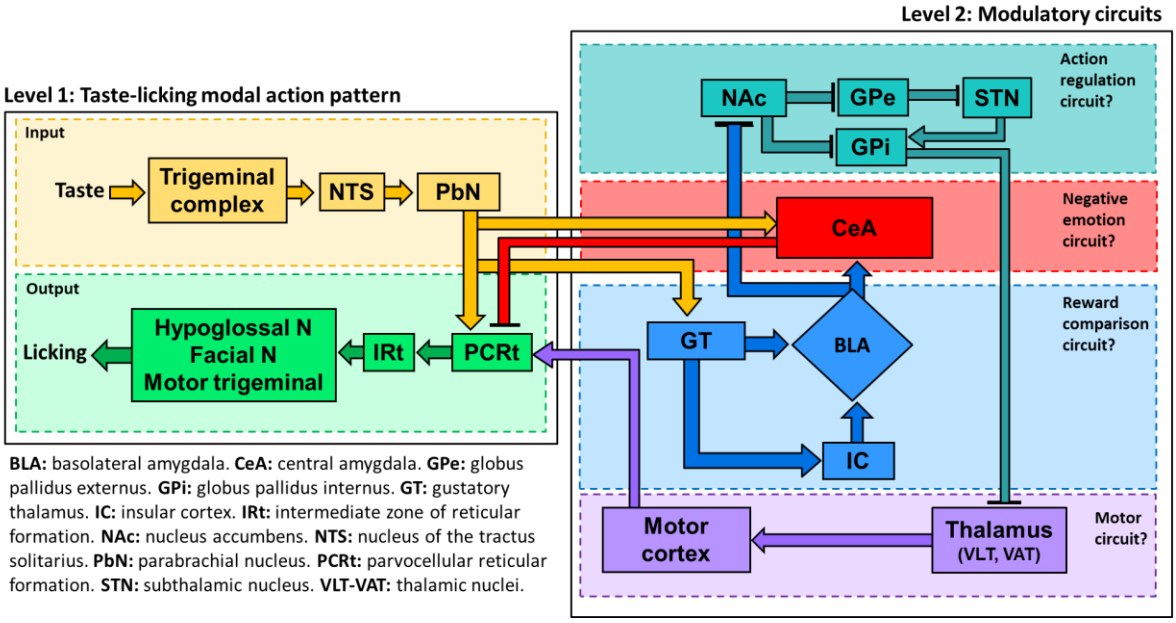
& Papini, 2017) make the cRD task an ideal animal model to study the neurobiology of frustrative nonreward (Ortega et al., 2017; Papini et al., 2015). Neural manipulations during exposure to reward shifts allows for the assessment of specific functions of brain regions and further understand and distinguish brain-behavior mechanisms acting on the immediate effects of unexpected reward downshift (early downshift sessions) and the following recovery phase (late downshift sessions).

Neurobiology of the cRD Effect

Neurobiological research on the cRD effect has primarily focused on the function of brain regions involved in the taste pathway, such as the parabrachial nucleus (PBN) and gustatory thalamus (GT, e.g., Grigson et al., 1994; Reilly & Trifunovic, 2003), and in brain regions modulating emotional activation, such as the prefrontal cortex and amygdala (e.g., Guarino et al., 2020; Kawasaki et al., 2015, 2017; Liao & Chuang, 2003; Lin et al., 2009; Ortega, Uhelski, et al., 2011; Ortega et al., 2013). This research has been critical to hypothesize a two-level neural circuitry underlying the cRD effect (Ortega et al., 2017). Figure 2 represents a modified and extended version of the neural circuitry hypothesized by Ortega et al., 2017.

Figure 2

Proposed Neural Circuitry Underlying the cRD Effect



Note. Modified from the Original Circuitry in Ortega et al., 2017.

The first level controls the taste-licking modal action pattern, which detects the taste of the substance and triggers its consumption (Jones et al., 2006). Taste receptors in the tongue detect the sucrose. Then, the impulses travel along cranial nerves to brainstem nuclei related to gustatory information and consummatory behavior. The nucleus of the tractus solitarius (NTS) is the first central relay region in the brainstem for processing taste information (Norgren, 1995). The NTS projects to the PBN, which receives ascending gustatory information and sends impulses to diencephalic and telencephalic regions in the second level of the hypothesized circuitry (Grigson et al., 1994). For example, the PBN projects to the GT, an area of the central gustatory system involved in taste detection and recognition (Reilly, 1998; Reilly & Trifunovic, 2003), and to the central amygdala (CeA), another area involved in the modulation of gustatory information (Lundy & Norgren, 2004). The second level involves interconnected

modulatory circuits, and it is suggested to modulate consummatory behavior based on memory and taste expectation. Studies involving neural manipulation procedures support an important function for amygdala nuclei in the mechanism involved in cRD.

A *reward comparison circuit* is hypothesized to be regulated by the basolateral amygdala (BLA), identified as key structure where information about current, downshifted low-value reward received from the GT, and the memory of the previously presented, high-value reward from the insular cortex (IC) converge, triggering a comparison process (Kawasaki et al., 2017). In this experiment, excitotoxic lesions of the BLA eliminated the effects of reward devaluation in the cRD task and anticipatory negative contrast (ANC) task. ANC involves access to the same rewards of the successive cRD task (4% and 32% sucrose solutions) but presented in close succession to one another in every session, a task that does not appear to be disrupted by administration of anxiolytics (Flaherty, 1996). In the ANC task, rats display lower response to the 4% sucrose when immediately followed by access to 32% sucrose compared to when followed by access to the same 4% sucrose. These two situations involve transitions in reward magnitude (reward devaluation and reward gain). However, BLA lesions did not affect activity in the open field (OF), a task not involved in reward comparison, but known to induce negative emotion. According to this pattern of results, detection of a reward discrepancy, as assessed by the cRD and ANC tasks, may be distinguished from the emotional response, as assessed by the OF. In the cRD task, for example, animals may distinguish two sucrose solution concentrations (reward comparison) but may not show cRD effect (emotional response). These results suggest a link between the BLA and other brain structures specifically involved in the modulation of negative emotions.

Among other brain regions, the BLA sends neural signals to the CeA and the nucleus accumbens (NAc), two other areas included in the hypothesized cRD postshift circuitry (Figure 2).

When the comparison process detects a significant discrepancy between the current and the expected reward, neural signals travel from the BLA to the CeA, activating the *negative emotion circuit* (Figure 2). Evidence suggesting the role of the CeA in reward loss is provided by several neural manipulations. For example, Kawasaki et al. (2015) reported that transient CeA inactivation by lidocaine microinfusions before the first downshift session attenuated the cRD effect and increased locomotion in the OF test, an effect suggesting reduced innate fear of an open space. Further, CeA inactivation did not affect ANC. This pattern of results suggested a function for the CeA during early cRD downshift sessions for the processing of information with a negative emotional valence. In addition, chemogenetic inhibition of the CeA during early downshift sessions attenuated the cRD effect (Guarino et al., 2020), a finding similar to that obtained using transient inactivation induced by lidocaine microinfusions (Kawasaki et al., 2015). Moreover, local CeA infusions of the benzodiazepine anxiolytic diazepam attenuated consummatory suppression in the cRD task, but the same pharmacological manipulation targeting the hippocampus did not disrupt consummatory response to reward downshift. Benzodiazepine receptors are widely distributed in the central nervous system, but they are particularly abundant in structures of the limbic system, such as the amygdala and the hippocampus. The effects of this manipulation specific to the CeA further support the role of this region in response to reward loss (Liao & Chuang, 2003).

In addition to sending neural projections to the CeA, the BLA makes synaptic contact with striatal regions, including the dorsomedial striatum (DMS) and the nucleus accumbens (NAc), both of which are key structures of the basal ganglia (BG) neural system and serve as input nuclei to downstream regions within the BG circuitry. In the hypothesized circuitry for cRD (Figure 2), the NAc is identified as the region receiving neural signals from the BLA, which triggers the activation of an *action regulation circuitry* involving BG pathways that modulate thalamo-cortical outputs. However, there is limited research investigating the role of brain regions within this circuitry during reward loss.

Basal Ganglia Neural System

The BG neural system includes the dorsal striatum (caudate nucleus and putamen; CP) and ventral striatum (nucleus accumbens core and shell; NAc), the globus pallidus externus (GPe) and internus (GPi), the substantia nigra pars compacta (SNc) and pars reticulata (SNr), and the subthalamic nucleus (STN). Cortical, hippocampal, amygdaloid, and thalamic excitatory fibers make synaptic connections with striatal medium spiny neurons (MSNs) in the CP and NAc, which serve as the input regions, and further reach downstream structures within the BG system. Striatal MSNs account for about 90% of all cells in the striatum (Anderson & Hearing, 2019; Kauer & Malenka, 2007; Lanciego et al., 2012), are inhibitory neurons that use GABA as neurotransmitter, and are present in other BG regions (Dong et al., 2021). Striatal inhibitory MSNs innervate downstream BG regions via two pathways (Gerfen et al., 1990; Gerfen & Surmeier, 2011; Lanciego et al., 2012; Smith et al., 1998) that have opposite functional effects (Albin et al., 1989; DeLong, 1990). Striatal MSNs that make synaptic connections with the GPi activate the

direct pathway, whereas striatal MSNs that make synaptic connections with the GPe activate the *indirect pathway*. Activation of MSNs of the direct pathway inhibits GPi neurons, inducing a reduction of neuronal firing. By contrast, activation of MSNs of the indirect pathway first inhibits GPe neurons, decreasing the GPe inhibitory output, thus disinhibiting STN neurons, which then excites GPi neurons. The GPe also sends axons to the SNr, which together with the GPi are the main outputs from the BG. These output MSNs inhibit activity in the ventrolateral (VLT) and ventroanterior (VAT) thalamic nuclei that send fibers back to the striatum, and excite neurons in the motor and premotor cortex, and in brainstem motor nuclei, such as the pedunculopontine nucleus (Nambu, 2008; Wei & Wang, 2016). The influence of BG output on the thalamo-cortical and brainstem connections is the result of an interplay between the direct pathway, which is associated with the facilitation of action, and the indirect pathway, which is associated with the inhibition of action. These two pathways exert opposite effects on a variety of behaviors, including psychomotor sensitization as well as behaviors related to drug seeking and taking (Yager et al., 2015). It must also be noted that there is an abundance of reciprocal connections between BG areas, and also a small percentage of GABAergic and cholinergic interneurons that modulate MSNs. Moreover, MSNs express a variety of receptors, including dopamine D1 and D2 receptors, glutamate receptors, and opioid receptors (Russo & Nestler, 2013). Important for the current research, striatal MSNs projecting to the GPe express the dopamine D2 receptor, which inhibits intracellular adenyl-cyclase trough G-protein signaling, and triggers activation of the indirect pathway. On the other hand, striatal MSNs projecting directly to GPi and SNr contain

dopamine D1 receptor, which activate adenyl-cyclase signaling, and activates the direct pathway (Lanciego et al., 2012).

The BG are a major neural system of interconnected pathways with emotional (limbic), cognitive (prefrontal cortex), and behavioral (motor cortex) functions (Avila et al., 2020; Baunez & Lardeux, 2011; Leisman et al., 2014; Rossi et al., 2015; Wei et al., 2016; Weintraub & Zaghloul, 2013; Yager et al., 2015). These are critical functions of the cRD task (Flaherty, 1996; Papini et al., 2015). However, limited research has been conducted on the function of the BG in reward loss.

Basal Ganglia and Reward Loss

The function of BG structures in reward loss has been studied with a variety of procedures. Immunohistochemistry studies found enhanced NAc neural activity as measured by c-Fos expression, a marker of neuronal depolarization, after a 32-to-4% sucrose downshift in the cRD task compared to unshifted controls (Pecoraro & Dallman, 2005). The same behavioral paradigm failed to produce evidence of increased pCREB expression, a marker of synaptic plasticity, in either the shell or core sections of the NAc, although pCREB did increase in the DMS (Glueck et al., 2015). Additionally, a microdialysis study reported that the cRD task was accompanied by a lower release of dopamine (DA) by NAc neurons during early downshift sessions in rats exposed to the 32-to-4% sucrose downshift relative to 4-to-4% controls (Genn, Ahn et al., 2004).

Further, evidence of low levels of DA in the NAc of rats following a delay between delivery of reward and extinction suggested that this decrease in DA release in the NAc during extinction may be related to an emotional response to the failure to obtain the expected reward (Biesdorf et al., 2015). However, lesion studies have produced

inconclusive evidence regarding the role of NAc neurons in the cRD task. For example, electrolytic lesions of the NAc failed to affect the cRD, although they impaired runway performance using the iRD paradigm (Leszczuk & Flaherty, 2000), suggesting that the NAc is more involved with anticipatory behavior than with consummatory behavior. Moreover, excitotoxic lesions of the DMS revealed no effects on cRD, although they abolished the enhancement of lever pressing induced by partial reinforcement training in the rat autoshaping paradigm (Torres et al., 2017).

Goals of the Current Dissertation

The unclear results discussed thus far led to this dissertation research. This dissertation was designed to contribute to the current knowledge on the functions of BG structures in the cRD situation. Because of the major role of BG system in modulating motor activity, the current research utilized open field (OF) activity to assess potential motor effects unrelated to reward downshift.

Using the cRD task to induce negative emotion, *Experiment 1* was designed to confirm that 32-to-2% and 8-to-2% as preshift-to-postshift sucrose downshifts produced consummatory suppression relative to 2% unshifted controls. Manipulation of the reward discrepancy to produce large (32-2% sucrose downshift) or small (8-2% sucrose downshift) effects of reward downshifts were then used in subsequent experiments to facilitate the observation of the effects of neural manipulation. Specifically, using chemogenetic inhibition and excitation, *Experiment 2a* and *2b* extended the current knowledge on the role of the NAc in the cRD circuitry, as well as its potential action-regulating function on downstream structures modulating the indirect pathway of the BG system. *Experiment 3* was the first attempt to investigate the function of the GPe in the

cRD circuitry. Research investigating the role of the GPe has received limited attention beyond its motor functions and has never been explored in the context of the cRD task.

Experiment 1

The aim of Experiment 1 was to determine whether consummatory suppression effect is observed with 32-to-2% and 8-to-2% sucrose downshifts. To match the conditions used in Experiments 2-3, vehicle injections were administered after each postshift session. Based on prior research (Papini & Pellegrini, 2006; Pellegrini & Papini, 2007), we expected the 32-to-2% and 8-to-2% sucrose downshifts to induce a significant suppression, but only the former to drive behavior below the level of the 2-to-2% sucrose control.

Method

Subjects. The subjects were 25 female Wistar rats, experimentally naïve and 90 days old at the start of the experiment. These animals were bred in our colony from parents purchased from Charles River Laboratories. Pups were weaned at about 21 days of age and transfer to single-sex polycarbonate cages in groups of at least two animals. At around 40 days of age, they were transferred to wire-bottom cages, each containing a rodent retreat for enrichment, where they remained until the end of the experiment. Developmental conditions are therefore common to all animals used in our experiments. Their mean (±SEM) ad libitum weight was 266.4 g (±4.4). Food was restricted until animals reached were at 81-84% of their ad libitum weights. All animals received food every day, but the amount was determined individually to keep weights constant across the experiment. Although females were used in this experiment, whereas males were used in Experiments 2-3, there is no evidence that sex affects the cRD task (Flaherty, 1996).

The use of females was determined by animal availability. Animals were fed with standard laboratory rat chow, freely available until animals were approximately 90 days of age when food restriction was implemented. Subjects had free access to water throughout the experiment. During the experiment, animals were under a 12 h light/12 h dark schedule (lights on at 07:00 h), in a colony room with constant temperature (22-23 °C) and humidity (45-65%). The research reported in this dissertation was carried out with IACUC approval in a USDA-inspected facility.

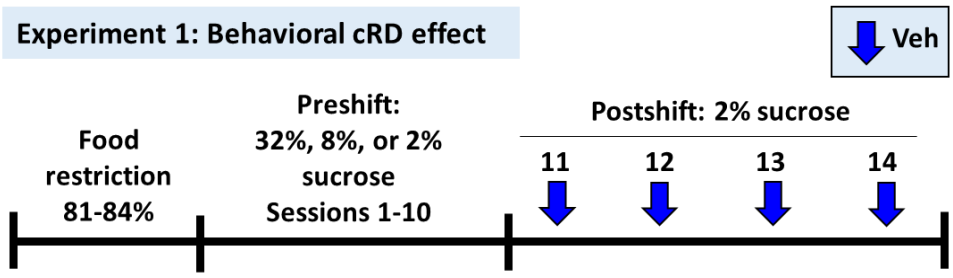
Apparatus. The cRD task was conducted in eight conditioning boxes (MED Associates, St. Albans, VT) made of aluminum and Plexiglass (29.3 × 21.3 × 26.8 cm, L × H × W). Each chamber was placed in a sound-attenuating enclosure equipped with a speaker generating white noise and a ventilating fan, both of which collectively administered noise with an intensity of 80.1 dB (SPL, scale C). A house light in the center of each enclosure's ceiling produced diffuse light during the cRD task. A tray of corncob bedding for the absorption of feces and urine was placed beneath the floor of the operant chamber. The floor was made of steel rods parallel to the feeder wall, which was punctured with three holes for the delivery of the sipper tubes. Specifically, the three holes were each 1 cm wide, 2 cm long, and 4 cm from the floor, positioned either exactly in the center of the wall, in the center of the right third of the wall, or in the center of the left third of the wall. During the cRD task, a sipper tube attached to a bottle containing sucrose solutions was inserted through the hole in the center to deliver the sucrose. The sipper tubes were each 1 cm in diameter and equipped with a ball bearing to minimize leakage. A computer located in an adjacent room controlled insertion and retraction of the sipper tubes through the hole. Once rats started licking the sipper tube, a circuit involving

the steel rods in the floor closed and the lick frequency was automatically recorded. A trial lasted 5 min from the animal's recorded first contact with the sipper tube, after which the computer retracted the sipper tube.

Procedure. The procedure is described in Figure 3. Animals were tested in the cRD task and vehicle (Veh) injections (dimethyl sulfoxide, DMSO, dissolved in 95% sterile saline, i.p., at a volume of 1 ml/kg) were administered 30 min before postshift sessions 11-14 to match the conditions of Experiments 2-3. Preparation of the sucrose solutions (32%, 8%, and 2%) involved mixing 68 g (92 g or 98 g) of deionized water for every 32 g (8 g or 2 g) of commercial sugar. The mixture containers were shaken until the sugar was completely dissolved; the containers were then kept at room temperature between cRD sessions.

Figure 3

Experimental Procedure for Experiment 1



Note. Experiment 1 involved a comparison between downshifted (32-to-2% and 8-to-2% sucrose) vs. unshifted controls (2% sucrose). Vehicle injections were included to match the conditions of Experiments 2-3.

cRD training started once all animals were within 81-84% of their ad libitum weights. Animals were randomly assigned to one of three groups. In Group 32 (n = 8), animals received 10 sessions of access to 32% sucrose (preshift), followed by 4 sessions of access to 2% sucrose. In Group 8 (n = 9), rats had access to 8% sucrose on sessions 1-

10 and to 2% sucrose on sessions 11-14. In Group 2 (n = 8), animals had access to 2% sucrose during both preshift and postshift sessions. Postshift sessions started 30 min after a Veh injection but were otherwise similar to other sessions. All animals received training simultaneously, 7 days per week, at approximately the same time every day. All sessions lasted 5 min from the first detection of a sipper-tube contact. Lick frequency, defined as the total number of licks recorded during the 5-min session, was the dependent variable. Animals were transported back to their home cages and to the colony room after each cRD session. A wet paper towel was used to wipe the conditioning boxes after each session. Feces were removed and bedding replaced as needed.

Experimental design and statistics. This was a Group by Session experiment. Groups differed in the concentration of sucrose accessible during preshift sessions 1-10, either 32%, 8%, or 2% sucrose. Independent comparisons involved Groups 32 vs. 2 and Groups 8 vs. 2, with the unshifted control, Group 2, being common to both comparisons. Lick frequency data were subjected to analysis of variance (ANOVA) with the alpha value set at the 0.05 level. Group was an independent-sample factor, whereas Session was always a repeated-measure factor. Effect size was indexed with the partial eta squared (η^2) statistic. Pairwise comparisons using the Bonferroni test were derived from the main analysis whenever justified by significant interactions. IBM SPSS Version 26 package was used to compute all the statistics.

Results

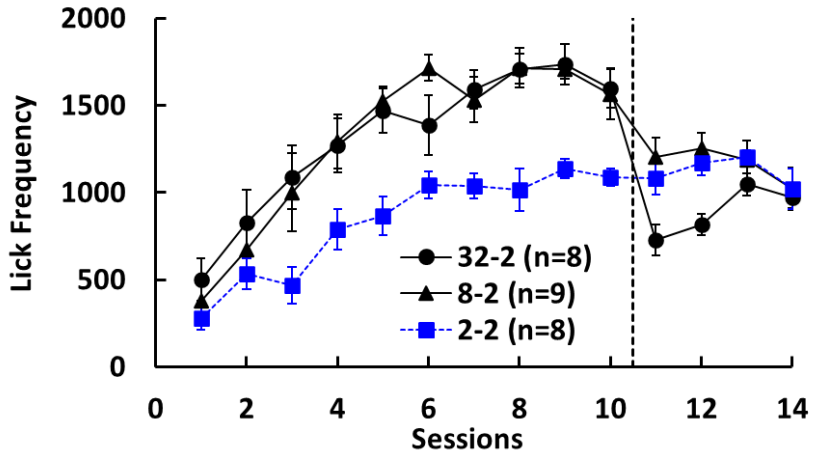
The results of Experiment 1 are presented in Figure 4. Because the aim of this experiment was to determine whether these two downshifts lead to consummatory suppression, separate analyses were computed for the 32-to-2% and 8-to-2% sucrose

conditions, in both cases relative to the unshifted 2% sucrose control. For preshift sessions, Group by Session (1-10) analyses indicated significant main effects of Group and Session, Fs > 20.83, ps < 0.001, η^2s > 0.60. The interaction was significant only in the comparison between Groups 8-2 vs. 2-2, $F(9, 135) = 2.09$, $p < 0.04$, $\eta^2 = 0.12$. Pairwise Bonferroni tests indicated that Group 2-2 performed significantly below Group 32-2 on sessions 3-5, and 7-10, ps < 0.03, and significantly below Group 8-2 on sessions 4-10, ps < 0.03. The significant main effect of Group indicated that access to 32% or 8% sucrose promoted higher lick frequencies than access to 2% sucrose.

For postshift sessions, separate Group by Session (10-14) analyses comparing Groups 32-2 vs. 2-2 and Groups 8-2 vs. 2-2 revealed the following results. A significant interaction was found for Groups 32-2 vs. 2-2, $F(4, 56) = 11.98$, $p < 0.001$, $\eta^2 = 0.46$. Pairwise Bonferroni tests confirmed that Group 32-2 licked significantly more than Group 2-2 on session 10 (last preshift session), $p < 0.002$, but Group 32-2 licked significantly below Group 2-2 on sessions 11 and 12, ps < 0.02, and was marginally different on session 13, $p = 0.052$. A similar analysis involving Groups 8-2 and 2-2 also found a significant interaction, $F(4, 60) = 2.92$, $p < 0.03$, $\eta^2 = 0.16$, which was also the result of a significantly different lick frequency on session 10, before the downshift, $p < 0.01$. Finally, pairwise Bonferroni tests also indicated that Group 32-2, $p < 0.001$, and Group 8-2, $p < 0.02$, significantly suppressed consummatory behavior from session 10 to 11.

Figure 4

Results of 32-to-2%, 8-to-2%, and 2-to-2% Sucrose Shifts in the cRD Task



Note. Mean (±SEM) of lick frequency in animals that received access to 32% sucrose (Group 32-2), 8% sucrose (Group 8-2), and 2% sucrose (Group 2-2) across preshift sessions 1-10. All animals received access to 2% sucrose during postshift sessions 11-14.

Experiments 2-3

Neural Manipulation via Chemogenetics

Chemogenetics involves the use of engineered receptors that are activated by otherwise inert substances (Roth, 2016). G protein-coupled receptors are the most common types to have been chemogenetically engineered to explore brain circuit mechanisms. Designer receptors exclusively activated by designer drugs (DREADDs) are the most widely used class of these engineered receptors employed in chemogenetic research (Roth, 2016; Urban & Roth, 2015).

DREADD Approach

DREADDs are engineered G protein-coupled receptors that have been modified to respond specifically to the synthetic compound clozapine N-oxide (CNO), but no longer to their endogenous ligand (i.e., acetylcholine; Urban & Roth, 2015). CNO

undergoes reversed metabolism to convert back to clozapine, which enters the blood brain barrier and binds to the designer receptor to inhibit or excite neural activity. DREADDs are delivered into brain regions via intracranial infusion of an adeno-associated virus (AAV) capable of infecting cells but shown to be safe for humans due to its inability to replicate, therefore preventing harmful spread. DREADDs are maximally expressed 2-3 weeks post-infusion (Smith et al., 2016; Zhu & Roth, 2014) and can be selectively activated by systemic administration of CNO. A common DREADD variant used for neural inhibition is hM4D(Gi), an engineered version of the M4 muscarinic receptor. Activation of this inhibitory DREADD activates potassium channels, leading to an efflux of potassium ions (i.e., membrane hyperpolarization), and it inhibits neurotransmitter release, leading to synaptic silencing (Armbruster et al., 2007; Rogan & Roth, 2011; Urban & Roth, 2015). Another commonly used DREADD variant used for neuronal excitation is hM3D(Gq), an engineered version of the M3 muscarinic receptor. Activation of this excitatory DREADD releases intracellular calcium level, which produces membrane depolarization, and leads to a burst-like firing of neurons (Armbruster et al., 2007; Rogan & Roth, 2011).

Two steps are necessary to employ the DREADD technique to remotely control neuronal activity: (1) an intracranial infusion of a viral construct to deliver the DREADD to a specific brain region, and (2) an intraperitoneal injection of CNO, the activator drug for DREADDs. Compared to pretraining irreversible lesions, DREADDs allow to train animals under normal neural activity conditions before and after the administration of CNO to activate the DREADDs. Compared to reversible lesions, the DREADD approach does not require cannula implants for microinfusions prior to behavioral testing.

Possible Effects of CNO on Behavior

DREADDs are nontoxic, allowing for extensive manipulations of neuronal activity while keeping the neurons healthy (Roth, 2016). However, CNO is a metabolite of clozapine, an antipsychotic drug and agonist of serotonin and dopamine receptors. Several studies have reported that to activate DREADDs, CNO has to convert back to clozapine, which has high affinity for DREADDs (Armbruster et al., 2007, Gomez et al., 2017, Ilg et al., 2018; Manvich et al., 2018). It is therefore important to carefully control for any off-target CNO/clozapine effects on behavior that are independent of binding to DREADDs. Clozapine has been shown to affect behavior in several tasks (Ilg et al., 2018), although, CNO injections (i.p., 1 and 3 mg/kg) administered prior to the first and second 32-to-2% sucrose downshift sessions did not have measurable effects (Guarino et al., 2020). Other antipsychotics have been found to have dose and task dependent effects on behavior. For example, a high dose (60 mg/kg), but not a low dose (10 mg/kg) of amisulpride reduced the cRD effect (Genn et al., 2002); also, high doses (0.2 and 0.4 mg/kg) of haloperidol impaired locomotion, consummatory behavior, and appetitive responses (0.4 mg/kg), but lower doses of haloperidol (0.025 to 0.1 mg/kg) did not affect these behaviors (Huang et al., 2010).

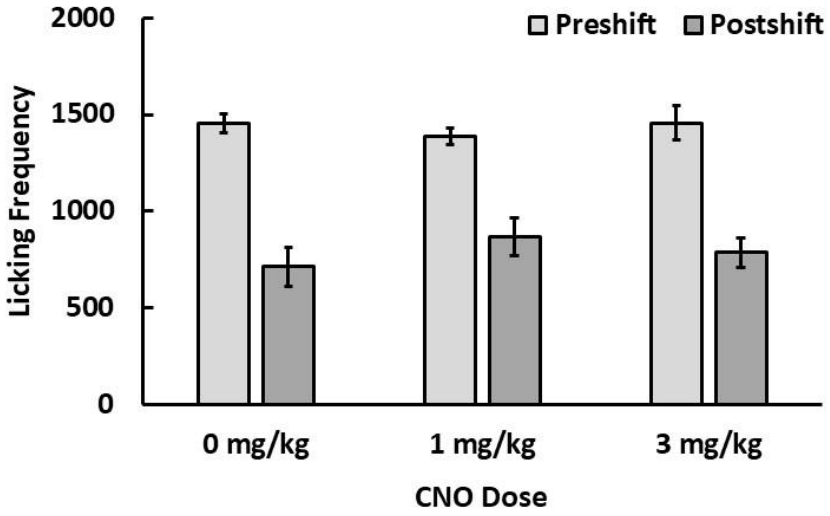
Additionally, studies controlling for CNO effects on behavior reported that CNO (up to 10 mg/kg) had no effects on various motivated behaviors in non DREADD-expressing animals when tested within 30-150 min after the systemic injection (Mahler & Aston-Jones, 2018; Roth, 2016). CNO did not affect sensorimotor gating (1 mg/kg, MacLaren et al., 2016), spontaneous locomotion (1, 2, 5 mg/kg, MacLaren et al., 2016; 2.5 and 10 mg/kg, Wirtshafter & Stratford, 2016), amphetamine-induced

hyperlocomotion and the associated release of dopamine (DA) in the nucleus accumbens (2 mg/kg, MacLaren et al., 2016), or cocaine-induced locomotion (3 mg/kg; Padovan-Hernandez & Knackstedt, 2018). However, in rats trained to discriminate clozapine (1.25 mg/kg) from vehicle, CNO (10 mg/kg) produced partial substitution for clozapine stimulus in a choice situation, although CNO did not affect baseline levels of lever pressing (Manvich et al., 2018).

Two experiments from our lab involving i.p. administration of CNO in intact animals and in DREADD-expressing animals tested in consummatory reward devaluation tasks indicated that: (1) administration of 1 and 3 mg/kg of CNO in intact animals produced consummatory suppression similar to those of vehicle controls (Figure 5; data from Guarino et al., 2020), and (2) administration of 3 mg/kg of CNO in DREADD-expressing animals did not disrupt consummatory behavior of unshifted controls (Figure 6; data from Guarino et al., 2020). These results suggest that the amount of clozapine back-metabolized from the highest CNO dose tested in our lab (i.e., 3 mg/kg) remained within the levels of specificity to activate the DREADD, but below the threshold for altering consummatory behavior in the consummatory reward downshift situation.

Figure 5

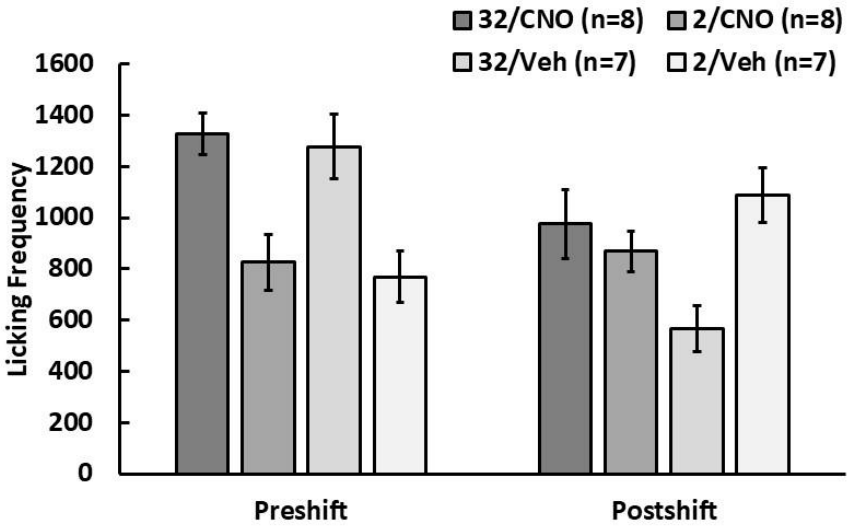
Effects of 0, 1, or 3 mg/kg CNO Prior to 32-to-2% Sucrose Downshift



Note. Data from Experiment 1 in Guarino et al., 2020.

Figure 6

Effects of CeA Chemogenetic Inhibition Prior to cRD Downshift



Note. Data from Experiment 2 in Guarino et al., 2020).

Control Procedures in Chemogenetic Experiments

There are several control procedures that can be applied to chemogenetic experiments on reward loss. Experiments 2-3 of the current dissertation did not include controls for CNO administration in intact animals. As mentioned previously, such condition, tested in an experiment also involving a 32-to-2% sucrose downshift, provided no evidence that systemic CNO administration interfered with consummatory suppression in the cRD task (Figure 5; Guarino et al., 2020). However, Experiments 2-3 included viral vector control (VVC) animals that were infused with a viral vector lacking the engineered receptors and treated with either CNO or Veh injections. The inclusion of these animals provided additional control for the effects of these injections in the absence of DREADDs. It was expected that these animals would behave similarly to those infused with DREADD receptors, but treated with Veh, rather than CNO. Moreover, the effects of excitatory and inhibitory DREADDs were independently assessed by c-Fos immunohistochemistry on tissue samples of animals sacrificed after the last CNO vs. Veh injection. c-Fos is expressed in neurons that have been recently depolarized (Chung, 2015). We expected that inhibitory DREADDs would lead to similar or lower expression of c-Fos, whereas excitatory DREADDs would augment c-Fos expression, in both cases relative to Veh and VVC controls.

Ideally, unshifted animals should be included in each experiment to provide control for the possibility that the neural manipulation acts on other systems (i.e., the motor system in the case of BG structures), affecting behavior independently of reward downshift. However, unshifted controls double the number of animals required in an experiment. To minimize the number of animals used in these experiments (Blakemore et

al., 2012), it was decided to exclude unshifted controls from Experiments 2-3. Instead, both experiments used the OF task to provide an independent assessment of DREADD's potential motor effects unrelated to reward downshift.

Experiment 2a

Experiment 2a aimed at testing the effects of chemogenetic neural manipulation of the NAc in the cRD task. Research on the function of the NAc on cRD assessed using various approaches produced ambiguous results. Additionally, to our knowledge, the effects of NAc neural inhibition and excitation in the cRD task has not been tested using a chemogenetic approach.

As reviewed in the introduction, an intact NAc does not seem necessary to observe the consummatory suppression induced by RD (Leszczuk & Flaherty, 2000). However, increased NAc neural activity during sucrose downshift (Pecoraro & Dallman, 2005) accompanied by decreased DA release (Genn, Ahn, et al., 2004) suggests that the NAc is involved in the response to RD. NAc activation may be mediated by inputs from the paraventricular nucleus of the thalamus (PVT) and the BLA (Do-Monte et al., 2017; Lafferty et al., 2020). For example, photoinhibition of the anterior PVT projections to the NAc increased sucrose seeking during reward omission, whereas photoactivation of these projections decreased sucrose seeking (Do-Monte et al., 2017). Moreover, activity of NAc neurons expressing dopamine D1 and D2 receptors is elevated during behavioral suppression induced by appetitive extinction (Lafferty et al., 2020).

Taken together, these findings suggest that chemogenetic inhibition and excitation should have asymmetric effects on consummatory behavior during cRD. Based on the lack of evidence that accumbens lesions interfere with cRD, we expected that

chemogenetic inhibition would have little or no effect on consummatory behavior during cRD. By contrast, based on c-Fos expression data, electrophysiological evidence, and optogenetic photoactivation results, we predicted that chemogenetic excitation would further suppress consummatory behavior during RD. Additionally, to disentangle motor and emotional effects and potential involvement of the NAc in the BG motor circuitry, chemogenetic inhibition and excitation of the NAc were also induced during an OF test that assessed locomotion.

Method

Subjects. The subjects were 58 male Wistar rats experimentally naïve to the procedures administered, and about 90 days old at the start of the experiment. Rats were bred in our colony as described in Experiment 1. Rearing, maintenance conditions, and food restriction were as described in Experiment 1. In preparation for surgery, all rats were gradually food deprived to 90% of their free-food weights estimated as the mean weight from two consecutive days. The mean (±SEM) ad lib weight of the 49 animals selected for analysis was 478.8 g (±6.0 g).

Viral vector constructs. Inhibitory [hM4D(Gi)] and excitatory [hM3D(Gq)] DREADDs were delivered into the NAc via intracranial infusions of an adeno-associated virus (AAV8). The viral vector constructs (inhibitory: pAAV-hSyn-hM4D(Gi)-mCherry 3×10^{12} virus molecules/ml; excitatory: pAAV-hSyn-hM3D(Gq)-mCherry 3×10^{12} virus molecules/ml; Addgene, Cambridge, MA) contain a red, fluorescent reporter (i.e., mCherry) and a DNA fragment for an engineered muscarinic receptor (M4 for inhibitory DREADD and M3 receptor for excitatory DREADD) that reacts to CNO. Non-DREADD viral vector control (VVC) groups, receiving bilateral infusions of a viral vector (pAAV-

hSyn-EGFP 7×10^{12} virus molecules/ml; Addgene, Cambridge, MA) containing a green, fluorescent reporter (i.e., enhanced green fluorescent protein, or EGFP), but not the engineered receptor, were also included.

Surgical procedure and DREADD infusions. To induce anesthesia, animals were placed in a chamber filled with a mixture of breathing air and isoflurane vapor, 5% for induction and 1-2% for maintenance. Once breathing deepened and slowed, the animal was positioned in a stereotaxic frame (Angle Two, Leica, program version 3.0.0) that maintained the delivery of isoflurane vapor to keep the animal under anesthesia during surgery. The frame was fixed with blunt-tipped ear bars, a bite bar, and a mask. Once the animal was situated in the frame, its eyes were covered with Vaseline to prevent eye dryness and possible harm from the microscope light. The area of the scalp to be incised was shaved and glazed with Betadine (povidone-iodine topical solution, 10%), after which an incision was made at midline of the scalp. Blunted hooks were used to separate the two sides of the incision and bare the skull, which was then carefully cleaned, protective layers peeled back from the surface. After assessing and adjusting the position of the skull for flatness, the location of the NAc was determined based on the atlas of Paxinos and Watson (2013) and marked on both sides of the skull. Each marked site was drilled and infused with the viral construct using a 10-μl Hamilton syringe fixed on a stereotaxic injector (Quintessential Stereotaxic Injector, Stoelting, Wood Dale, IL), administering 1 μl of virus per side at a rate of 0.15 μl/min at NAc coordinates (anterior/posterior, AP: -2.2; medial/lateral, ML: 4.3; dorsal/ventral, DV: -7.95). The Hamilton syringe was let to remain in place for an additional ten minutes for the fluid to diffuse in brain tissue, after which the syringe was slowly withdrawn, and the scalp was

stapled back together to facilitate healing. The animal was then removed from the stereotaxic frame and injected half of the required dose of buprenorphine hydrochloride (0.05 mg/kg, subcutaneous) to dampen surgery-induced pain; the other half was administered 24 h after surgery. During the ensuing 5-day recovery period, animals stayed individually in polycarbonate cages, where they had access to food at a 90% deprivation level, including typical lab rodent chow and supplementary recovery gel, as well as readily available water. At the end of the recovery period, animals were returned to their typical wire-bottomed home cages.

Experimental design. Based on neural manipulation condition, animals were assigned to one of three groups, inhibition (Inh), excitation (Exc), and viral vector control (VVC). Specifically, Group Inh received bilateral infusion of inhibitory DREADDs, whereas Group Exc group received bilateral infusion of excitatory DREADDs into the NAc. Group VVC received bilateral infusion of an AVV vector that did not carry the engineered receptor activated by CNO. Within each group, subjects were further categorized based on the injection they received on the four postshift sessions. Half the animals were injected with CNO and the other half with vehicle (Veh). Six groups of animals were included: Exc/CNO, Exc/Veh, Inh/CNO, Inh/Veh, VVC/CNO, and VVC/Veh.

Post-surgery food restriction procedure. After recovery from surgery and in preparation for behavioral testing, animals were further food-restricted and received a regulated daily amount of rat chow to maintain their weight at 81-84% of their average initial free-food weight, for the duration of the experimental phases. During the period of

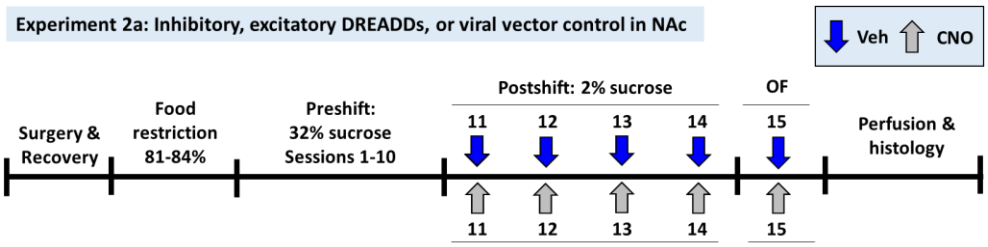
behavioral testing, food was provided each day at about the same time, at least thirty min following behavioral testing.

CNO preparation and injection procedure. CNO (NIDA Drug Supply Program) was dissolved in 5% dimethyl sulfoxide (DMSO) and 95% sterile saline. The vehicle solution used for Veh groups was the same volume (1 ml/kg) and content, except CNO was not included. Injections were administered thirty min prior to behavioral testing in a room different from those where the tests were performed.

cRD: Apparatus and procedure. The apparatus and the preparation of the sucrose solutions were as described in Experiment 1. The procedure is described in Figure 7. Behavioral testing began 10 days after viral infusion to optimize use of DREADD expression, which was also when the weight of all animals was within the target range. Testing took place approximately at the same time of the day every day throughout the experiment. All animals received access to a 32% sucrose solution during the 10-day preshift and a 2% sucrose solution during the four postshift sessions. However, the animals differed on the injections they received in the postshift stage, with half of them receiving a CNO injection and the other half receiving a Veh injection prior to each of the four sessions.

Figure 7

Experimental Procedure for Experiment 2a



Note. In Experiment 2a, all animals were exposed to a 32-to-2% sucrose downshift. Open field (OF) testing was scheduled on session 15.

OF: Apparatus and procedure. OF testing was carried out in four units (43 x 30 x 43 cm, L x H x W; MED Associates, St. Albans, VT, USA). Two 150 W LUMAPRO Clamp-On lights (Grainger, Fort Worth, TX) with 15 W LUMAPRO LED warm white lamp bulbs (Grainger, Fort Worth, TX) illuminated the OF arenas during testing. A Color Gigabyte Ethernet Camera (Noldus, Leesburg, VA) placed directly above the arenas and connected to EthoVision XT Version 11 Software (Noldus, Leesburg, VA) recorded the activity of the animals.

OF testing occurred the day after the last cRD session. A single 15-min session of exposure to the OF was administered. Animal assignment to either the CNO or Veh condition was consistent with the injection received during cRD postshift sessions. All injections were administered 30 min before the start of the OF test. Animals were placed in the center of the arena and allowed free movement. Using a computer located in an adjacent room, EthoVision XT was used to assess the distance traveled (in centimeters). Each field was cleaned immediately after each session, before testing the next group of rats. Immediately after each session, animals were placed back in their home cages and transported back to the colony room.

Perfusion. Rats were anesthetized with sodium pentobarbital and transcardially perfused with 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4. Perfusions started 120 min after the last administration of CNO or Veh injection to optimize the expression of c-Fos. For 26 animals, perfusions occurred on the same day of the OF session (day 15, Figure 4), 120 min after CNO (or Veh) administration. However, due to a unusual freezing weather conditions that affected the entire North Texas region in February 2021, disrupted access to the vivarium, and

interfered with data collection, 19 animals were perfused the day after the OF test, and 13 animals were perfused one week after the OF test. These 32 animals received an additional injection of CNO (or Veh). Immediately after extraction, brains were placed in 4% PFA for at least 3 days and then placed in 30% sucrose for at least 2 days for cryoprotection.

Histology. Brains were sectioned coronally in 40-μm slices using a cryostat (Leica Biosystems, Buffalo Grove, IL). Brain sections were collected for both localization of DREADD expressions and for immunohistochemistry to assess the expression of c-Fos, a protein expressed in recently-depolarized neurons that provides an index of neural activity (e.g., Chung, 2015).

DREADDs localization. Sections collected for the assessment of DREADD expression were placed directly onto slides, Fluoromount-G mounting medium and cover slips were applied on the slides to preserve the fluorescent tags, mCherry or EGFP, and be able to localize the virus at a later time. The location of the virus was assessed via fluorescence microscopy (Nikon eclipse Ti inverted microscope, Nikon, Melville, NY) and images were processed using CRi Nuance FX multispectral imaging system (Caliper Life Sciences, Hopkinton, MA) and Nuance 3.0 imaging software (Caliper Life Sciences, Hopkinton, MA). The area of maximal fluorescence expression was determined for each animal by connecting the most external points in each image. The polygons thus created were mapped into a representation from the Paxinos and Watson (2013) atlas, as represented in the left column of Figure 8.

c-Fos assessment. Sections collected for c-Fos immunohistochemistry were transferred to 0.1 M PBS. Brain sections were first treated with 3% hydrogen peroxide to

inhibit endogenous peroxide activity, then washed and incubated for 1 h in a PBS solution containing 0.3% Triton X100 and 2% normal goat serum to minimize nonspecific binding. Sections were then incubated for 48 h at 4 °C with a rabbit polyclonal antibody against the c-Fos protein (ab190289, 1:10000, Abcam, Cambridge, MA) in PBS containing 0.3% Triton X100, 0.25% bovine serum albumin. Biotinylated anti-rabbit antibody (31820, 1:400, Invitrogen, 247 Carlsbad, CA), Vectastain ABC kit (PK-6100, Vector Laboratories, Burlingame, CA), and a DAB substrate kit (SK-4100, Vector Laboratories, Burlingame, CA) were used to visualize c-Fos expressive cells. After sections were mounted, dehydrated, and cleared, sections were visualized using a Nikon Eclipse 90i microscope equipped with a DS-Fi1 digital camera. Two-dimensional counts of immune-positive nuclei from each image were counted using NIS-Elements AR 4.6 software (Nikon, Melville, NY) without knowledge of the experimental condition. Counts were obtained from both core and shell regions of the NAc from each hemisphere, and then averaged to reach a final count. Data were quantified at +1.7 mm AP using boundaries set by the Paxinos and Watson (2013) atlas.

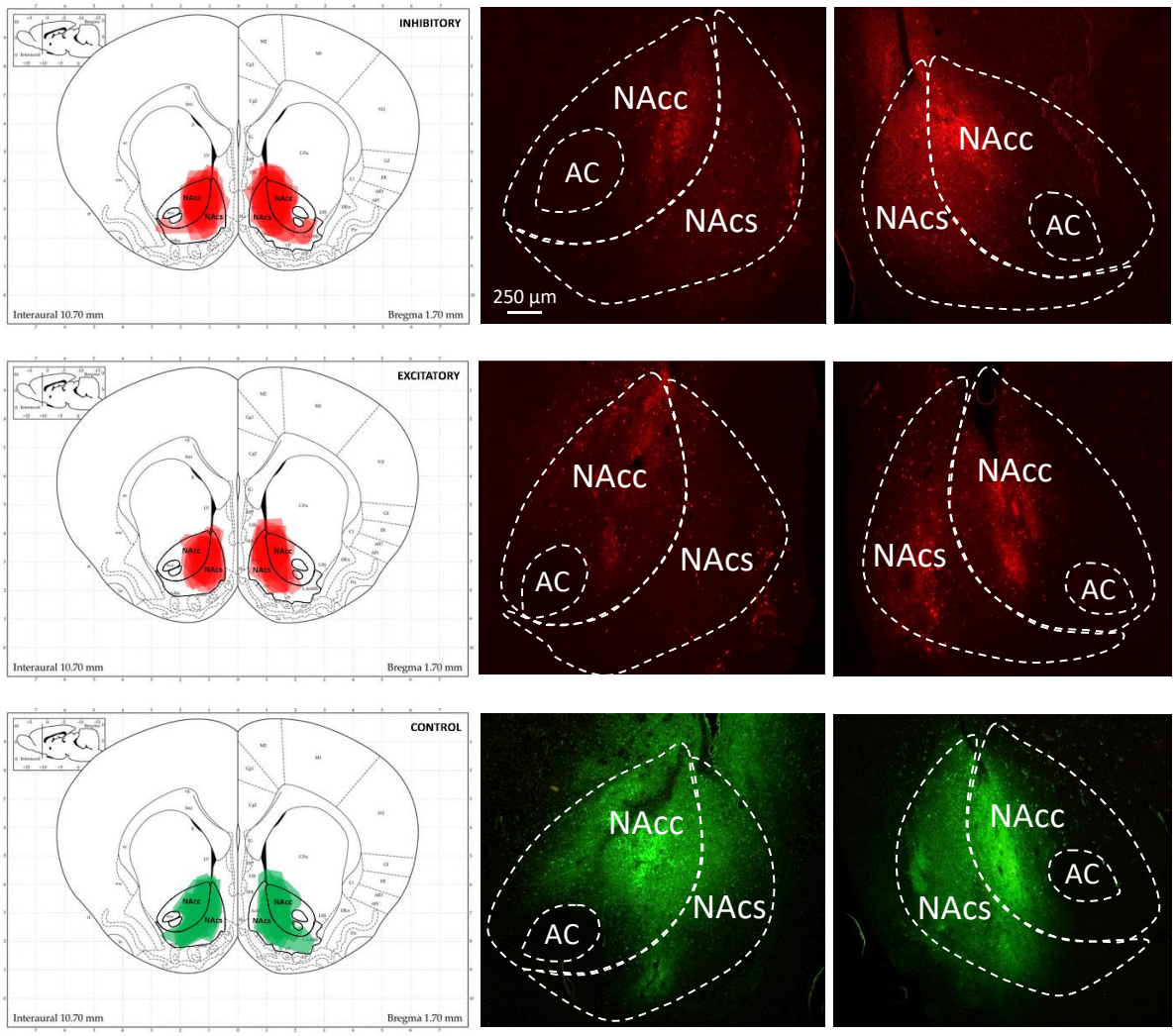
Statistical analyses. Lick frequency (cRD task), distance traveled (OF task), and cell count (c-Fos expression) data were analyzed with IBM SPSS Statistics 26. Behavioral data were analyzed as described in Experiment 1. The results of the OF task and cell count data for c-Fos expression were analyzed with one-way ANOVAs. All pairwise comparisons were based on the Bonferroni test. In all cases, an alpha value lower than 0.05 was adopted for inferences of statistical significance.

Results

DREADDs localization. Animals that lacked viral expression in both hemispheres (n = 3) or had viral expression significantly extended beyond the NAc (n = 6) were excluded from all analyses. Animals included in the final sample (n = 49) exhibited viral expression in the NAc and in both brain hemispheres. This final sample was distributed as follows: Inh/CNO (n = 9), Inh/Veh (n = 8), Exc/CNO (n = 8), Exc/Veh (n = 8), VVC/CNO (n = 8), and VVC/Veh (n = 8). Figure 7 shows the expression of the hM4Di (Inh) and hM3Dq (Exc) DREADDs, and the viral vector control (VVC) in the NAc in both hemispheres (middle and right columns), and the area of maximal fluorescence on the Paxinos and Watson (2013) atlas (left column). The overlap indicates that the infusions were on target for the selected animals, although it also extended to adjacent regions in some of the animals.

Figure 8

DREADD Expression for 32-to-2% NAc Groups



Note. The left column shows the overlay of polygons drawn by connecting the external points of fluorescent markers from each brain and mapping them into a representation from the Paxinos and Watson (2013) atlas. The figure shows fluorescent areas in animals from Groups Inh/CNO (inhibitory; top), Exc/CNO (excitatory; middle), and VVC (control; bottom). The other two columns show actual fluorescent images from selected brains. DREADDs were generally on target, although they were nonselective in terms of core (NAcc) and shell (NAcs) regions of the NAc. AC: anterior commissure. The A/P coordinate of this slice is +1.70.

Controls. Animals that received the VVC and were treated with either CNO (n = 8) or Veh (n = 8) were combined into a single Group VVC. Performance of Group VVC

was compared with animals receiving Inh/Veh (n = 9) and Exc/Veh (n = 8) in separate analyses for each of the dependent variables. A Group (Inh/Veh, Control) by Session (10-14) analysis for the cRD task indicated nonsignificant effect of Group and Group by Session interaction, Fs < 1. The effect of Session was significant, $F(4, 84) = 19.49$, $p < 0.001$, $\eta^2 = 0.48$. Another Group (Exc/Veh, Control) by Session (10-14) analysis also indicated nonsignificant effect of Group and Group by Session interaction, Fs < 1. This analysis revealed a significant change across sessions, $F(4, 84) = 23.46$, $p < 0.001$, $\eta^2 = 0.53$. A one-way analysis assessing OF activity involving animals in Inh/Veh (n = 3), VVC/CNO (n = 4), and VVC/Veh (n = 3) groups indicated nonsignificant differences, $F < 1$. A similar analysis assessing OF activity involving animals in Exc/Veh (n = 8), VVC/CNO (n = 4), and VVC/Veh (n = 3) groups also indicated nonsignificant difference, $F < 1$. A one-way analysis assessing c-Fos expression involving animals in Inh/Veh (n = 5), VVC/CNO (n = 6), and VVC/Veh (n = 6) indicated nonsignificant differences, $F < 1$. A similar analysis involving animals in Exc/Veh (n = 4), VVC/CNO (n = 6), and VVC/Veh (n = 6) also indicated nonsignificant difference, $F < 1$. Thus, animals that received DREADD infusions and treated with vehicle (Inh/Veh or Exc/Veh) and animals that received VVC infusions and treated with either CNO or Veh were pooled into a single Group Control for each DREADD manipulation condition, for statistical purposes for all dependent variables. Means (±SEMs) for each of the groups pooled into Group Control are presented in Table 1. Data for Groups Inh/Veh, Exc/Veh, and VVC are presented separately in Figures 9-11.

Table 1

Lick frequency (cRD), distance traveled (OF), and cell count (c-Fos) for Controls

		cRD				OF	
Exp	Group	11	12	13	14	15	c-Fos
2a	Inh/Veh	926.0 (±94.0)	1036.5 (±131.8)	1100.3 (±131.6)	1041.4 (±93.7)	3946.4 (±440.3)	384.5 (±93.6)
	Exc/Veh	868.9 (±104.6)	1088.9 (±99.7)	1276.9 (±61.7)	1217.3 (±109.3)	3734.3 (±249.2)	473.5 (±89.8)
	VVC/CNO	1368.3 (±120.1)	1508.7 (±100.4)	1243.3 (±146.9)	1331.7 (±81.4)	3926.6 (±417.5)	461.3 (±117.4)
	VVC/Veh	1519.7 (±95.0)	1511.0 (±77.0)	1538.3 (±119.0)	1340.3 (±92.9)	3579.2 (±315.5)	325.9 (±49.3)
2b	Inh/Veh	1405.0 (±71.9)	1359.1 (±116.8)	1404.1 (±88.4)	1387.3 (±148.8)	3432.9 (±188.0)	527.9 (±129.7)
	VVC/CNO	1368.3 (±120.1)	1508.7 (±100.4)	1243.3 (±146.9)	1331.7 (±81.4)	3089.6 (±302.5)	385.8 (±51.1)
	VVC/Veh	1519.7 (±95.0)	1511.0 (±77.0)	1538.3 (±119.0)	1340.3 (±92.9)	2900.9 (±538.5)	541.2 (±54.8)
3	Inh/Veh	929.4 (±158.5)	1061.7 (±66.6)	1035.9 (±78.5)	1188.9 (±70.8)	3534.5 (±218.2)	64.0 (±13.6)
	VVC/CNO/Inh	996.0 (±34.0)	1174.0 (±56.0)	970.3 (±163.8)	1013.3 (±158.3)	1713.8 (±452.6)	
	VVC/Veh/Inh	889.3 (±112.8)	857.0 (±33.7)	1271.0 (±43.0)	1249.0 (±140.0)	1930.3 (±391.2)	
	Exc/Veh	969.8 (±89.7)	1127.6 (±132.8)	1357.4 (±47.2)	1291.9 (±66.6)	3225.4 (±206.1)	80.9 (±11.6)
	VVC/CNO/Exc	1077.3 (±216.6)	1312.3 (±76.9)	1274.0 (±34.0)	1294.0 (±58.0)	1997.5 (±234.4)	
	VVC/Veh/Exc	1125.5 (±212.5)	1370.0 (±72.0)	1427.3 (±181.4)	1271.7 (±252.7)	2181.2 (±799.4)	

Note. Means (±SEM) for cRD sessions 11-14, OF task (session 15), and c-Fos cell counts

cRD task. All animals in Experiment 2a had access to 32% sucrose during 10 preshift sessions and control animals were common to both inhibitory and excitatory DREADD conditions. Two separate Group by Session (1-10) analyses to assess preshift performance in each DREADD condition were conducted. For inhibitory DREADD condition, a Group (Inh, Control) by Session (1-10) analysis confirmed that the main effect of Group and the Group by Session interaction were nonsignificant, Fs < 1.82, ps > 0.18, η^2s < 0.05. Lick frequency increased significantly across sessions, $F(9, 279) = 30.55$, $p < 0.001$, $\eta^2 = 0.50$. For excitatory DREADD condition, a Group (Exc, Control) by Session (1-10) analysis also confirmed that the main effect of Group and the Group by Session interaction were nonsignificant, Fs < 1. Lick frequency increased significantly across sessions, $F(9, 270) = 34.82$, $p < 0.001$, $\eta^2 = 0.54$.

Consummatory behavior during the last preshift session (session 10) and the four downshift sessions (sessions 11-14) is presented separately for inhibitory DREADD and excitatory DREADD manipulations in Figure 9. Group Control for each DREADD manipulation included some animals unique to each DREADD treatment (Inh/Veh and Exc/Veh) and some animals common to both DREADD conditions (VVC/CNO and VVC/Veh). Two analyses evaluated the consummatory behavior during these sessions in each of the two DREADD treatments.

Postshift inhibitory DREADD analyses (Figure 9, left). A Group (Inh, Control) by Session (10-14) analysis was conducted to assess suppression of consummatory behavior and any effects of NAc chemogenetic inhibition on cRD. A significant effect of Session was detected, $F(4, 124) = 36.69$, $p < 0.001$, $\eta^2 = 0.54$. The Group by Session interaction was also significant, $F(4, 124) = 2.69$, $p < 0.05$, $\eta^2 = 0.08$. The main effect of Group was

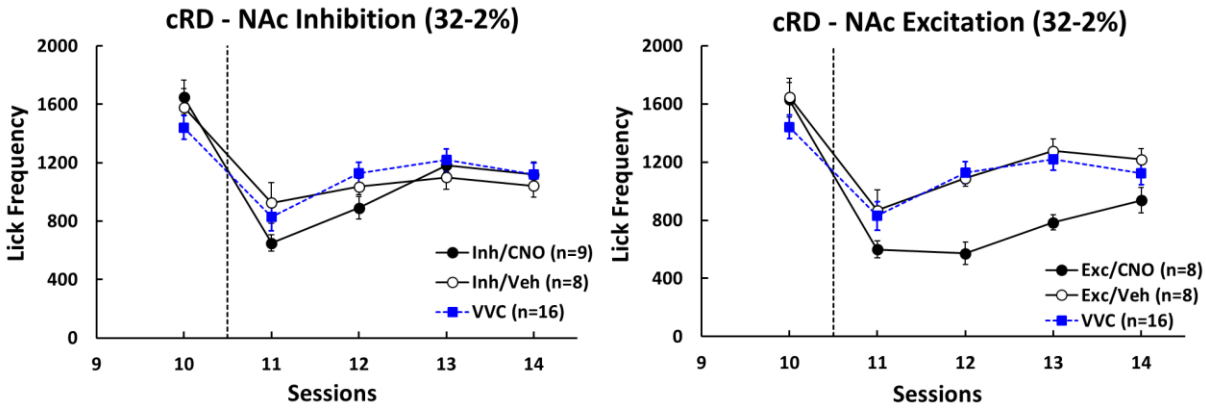
nonsignificant, $F < 1$. Pairwise Bonferroni tests indicated that both groups significantly suppressed lick frequency from session 10 to 11, ps < 0.001. Consummatory behavior of Groups Inh and Control did not differ on session 10 or on any of the downshift sessions, ps > 0.11, suggesting that NAc chemogenetic inhibition did not affect cRD performance during downshift sessions.

Postshift excitatory DREADD analyses (Figure 9, right). A Group (Exc, Control) by Session (10-14) analysis was conducted to assess suppression of consummatory behavior and any effects of NAc chemogenetic excitation on cRD. Significant main effects of Session, $F(4, 120) = 40.80$, $p < 0.001$, $\eta^2 = 0.58$, and Group, $F(1, 30) = 8.74$, $p < 0.006$, $\eta^2 = 0.23$, were detected. The Group by Session interaction was also significant, $F(4, 120) = 6.36$, $p < 0.001$, $\eta^2 = 0.17$. Pairwise Bonferroni tests indicated that both groups significantly suppressed lick frequency from session 10 to 11, ps < 0.001. Additionally, lick frequency of Group Exc was significantly below Group Control on sessions 12 and 13, ps < 0.001, suggesting that NAc chemogenetic excitation affected the recovery response from cRD.

As expected, the effects of NAc inhibition and NAc excitation were asymmetrical. The 32-to-2% sucrose downshift led to significant suppression of behavior in both DREADD conditions. However, NAc chemogenetic inhibition did not interfere with the recovery of consummatory behavior after cRD, whereas NAc chemogenetic excitation enhanced consummatory suppression during downshift sessions.

Figure 9

Results for cRD Task for 32-to-2% NAc Groups

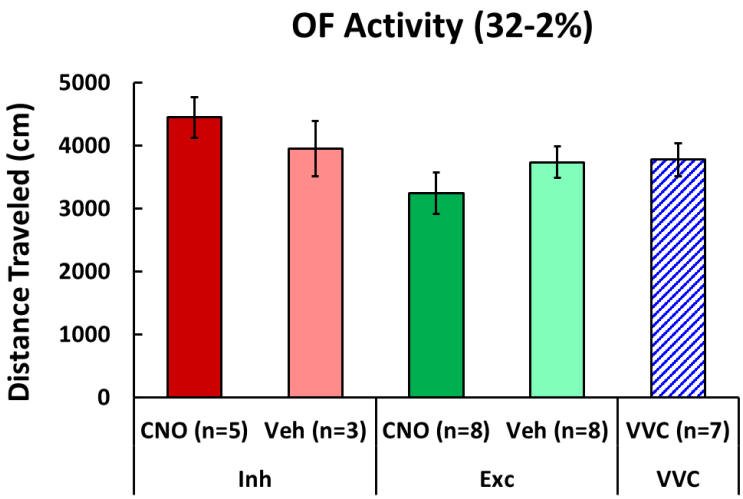


Note. Mean (±SEM) of lick frequency in animals receiving inhibitory, excitatory DREADDs, or VVC into the NAc. Group VVC (viral vector control; dashed lines) is common to both DREADD conditions. All animals received access to 32% sucrose during ten 5-min preshift sessions (only session 10 is shown in these figures) followed by access to 2% sucrose for an additional 4 postshift sessions.

OF task. The unusual freezing weather conditions described in the perfusion section affected OF data collection for 18 animals in the Inh (n = 9) and VVC (n = 9) conditions that were included in the cRD analyses. Data for these animals were not included in OF analyses. Total activity in the OF test in terms of distance traveled (cm) is presented in Figure 10. Separate one-way analyses were conducted for NAc inhibition and NAc excitation conditions, with Group Control including animals treated with vehicle injections within each DREADD condition (Inh/Veh and Exc/Veh) and a set of animals common to both groups (VVC/CNO and VVC/Veh). In both cases, statistical analyses found no significant differences between groups, Fs < 2.68, ps > 0.12, η^2s < 0.18. Thus, there was no evidence that these chemogenetic manipulation affected locomotor behavior.

Figure 10

Results for OF Test for 32-to-2% NAc Groups

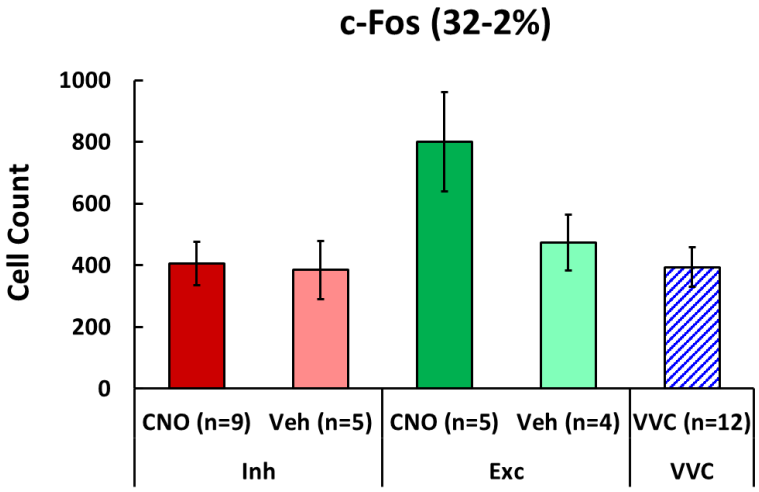


Note. Mean (±SEM) of distance traveled (cm) during 15-min OF test in animals receiving inhibitory (red bars), excitatory DREADDs (green bars), or viral vector control (VVC; blue bar) into the NAc.

c-Fos assessment. c-Fos expression for 14 animals included in the analyses for the cRD task was not assessed. Sections collected for c-Fos immunohistochemistry for these animals were compromised due to delays in brain tissue processing related to the COVID-19 pandemic. Thus, c-Fos data for these animals could not be collected. c-Fos expression data in terms of cell count is presented in Figure 11. One-way analyses indicated that whereas the difference between groups was nonsignificant for inhibitory DREADD animals, $F < 1$, c-Fos expression was significantly higher in excitatory DREADD animals relative to Group Control, $F(1, 19) = 9.21$, $p < 0.008$, $\eta^2 = 0.33$.

Figure 11

Results for c-Fos Expression in 32-to-2% NAc Groups



Experiment 2b

In Experiment 2a, NAc chemogenetic inhibition using a 32-to-2% sucrose downshift caused a mild, nonsignificant, suppression in consummatory behavior in the cRD task relative to Veh and VVC control groups (see Figure 9). The possibility that the large reward discrepancy (32-to-2% sucrose downshift) between preshift and postshift sessions prevented the emergence of an effect was worth exploring. To verify that a potential floor effect did not obscure the effects of chemogenetic inhibition, Experiment 2b replicated the NAc inhibition condition in the cRD task but using 8-to-2% sucrose downshift, a milder preshift-to-postshift reward discrepancy. If the absence of effects was related to a floor effect, a smaller reward disparity (8-to-2% sucrose downshift), producing little lick disruption during early downshift sessions, would facilitate the observation of behavioral effects during recovery sessions. As shown in Experiment 1 (see Figure 4), rats adjust to the downshift, but without producing a consummatory suppression significant below unshifted controls. In addition to Inh/CNO and Inh/Veh

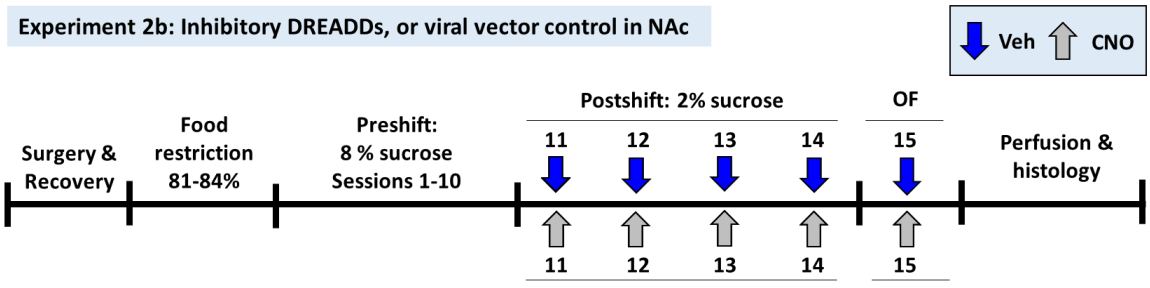
conditions, a few VVC animals were included, as were an OF task and a c-Fos expression assay.

Method

Subjects, apparatus, and behavioral tasks. The subjects were 25 male Wistar rats experimentally naïve to the procedures administered, about 90 days old at the start of the experiment. The mean (SEM) ad libitum weight of the 21 animals selected for analyses was 512.4 g (±12.9 g). Maintenance conditions, experimental procedures, and statistical analyses were as described in Experiments 1 and 2a. Surgery procedure for DREADD infusions, viral vectors, perfusion procedure, histological procedures were as described in Experiment 2a. Animals were randomly assigned to four groups: Inh/CNO, Inh/Veh, VVC/CNO, and VVC /Veh. In the cRD task, training conditions were as described in Experiment 1 for Group 8-2, that is, 10 preshift sessions of access to 8% sucrose followed by 4 postshift sessions of access to 2% sucrose. Figure 12 shows the sequence of events in Experiment 2b.

Figure 12

Experimental Procedure for Experiment 2b



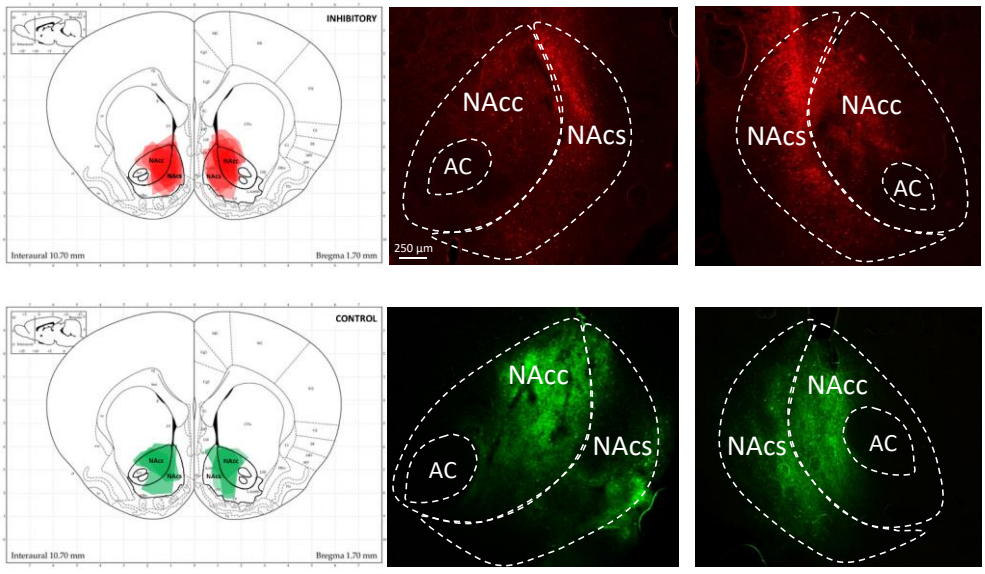
Note. In Experiment 2b, all animals were exposed to an 8-to-2% sucrose downshift. Open field (OF) testing was scheduled on session 15.

Results

DREADDs localization. Animals that lacked viral expression at the region of interest in both hemispheres (n = 2), or expression of the viral construct extended beyond the target region (n = 2) were excluded from all further analyses. Animal selected for the final sample were distributed as follows: INH/CNO (n = 8), INH/Veh (n = 7), VVC/CNO (n = 3), and VVC/Veh (n = 3). Figure 13 shows the expression of the hM4Di (Inh) DREADDs and the viral vector control (VVC) in the NAc in both hemispheres (middle and right columns), area of maximal fluorescence on the Paxinos and Watson (2013) atlas (left column). The overlap indicates that the infusions were on target for the selected animals, although it also extended to adjacent regions in most of the animals.

Figure 13

DREADD Expression for 8-to-2% NAc Groups



Note. The left column shows the overlay of polygons drawn by connecting the external points of fluorescent markers from each brain and mapping them into a representation from the Paxinos and Watson (2013) atlas. The figure shows fluorescent areas in animals from Groups Inh/CNO (inhibitory; top), and VVC (control; bottom). The other two columns show actual fluorescent images from selected brains. DREADDs were generally on target, although they were nonselective in terms of core (NAcc) and shell (NAcs) regions of the NAc. AC: anterior commissure. The A/P coordinate of this slice is +1.70.

Controls. As in Experiment 2a, animals that received the VVC and were treated with either CNO (n = 3) or Veh (n = 3) were combined into a single Group VVC. Performance of Group VVC was compared with animals receiving Inh/Veh (n = 7) for each of the dependent variables. A Group (Inh/Veh, VVC) by Session (10-14) analysis for the cRD task indicated nonsignificant effect of Group and Group by Session interaction, Fs < 1.14, ps > 0.36, η^2s < 0.19. The effect of Session was significant, $F(4, 40) = 2.93$, $p < 0.04$, $\eta^2 = 0.23$. One-way analyses comparing OF activity and c-Fos expression in Groups Inh/Veh and VVC indicated nonsignificant difference between these groups, Fs < 1. Thus, animals that received inhibitory DREADD infusions and treated with vehicle (Veh) animals that received VVC infusions and treated with either CNO or Veh were pooled into a single Group Control for statistical purposes for all dependent variables. Means (±SEMs) for all measures for each of the groups pooled into Group Control are presented in Table 1. Data for Groups Inh/Veh and VVC are presented separately in Figures 14-16.

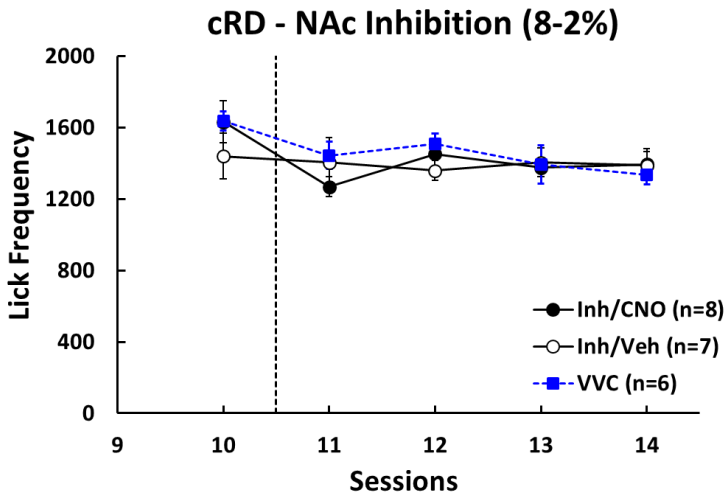
cRD task. All animals in Experiment 2b had access to 8% sucrose during 10 preshift sessions. Group Control included Inh/Veh and VVC animals. A Group (Inh, Control) by Session (1-10) analysis for preshift was conducted to assess whether there were differences before the downshift event. This analysis confirmed that the effect of Group and Group by Session interaction were nonsignificant, Fs < 1.92, ps > 0.12, η^2s < 0.09. Lick frequency increased significantly across sessions, $F(9, 171) = 54.93$, $p < 0.001$, $\eta^2 = 0.74$.

A Group (Inh, Control) by Session (10-14) analysis was conducted to assess suppression of consummatory behavior and any effects of NAc chemogenetic inhibition

on cRD (Figure 14). A significant effect of Session was detected, $F(4, 76) = 6.49$, $p < 0.001$, $\eta^2 = 0.26$. The main effect of Group and the Group by Session interaction were nonsignificant Fs < 1.70, $p > 0.16$, $\eta^2 < 0.09$. However, pairwise Bonferroni tests indicated that the effect of Session was due to a significant suppression of lick frequency from session 10 to 11, $p < 0.001$. Consummatory behavior of Groups Inh and Control did not differ on session 10 or on any of the downshift sessions, ps > 0.11, excluding the possibility that the lack NAc inhibition effects on cRD in Experiment 2a reflected a floor effect of consummatory behavior.

Figure 14

Results for cRD task for 8-to-2% NAc Groups



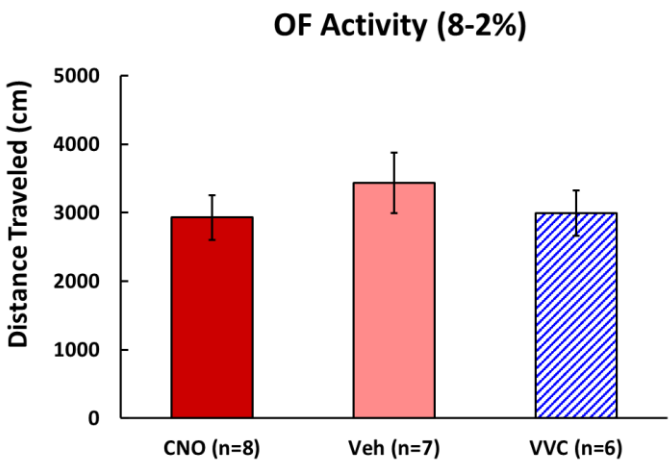
Note. Mean (±SEM) of lick frequency in animals receiving inhibitory DREADDs, or VVC (blue function) into the NAc. All animals received access to 8% sucrose during ten 5-min preshift sessions (only session 10 is shown in this figure) followed by access to 2% sucrose for an additional 4 postshift sessions.

OF task. Total activity in the OF test in terms of distance traveled (cm) is presented in Figure 15. A one-way analysis confirmed a nonsignificant difference

between groups, $F(1, 19) = 1.27$, $p > 0.27$, $\eta^2 = 0.06$, confirming that NAc chemogenetic inhibition did not affect locomotor behavior.

Figure 15

Results for OF Test for 8-to-2% NAc Groups

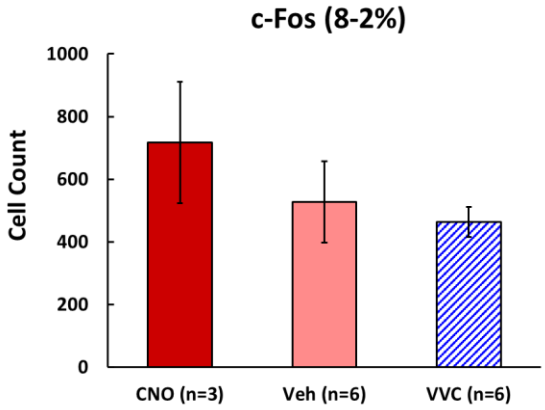


Note. Mean (±SEM) of distance traveled (cm) during 15-min OF session in animals receiving inhibitory (red bars) or viral vector control (VVC; blue bar) into the NAc.

c-Fos assessment. c-Fos expression for 6 animals included in the analyses for the cRD task was not assessed. Sections collected for c-Fos immunohistochemistry for these animals were compromised due to delays in brain tissue processing related to the COVID-19 pandemic. Thus, c-Fos data for these animals could not be collected, reducing the sample sizes for Groups Inh ($n = 3$) and Control ($n = 12$). c-Fos expression data in terms of cell count is presented in Figure 16. As in Experiment 2a, there was no evidence that activation of inhibitory DREADDs led to a reduction in c-Fos expression in the NAc. A one-way analysis indicated a nonsignificant group difference, $F(1, 13) = 1.89$, $p > 0.19$, $\eta^2 = 0.13$.

Figure 16

Results for c-Fos Expression in 8-to-2% NAc Groups



Experiment 3

NAc chemogenetic excitation, but not inhibition, had a clear behavioral effect on cRD. The reduction in lick frequency in the aversive context of a sucrose downshift produced by NAc excitation supported an inhibitory role for NAc neurons on projection regions. Of the several efferent pathways from the NAc, we focused on exploring its influence within the basal ganglia (BG) neural system. As reviewed in the introduction, activation of the BG direct pathway is associated with behavioral facilitation, whereas activation of the BG indirect pathway is associated with behavioral suppression (Aron et al., 2016; Lanciego et al., 2012; Nambu et al., 2002; Saint-Cyr, 2003).

Thus, based on the results of Experiment 2a, we hypothesized that NAc chemogenetic excitation activated inhibitory synapses connecting the NAc to the GPe – the indirect pathway. The GPe functions as a central relay station in the BG indirect pathway (Benhamou et al., 2012), creating a complex system of neural connections with the other BG structures for processing motor and non-motor information (Albin et al., 1989; Calabresi et al., 2014). Efferent projections from the GPe inhibit STN

glutamatergic neurons, which in turn excite GABAergic neurons in the GPi, a series of effects that interferes with thalamo-cortical circuits, thus suppressing behavior. Despite the complexity of these neural interactions, research supports these behavioral effects (Avila et al., 2020; Saunders et al., 2016; Stephenson-Jones et al., 2016; Yager et al., 2015).

If the effects of NAc chemogenetic excitation enhanced consummatory suppression during recovery from the cRD (decreased lick frequency relative to controls; Figure 9) because of activation of this indirect pathway, then chemogenetic excitation of the GPe, a key downstream nucleus in the indirect pathway, should produce the opposite effect, namely a reduction or elimination of consummatory suppression (increased lick frequency relative to controls) during RD. This is because excitation of GPe GABAergic neurons should lead to a strong inhibition of STN neurons, which should reduce excitatory input into the GPi, thus facilitating behavior resulting from thalamo-cortical pathway disinhibition. Although, based on the results of Experiment 2a and 2b, we expected GPe chemogenetic inhibition to lead to no observable effects, this condition was still included in Experiment 3.

The use of different reward disparities for GPe inhibition and excitation was designed to facilitate the observation of behavioral effects. Predicting that consummatory suppression would be enhanced with GPe inhibition or reduced with GPe excitation would seem to require small and large reward discrepancies, respectively, to minimize floor and ceiling effects. However, because reward discrepancy between preshift and postshift concentrations is directly related to the degree of consummatory suppression (Papini & Pellegrini, 2006; Pellegrini & Papini, 2007), we chose to produce strong and

weak levels of lick reduction for GPe inhibition and GPe excitation, respectively. A small reward discrepancy may have made it difficult to observe the effects of GPe inhibition as they could be obscured by little lick disruption, whereas a large reward discrepancy may have made it difficult to observe the effects of GPe excitation as they could be obscured by intense response suppression. The effect of GPe chemogenetic inhibition was therefore assessed using a 32-to-2% sucrose downshift, whereas the effect of GPe chemogenetic excitation was assessed using an 8-to-2% sucrose downshift.

Experiment 3 was also designed to attempt exploring the effects of GPe neural manipulation during early and late reward downshift sessions. In both chemogenetic manipulations, half of the animals received CNO administration before sessions 11-12 and Veh administration before sessions 13-14, whereas the other half of the animals received Veh administration before sessions 11-12 and CNO administration before sessions 13-14. This design would help explore the issue of whether the GPe is important in the initial response to the downshift vs. the recovery from the downshift. The BG may shift its control on behavior as a function of the context in which DREADDs are activated, whether during the more negative valence of the early downshift sessions or during the more positive valence of the late downshift sessions when consummatory behavior is recovering from the RD event (Soares-Cunha et al., 2020). As in Experiments 2a and 2b, animals were also tested in the OF task to assess potential motor effects, Group VVC was included, and brain tissue was assessed for c-Fos expression to validate DREADD activation.

Method

Subjects, apparatus, and behavioral tasks. The subjects were 60 male Wistar rats experimentally naïve to the procedures administered, about 90 days old at the start of the experiment. The mean (±SEM) ad lib weight of the 40 animals selected for analyses was 496.1 g (±7.9 g). Maintenance conditions, experimental procedures, and statistical analyses were as described in Experiments 1 and 2a. Surgery procedure for DREADD infusions, viral vectors, perfusion procedure, histological procedures were as described in Experiment 2a, unless explicitly noted.

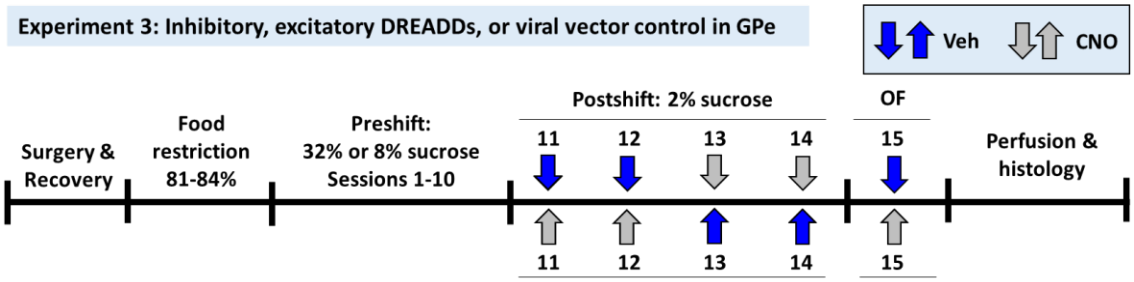
cRD task. The cRD task involved access to sucrose solutions of different concentrations as described in Experiment 1. Inhibitory DREADD animals and half of the VVC animals were exposed to a 32-to-2% sucrose downshift, whereas excitatory DREADD animals and the other half of the VVC animals were exposed to an 8-to-2% sucrose downshift. During 10 preshift sessions, animals with the inhibitory DREADDs had access to 32% sucrose, whereas animals with the excitatory DREADDs received access to 8% sucrose. All animals had access to 2% sucrose during the 4 postshift sessions.

Experimental design. Animals received an injection of CNO (3 mg/kg, intraperitoneal) or Veh (5% DMSO, 95% sterile saline, equal volume, intraperitoneal) 30 min before the start of downshift sessions 11-14. Within each DREADD condition (Inh and Exc), half of the animals received a CNO injection on sessions 11-12 and a Veh injection on sessions 13-14 (Groups Inh/11-12, Exc/11-12). The other half of the animals received a Veh injection on sessions 11-12 and a CNO injection on session 13-14 (Groups Inh/13-14, Exc/13-14). In Groups Inh/VVC and Exc/VVC, animals received the

same drug treatments described for the other groups, but CNO and Veh animals were pooled together as no differences were observed. During the OF test, animal assignment to either the CNO or Veh condition was consistent with the injection received during cRD downshift sessions 11-12. Figure 17 describes these procedures.

Figure 17

Experimental Procedure for Experiment 3



Note. In Experiment 3, animals in the GPe inhibition groups were exposed to a 32-to-2% sucrose downshift, whereas animals in the GPe excitation groups were exposed to an 8-to-2% sucrose downshift. Groups differed in the schedule of CNO vs. Veh injections during postshift sessions, with animals receiving CNO or Veh on sessions 11-12 and the opposite injection on sessions 13-14. Open field (OF) testing was scheduled on session 15. Injection condition during OF testing was consistent with the injection received on sessions 11-12.

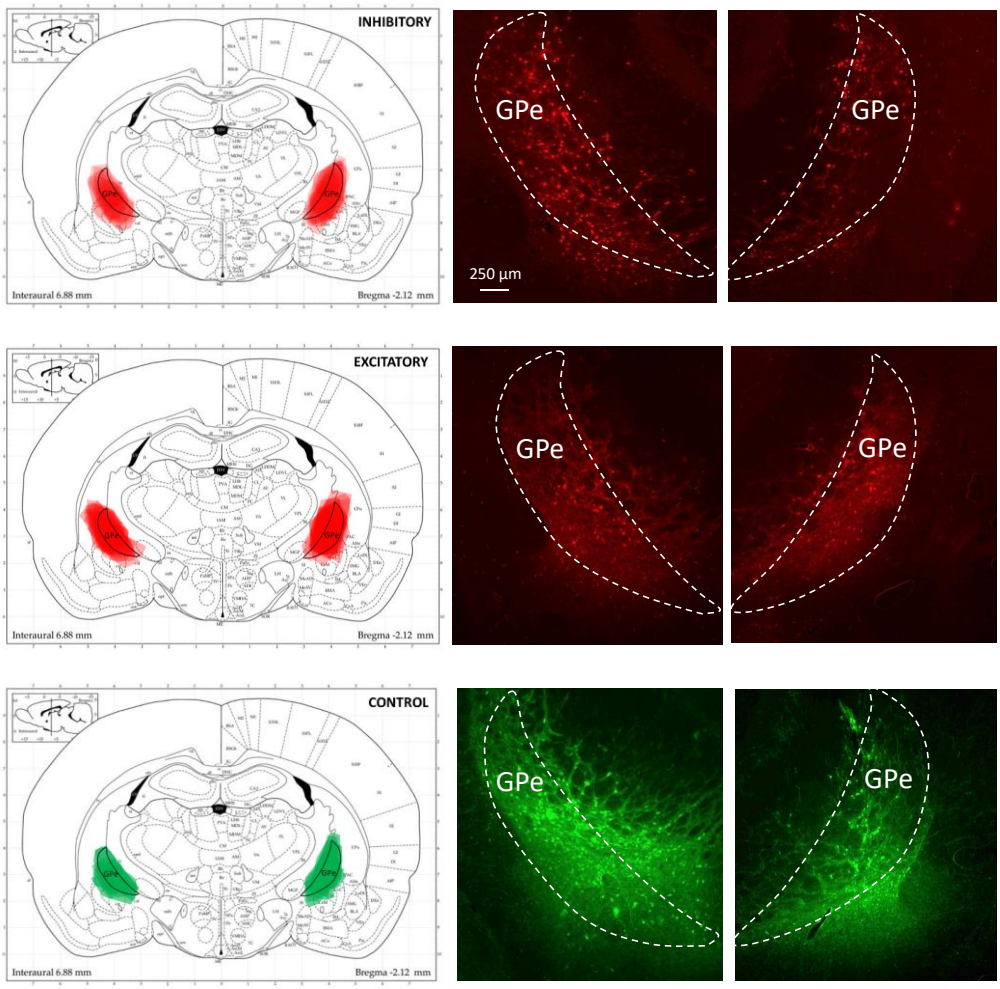
Results

DREADDs localization. Animals that lacked viral expression in both hemispheres (n = 5) or had viral expression significantly extended beyond the GPe (n = 15) were excluded from all analyses. Animals included in the final sample (n = 40) exhibited viral expression in the GPe and in both brain hemispheres. This final sample was distributed as follows: Inh/11-12 (n = 7), Inh/13-14 (n = 7), Inh/VVC (n = 5), Exc/11-12 (n = 8), Exc/13-14 (n = 8), and Exc/VVC (n = 5). Figure 18 shows the expression of the hM4Di (Inh) and hM3Dq (Exc) DREADDs, and the viral vector control (VVC) in the GPe in both hemispheres (middle and right columns), and the area of

maximal fluorescence on the Paxinos and Watson (2013) atlas (left column). The overlap indicates that the infusions were on target for the selected animals, although it also extended to adjacent regions in most of the animals.

Figure 18

DREADD Expression for GPe Groups



Note. The left column shows the overlay of polygons drawn by connecting the external points of fluorescent markers from each brain and mapping them into a representation from the Paxinos and Watson (2013) atlas. The figure shows fluorescent areas in animals from Groups Inh/CNO (inhibitory; top), Exc/CNO (excitatory; middle), and VVC (control; bottom). The other two columns show actual fluorescent images from selected brains. The figure shows a substantial overlap in the location of the fluorescence in the GPe region. Data from Experiment 3. The A/P coordinate of this slice is -2.12.

Controls. Performance of animals that received inhibitory DREADD infusion and treated with vehicle (Inh/Veh; n = 7), VVC/CNO (n = 2), and VVC/Veh (n=3) was analyzed using a Group by Session (10-12) design. The main effect of Group and Group by Session interaction were nonsignificant, Fs < 1, but the main effect of Session was significant, $F(2, 18) = 9.83$, $p < 0.001$, $\eta^2 = 0.52$. Similarly, performance of animals that received excitatory DREADD infusion and treated with vehicle (Exc/Veh; n = 7), VVC/CNO (n = 3), and VVV/Veh (n = 2) was analyzed using a Group by Session (10-12) design. The main effect of Group and Group by Session interaction were nonsignificant, Fs < 1, but the main effect of Session was significant, $F(2, 20) = 27.30$, $p < 0.001$, $\eta^2 = 0.73$. The analyses for OF performance in inhibitory DREADD animals, including Inh/Veh (n = 7), VVC/CNO (n = 2), and VVC/Veh (n = 3), revealed nonsignificant differences, $F < 1$. A similar analysis for animals excitatory DREADD animals, including Exc/Veh (n = 8), VVC/CNO (n = 3), and VVC/Veh (n = 2) also revealed nonsignificant differences, $F < 1$. Unfortunately, samples for all VVC animals collected for c-Fos assessment were lost due to lab access restrictions related to the COVID-19 pandemic. Data from these groups were pooled into a single Group Control for each DREADD manipulation condition for cRD and OF analyses. Means (±SEMs) for each of the groups pooled into Group Control are presented in Table 1, but their data is shown separately in Figures 19-21.

cRD task. Preshift performance (sessions 1-10) was analyzed separately for inhibitory DREADD animals (with access to 32% sucrose) and excitatory DREADD animals (with access to 8% sucrose). Group by Session (1-10) analyses indicated only a significant increase in licking across sessions, Fs > 63.83, ps < 0.001, η^2s > 0.79. The

main effect of Group effect and Group by Session interaction were nonsignificant, Fs < 1.06, ps > 0.40, η^2s < 0.06.

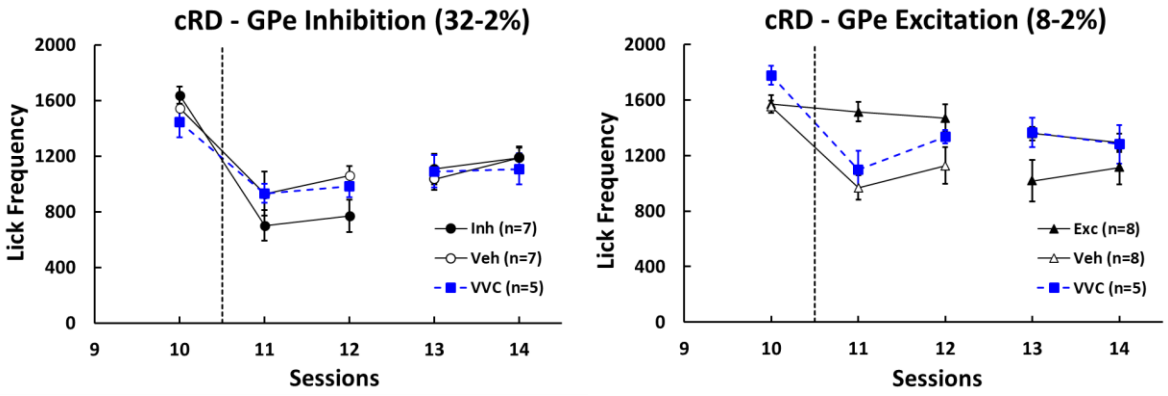
All animals had access to 2% sucrose during postshift sessions. Half the animals received CNO injections before sessions 11-12 and Veh before sessions 13-14; the other half received CNO injections before sessions 13-14 and Veh before sessions 11-12. This design allowed for a comparison of chemogenetic effects either early or late in downshift sessions. In the statistical analyses, animals that received DREADD infusions and treated with vehicle, and animals that received the VVC and treated with CNO or VVC were pooled into a single Control group.

Postshift inhibitory DREADD analyses (Figure 19, left). A Group (Inh, Control) by Session (10-12) analysis indicated a significant main effect of Session, $F(2, 34) = 50.72$, $p < 0.001$, $\eta^2 = 0.75$, and the Group by Session interaction was also significant, $F(2, 34) = 3.60$, $p < 0.04$, $\eta^2 = 0.18$. The Group effect was nonsignificant, $F(1, 17) = 2.64$, $p > 0.21$, $\eta^2 = 0.13$. Additionally, pairwise Bonferroni tests indicated that both Groups Inh and Control significantly suppressed consummatory behavior from session 10 to 11, ps < 0.001. The source of the interaction is the significant difference between Group Exc and Group Control on session 12, $p < 0.03$. A different set of analyses was conducted for downshift sessions 13-14, as different animals received CNO vs. Control treatments in early vs late downshift sessions. A Group (Inh, Control) by Session (13-14) analysis revealed nonsignificant effects for all three factors, Fs < 3.22, ps > 0.09, η^2s < 0.16. These results indicated that GPe chemogenetic inhibition enhanced consummatory suppression during early downshift sessions relative to Group Control, without affecting behavior on late downshift sessions.

Postshift excitatory DREADD analyses (Figure 19, right). A Group (Exc, Control) by Session (10-12) analysis indicated significant effects for all three factors, Fs > 6.46, ps < 0.02, η^2s > 0.25. Pairwise Bonferroni tests indicated no significant group differences on session 10, $p > 0.41$, but GPe excitation produced significantly less consummatory suppression relative to Group Control on session 11, $p < 0.001$. This difference was reduced on session 12, $p > 0.06$. Importantly, whereas Group Control significantly suppressed consummatory behavior from session 10 to 11, $p < 0.001$, there was no evidence of suppression in Group Exc, $p > 1$. As done for inhibitory DREADD analyses, a different set of analyses was conducted for downshift sessions 13-14. A Group (Exc, Control) by Session (13-14) indicated that Group Exc suppressed consummatory behavior significantly more than Group Control, $F(1, 19) = 5.17$, $p < 0.04$, $\eta^2 = 0.21$. The main effect of Session and Group by Session interaction were nonsignificant, Fs < 2.01, ps > 0.17, η^2s < 0.10. Pairwise Bonferroni tests indicated that Groups Exc and Control were significantly different on session 13, $p < 0.02$, but not on session 14, $p > 0.18$. These results indicated that GPe chemogenetic excitation reduced consummatory suppression during early downshift sessions, and enhanced consummatory suppression on late downshift sessions, in both cases relative to Group Control.

Figure 19

Results for cRD Task for GPe Groups

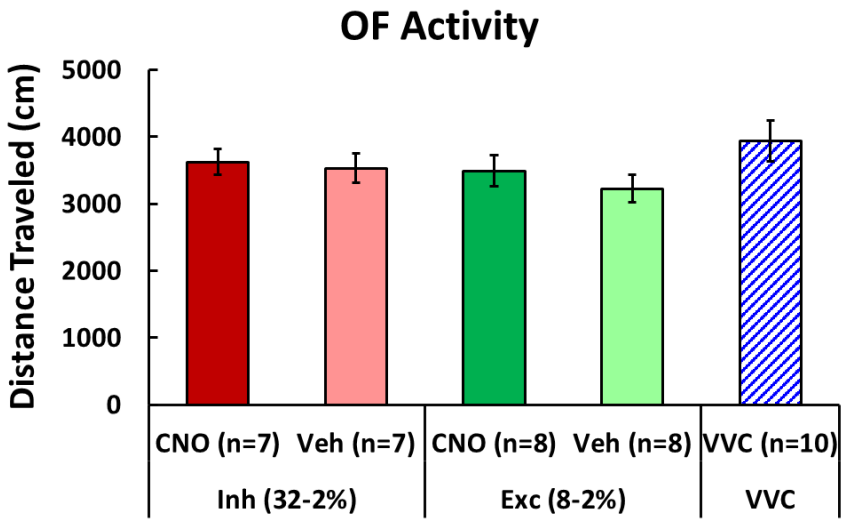


Note. Means (±SEM) of lick frequency for inhibitory (Inh) and excitatory (Exc) DREADD groups during the last preshift session (session 10) and postshift sessions 11-14. Group labels refer to the treatment received (either inhibitory DREADD, Inh; excitatory DREADD, Exc; or viral vector control, VVC) and the sessions in which they received clozapine N-oxide (CNO) injections (either 11-12 or 13-14; with vehicle, Veh, injections administered in the alternative postshift sessions). For VVC groups (dashed lines), animals that received CNO or vehicle were pooled together. During preshift sessions, animals in the inhibitory DREADD condition had access to 32% sucrose, whereas animals in the excitatory DREADD condition had access to 8% sucrose. All animals were exposed to 2% sucrose during postshift sessions 11-14.

OF task. Figure 20 shows that distance traveled in the OF test was similar across all five groups. Separate one-way analyses were computed for GPe inhibition and GPe excitation conditions, with Group Control pooling animals within each DREADD condition treated with vehicle injections (Inh/Veh and Exc/Veh) and a set of animals common to both groups (VVC/CNO and VVC/Veh). In both cases, statistical analyses found no significant differences between groups, $F < 1$. Thus, there was no evidence that these chemogenetic manipulation affected locomotor behavior.

Figure 20

Results for OF test for GPe Groups

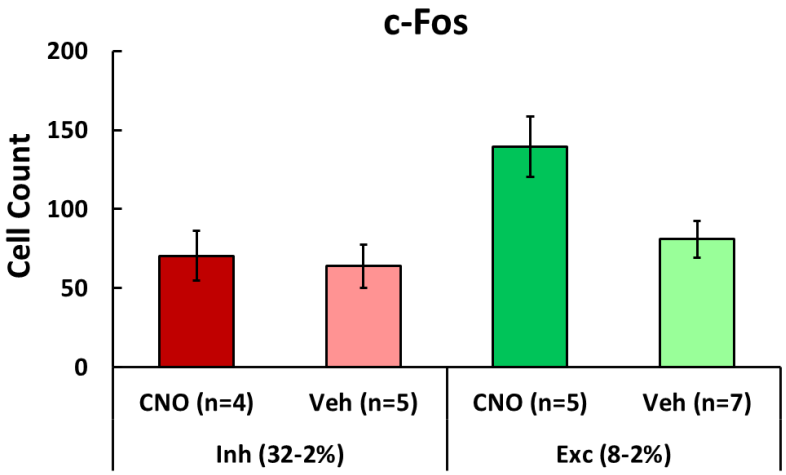


Note. Mean (±SEM) of distance traveled (cm) during 15-min OF session in animals receiving inhibitory (red bars), excitatory DREADDs (green bars), or viral vector control (VVC; blue bar) into the GPe.

c-Fos expression. Tissue samples were lost for 19 animals due to delays related to the COVID-19 pandemic. Counts for 1 animal in the VVC/CNO and for 1 animal in the VVC/Veh were available, so these data were added to the Exc/Veh condition. The final sample size for each of the groups was: 4, 5, 5, and 7 for Groups Inh/CNO, Inh/Veh, Exc/CNO, and Exc/Veh, respectively. The low sample size suggests taking these results with caution. Figure 21 shows that c-Fos expression was nondifferential for inhibitory DREADD groups, but there was higher c-Fos expression in excitatory DREADD animals relative to their respective control group. Bonferroni comparisons supported these conclusions, $p > 0.76$ and $p < 0.02$, respectively.

Figure 21

Results from c-Fos Expression GPe Groups



Discussion

This dissertation focused on the functions of the BG in a neural circuitry underlying frustrative nonreward. The BG involve a complex neural system of interconnected pathways hypothesized to modulate parallel and complementary circuits regulating motor, cognitive, and emotional functions (Eisinger et al., 2018). Dorsal and ventral striatal regions, including the CP and the nucleus accumbens NAc, send neural signals to a thalamo-cortical circuit via direct and indirect pathways. Activation of the direct pathway disinhibits the thalamo-cortical circuit via the GPi, thus increasing action, while activation of the indirect pathway suppresses thalamo-cortical circuit, thus reducing action, through GPi activation via the GPe and STN (Aron et al., 2016; Nambu et al., 2002; Saint-Cyr, 2003). The interplay of these pathways balances the output of the motor cortex necessary for normal movement. Knowledge of the role of the BG as predominantly modulating motor functions has been increasingly extended by evidence suggesting that these nuclei are also implicated in several non-motor domains, including

language processing, decision-making, learning, memory, and emotion (Avila et al., 2020; Saint-Cyr, 2003; Weintraub & Zaghloul, 2013). This dissertation specifically focused on studying the role of the NAc and GPe in the initial and recovery responses to unexpected reward loss in the cRD task, a situation that triggers emotional activation.

Importance of Preshift-to-postshift Sucrose Discrepancy

In neural-manipulation experiments involving sucrose downshifts, it is important to consider the preshift-to-postshift reward discrepancy to exclude the possibility that floor or ceiling effects obscure the outcomes of neural manipulations. In Experiment 1 of the current dissertation, intact animals were tested to provide evidence for a large (32-to-2% sucrose downshift) and a mild (8-to-2% sucrose downshift) preshift-to-postshift consummatory suppression induced by the sucrose concentrations used in subsequent neural manipulation experiments. Additionally, Experiment 1 provided behavioral evidence of consummatory performance in unshifted 2% sucrose controls and evidence of cRD effects after 32-to-2% sucrose downshift. Because unshifted 2% controls were not included in Experiments 2-3 to reduce the number of animals, results from Experiment 1 provided support for the assumption that the effects of the chemogenetic manipulations implemented in Experiments 2-3, under the same training conditions, are consistent with the effects observed in intact animals. The selection of sucrose concentrations expected to produce large or mild preshift-to-postshift consummatory suppression aimed to minimize possible floor or ceiling effects on licking behavior that could obscure the effects of chemogenetic manipulations. Based on this rationale, to facilitate the observation of enhanced suppression related to neural manipulation, I selected preshift-to-postshift sucrose concentrations that would not usually produce

pronounced consummatory suppression (i.e., 8-to-2% sucrose). By contrast, to facilitate the observation of reduced suppression, I selected preshift-to-postshift sucrose concentrations that are known to produce strong consummatory suppression (i.e., 32-to-2% sucrose; Papini & Pellegrini, 2006; Pellegrini & Papini, 2007). Although both sucrose downshifts produce significant preshift-to-postshift consummatory suppression from the last preshift session (session 10) to the first postshift session (session 11), only the 32-to-2% downshift produce significant suppression below the level of an unshifted control (2% sucrose).

Notice that emotional activation can occur in the absence of significant levels of consummatory suppression relative to the unshifted control (Ortega, Daniel, et al., 2011; Pellegrini et al., 2005). For example, one study using a 16-to-4% sucrose downshift, which did not produce consummatory suppression below an unshifted control (4% sucrose), did yield significant suppression in animals treated with a formalin injection in one foot. Formalin added peripheral pain to the downshift event, thus inducing the effect, but in the absence of a reward downshift in unshifted controls, formalin had no effect on consummatory behavior (Ortega, Daniel, et al., 2011). In hindsight, it would have even been more sensitive to use an 8-to-2%, milder reward disparity for other chemogenetic manipulations tested in this research. For example, the milder reward disparity may have enhanced the effects of NAc excitation in Experiment 2a, and an effect of GPe inhibition in Experiment 3 may have been enhanced. The opposite problem was to produce such a strong level of consummatory suppression that chemogenetic effects would be obscured. Thus, when predicting that chemogenetic excitation of the GPe would reduce consummatory suppression (see introduction to Experiment 3), I chose to use a milder

reward disparity (8-to-2% sucrose) to enhance chances of detecting an effect. This proved to be an appropriate decision (see Figure 19, right).

NAc Chemogenetic Excitation Disrupts Recovery from cRD

Experiment 2 showed, for the first time, that the NAc is involved in the modulation of the recovery response in the cRD task. Although chemogenetic inhibition of NAc neurons, using either a large 32-to-2% or a mild 8-to-2% sucrose downshift, did not disrupt licking, chemogenetic excitation of NAc neurons affected the recovery response to reward loss. Specifically, compared to control conditions, NAc excitation resulted in slower recovery of consummatory behavior after the sucrose reward was devalued from a 32%, high-value reward to a 2%, low-value reward, suggesting that activation of NAc neurons may enhance the aversive emotional state induced by reward downshift. The lack of evidence of increased consummatory suppression during the first downshift event could be the result of a floor effect due to a strong emotional response; however, it could also suggest that the NAc is involved in emotion regulation only after some experience with the aversive context associated with reward loss has occurred. It is well established that contextual cues associated with rewarding or aversive consequences produce changes in emotional states (Flagel et al., 2011; Namburi et al., 2015).

Various studies have identified the amygdala has a key structure involved in the processing and regulation of the emotional response to changes in contextual cues associated with reward shifts (Esber et al., 2015; Madarasz et al., 2016; Peck & Salzman, 2014; Tye et al., 2008). However, recent evidence indicated a similar function for the PVT in emotion regulation (Choi & McNally, 2017; Hsu et al., 2014). PVT neurons are activated when contextual cues are associated with reward outcomes (Choi et al., 2010;

Igelstrom et al., 2010; Li et al., 2016) or aversive outcomes (Do-Monte et al., 2015b; Penzo et al., 2015; Yasoshima et al., 2007). Distinct PVT pathways are activated by stimuli with opposite valence and are selectively recruited to modulate responses depending on the emotional context (Do-Monte et al., 2017). Important for this research is that the PVT is a major source of glutamatergic inputs to the NAc (Li & Kirouac, 2008; Moga et al., 1995). Photoinhibition of the efferent projections from the anterior PVT to the NAc did not affect lever pressing in a cued sucrose-seeking task when reward was available, but increased lever pressing when reward was omitted. By contrast, photoactivation of anterior PVT efferent projections to NAc reduced sucrose seeking and induced place aversion. Also, photoinhibition of the same projections did not produce observable effects on consumption of sucrose when freely available in a 5-min session or locomotion in an OF test (Do-Monte et al., 2017). This pattern of results suggests that the projection from the anterior PVT to the NAc was selectively recruited during the frustrative state induced by reward omission, a hypothesis consistent with the findings of Experiment 2. It is possible that the negative emotional state induced by the cRD context triggers activation of these projections and excitatory DREADDs in the NAc enhance this aversive emotional state of the animals, leading to enhanced rejection of the downshifted reward. The lack of effects on late downshift sessions of the cRD, when the aversive emotional state decreased, and on locomotor activity in the OF test provide further support to the idea that these effects are the result of specific interference with the circuitry modulating the emotional response to reward loss.

Further, MSNs in the NAc express both D1 and D2 receptors. Although research has shown that striatal D1-expressing MSNs encode reward, whereas D2-expressing

MSNs encode aversion (Tai et al., 2012; Kravitz et al., 2012), recent studies indicated that D1 and D2 MSNs subtypes may signal both reward and aversion depending on the activation pattern. For example, brief optogenetic stimulation of D1 and D2 MSNs in the NAc elicited positive reinforcement and enhanced cocaine conditioning, whereas prolonged activation induced aversion and decreased cocaine conditioning (Soares-Cunha et al., 2020). In relation to our research, one possibility is that excitatory DREADDs may induce a similar aversive state to that induced by prolonged optogenetic stimulation.

The lack of significance in the case of NAc inhibition provides support for the lack of effects of DMS lesions on cRD and OF tasks (Torres et al., 2016), and the lack of effects of NAc lesion on cRD (Leszczuk & Flaherty, 2000). Such neural manipulation of the two main input nuclei to the BG neural system affect both the direct and indirect pathways, disrupting the balance between the two, thus making it difficult to detect behavioral changes.

GPe Chemogenetic Manipulations Disrupts cRD effects

Experiment 3 showed, for the first time, that the GPe is involved in the modulation of licking behavior in the cRD task. Chemogenetic manipulations of GPe neurons produced a complex set of results. Chemogenetic inhibition of GPe neurons using a 32-to-2% sucrose downshift increased consummatory suppression during early downshift sessions, whereas chemogenetic excitation of GPe neurons using an 8-to-2% sucrose downshift reduced consummatory suppression also during early downshift experience, when the emotional response to reward loss is at peak. Although GPe inhibition did not affect late downshift sessions, GPe excitation unexpectedly produced enhanced consummatory suppression on late downshift session. Pharmacological and

lesion studies reported selectivity in terms of the postshift session on which they have effects. For example, benzodiazepines anxiolytics (Flaherty et al., 1990; Flaherty et al., 1986) and anterior cingulate cortex (ACC) lesions (Ortega, Uhelski, et al., 2011) reduced the effects of RD only after some experience with the downshifted reward, whereas opioids targeting delta receptors (DPDPE, naltrindole) affected the initial response to the sucrose downshift, but did not disrupt recovery of consummatory behavior (Wood et al., 2005). Findings from Experiment 3 are the first to provide support for a potential role for the GPe during the initial response to cRD, without disrupting behavioral during recovery in the case of GPe inhibition, but reversing the effects in the case of GPe excitation. These results provide evidence that the BG may shift its control on behavior depending on exposure to stimuli with negative valence during initial downshift sessions or more positive valence during recovery from cRD in late downshift sessions.

Overall, the effects of these manipulations of GPe neural activity on the cRD task are more consistent with an emotional effect induced by the reward loss than with a motor effect, since both chemogenetic manipulations affected early downshift sessions, when the emotional response to reward loss is higher. A motor hypothesis would have predicted a persistent effect on licking across all downshift sessions in both DREADD conditions, since motor effects would be independent of reward loss. Although there was no evidence that inhibiting GPe neurons affected performance in late downshift sessions, GPe excitation had opposite effects on early vs. late downshift sessions. The absence of evidence that these manipulations affected locomotor activity in the OF test, also supports the emotional activation hypothesis, suggesting that these two dependent variables are regulated by different mechanisms. Other experiments have shown that

these two tasks can be affected by the same neural manipulation. For example, reversible inactivation of the centromedial amygdala eliminated the cRD effect as well as avoidance of the central area in the OF task (Kawasaki et al., 2015). These results were interpreted as an emotional function of this amygdala nucleus in emotional behavior, involving frustrative nonreward in the cRD task and unconditioned fear in the OF task. However, lesion of the BLA affected cRD, but not the OF activity, thus showing that these behaviors can be dissociated, as in the present experiments (Kawasaki et al., 2017).

Despite the functional complexity of the BG circuitry, the ultimate effect of GPe activity would be to reduce inhibition of the thalamo-cortical pathway that promotes behavior. However, the results of Experiment 3 suggest that these behavioral effects depend on the state of the animal, including its emotional state. Increased GPe neural activity would enhance inhibition of glutamatergic STN neurons, thus reducing excitation GPi neurons and, in turn, disinhibiting the thalamo-cortical pathway that facilitates licking behavior. By contrast, GPe inhibition would allow for enhanced activity in STN glutamatergic neurons and thus increase the strength of the inhibitory influence of GPi neurons on the thalamo-cortical pathway thereby suppressing licking behavior. Consistent with this prediction, GPe excitation and inhibition produced opposite results in cRD during early downshift sessions.

In addition to its effects on the thalamo-cortical pathway, there are other components of the reward loss circuit that could explain the results of Experiment 3. Unexpected reward downshifts reduce approach to the site associated with a large reward, cause transient rejection of the downshifted reward, enhance search behavior, and facilitate the release of stress hormones, a set of effects related to negative emotion (see

Ortega et al., 2017; Papini et al., 2015). Since reduced dopamine in the GP is associated with increased anxiety behavior in the elevated plus maze (EPM; Avila et al., 2020), it seems possible that GPe chemogenetic inhibition could increase negative emotion and thus lead to a reduction in licking during RD. If GPi neurons have inhibitory connections with the lateral habenula (LHb) and/or the amygdala (Shammah-Lagnado et al., 1996; Zahm & Root, 2017), GPe inhibition could enhance neural activity in these areas, thus reducing licking during the reward downshift experience. Conversely, GPe excitation could reduce activity in these areas, thus attenuating the suppressive effects on behavior, potentially leading to an increase in licking during the reward downshift experience. The results from Experiment 3 encourage an assessment of the potential role played by BG projections to the LHb and amygdala to clarify their function in reward loss (see Stephenson-Jones et al., 2016).

Conclusions

The ability to recognize and differentiate stimuli predicting positive or negative outcomes adjusts behavior to situations that lead to nonreward, and re-direct behavior to alternative resources critical for survival. In the natural environment, animals must adjust their foraging behavior in response to changes in food availability. In situations involving reward loss, this behavioral adaptation may require decreasing behavior towards sites that no longer lead to food resources, thus increasing food seeking behavior towards alternative food resources (Amsel, 1992; Papini, 2003). In humans, unexpected losses have been linked to the onset and maintenance of psychiatric disorders, such as anxiety, depression, and substance use disorders (Huston et al., 2013; Papini et al., 2015). For example, the COVID-19 pandemic represented a combination of unexpected situations

involving reward loss, including lockdowns, social distancing, limited access to health care, loss of loved ones, and unemployment. These situations resulted in increased cases of psychological distress, mood disorders, and psychiatric disorders, including anxiety, depression, and post-traumatic stress disorder (Liu et al., 2020; Ransing et al., 2020; Smith et al., 2020), all of which can have significant consequences for the emotional well-being, addiction, collective health, and social functioning (Holingue et al., 2020; Twenge et al., 2020). A large amount of behavioral and physiological data has been collected about consequences of reward loss. This dissertation research contributes to the literature on reward loss and extends the current knowledge on the function of the BG neural circuit by highlighting the emotional underpinnings of this complex circuit that may serve as an interface between emotion, cognition, and action in situations involving reward loss.

References

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in neurosciences*, *12*, 366-375.

Amsel, A. (1958). The role of frustrative nonreward in noncontinuous reward situations. *Psychological bulletin*, *55*, 102-119.

Amsel, A. (1992). *Frustration theory: An analysis of dispositional learning and memory* (No. 11). Cambridge, UK: Cambridge University Press.

Anderson, E., & Hearing, M. (2019). *Neural circuit plasticity in Addiction.* In M. Torregrossa (Ed.), Neural mechanisms of addiction (pp. 35-60). Elsevier Inc.

Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S., & Roth, B. L. (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proceedings of the National Academy of Sciences*, *104*, 5163-5168.

Aron, A. R., Herz, D. M., Brown, P., Forstmann, B. U., and Zaghloul, K. (2016). Frontosubthalamic circuits for control of action and cognition. J. Neurosci. 36, 11489–11495.

Avila, G., Picazo, O., Chuc-Meza, E., & García-Ramirez, M. (2020). Reduction of dopaminergic transmission in the globus pallidus increases anxiety-like behavior without altering motoractivity. *Behavioural Brain Research*, *386*, 112589.

Baunez, C., and Lardeux, S. (2011). Frontal cortex-like functions of the subthalamic nucleus. *Frontiers in System Neuroscience*, *5*, 83.

Becker, H. C., & Flaherty, C. F. (1982). Influence of ethanol on contrast in consummatory behavior. *Psychopharmacology*, *77*, 253-258.

Becker, H. C., & Flaherty, C. F. (1983). Chlordiazepoxide and ethanol additively reduce gustatory negative contrast. *Psychopharmacology, 80,* 35-37.

Benhamou, L., Bronfeld, M., Bar-Gad, I., & Cohen, D. (2012). Globus Pallidus external segment neuron classification in freely moving rats: a comparison to primates. *PLoS ONE, 7,* e45421

Biesdorf, C., Wang, A. L., Topic, B., Petri, D., Milani, H., Huston, J. P., & de Souza Silva, M. A. (2015). Dopamine in the nucleus accumbens core, but not shell, increases during signaled food reward and decreases during delayed extinction. *Neurobiology of Learning and Memory, 123,* 125-139.

Blakemore, C., Clark, J. M., Nevalainen, T., Oberdorfer, M., & Sussman, A. (2012). Implementing the 3Rs in neuroscience research: A reasoned approach. *Neuron, 75,* 948-950.

Bower, G. H. (1961). A contrast effect in differential conditioning. *Journal of Experimental Psychology, 62,* 196-199.

Calabresi, P., Picconi, B., Tozzi, A., Ghiglieri, V., & Di Filippo, M. (2014). Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nature Neuroscience, 17,* 1022-1030.

Choi, D. L., Davis, J. F., Fitzgerald, M. E., & Benoit, S. C. (2010). The role of orexin-A in food motivation, reward-based feeding behavior and food-induced neuronal activation in rats. *Neuroscience, 167*(1), 11-20.

Choi, E. A., & McNally, G. P. (2017). Paraventricular thalamus balances danger and reward. *Journal of Neuroscience, 37,* 3018-3029.

Chung, L. (2015). A brief introduction to the transduction of neural activity into Fos signal. *Development & Reproduction*, *19*, 61-67.

Crespi, L. (1942). Quantitative variation of incentive and performance in the white rat. *American Journal of Psychology, 55,* 467-517.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences*, *13*, 281-285.

Do-Monte, F. H., Minier-Toribio, A., Quiñones-Laracuente, K., Medina-Colón, E. M., & Quirk, G. J. (2017). Thalamic regulation of sucrose seeking during unexpected reward omission. *Neuron*, *94*, 388-400.

Do-Monte, F. H., Quinones-Laracuente, K., & Quirk, G. J. (2015). A temporal shift in the circuits mediating retrieval of fear memory. *Nature*, *519*, 460-463.

Dong, J., Hawes, S., Wu, J., Le, W., & Cai, H. (2021). Connectivity and Functionality of the Globus Pallidus Externa Under Normal Conditions and Parkinson's Disease. *Frontiers in Neural Circuits*, *15*, 645287.

Eisinger, R. S., Urdaneta, M. E., Foote, K. D., Okun, M. S., & Gunduz, A. (2018). Non-motor characterization of the basal ganglia: Evidence from human and non-human primate electrophysiology. *Frontiers in Neuroscience, 12, 385.*

Elliot, M. H. (1928). The effect of change of reward on the maze performance of rats. *University of California Publications in Psychology, 4*, 19-30.

Esber, G. R., Torres-Tristani, K., & Holland, P. C. (2015). Amygdalo-striatal interaction in the enhancement of stimulus salience in associative learning. *Behavioral Neuroscience, 129*, 87-95.

Flagel, S. B., Cameron, C. M., Pickup, K. N., Watson, S. J., Akil, H., & Robinson, T. E. (2011). A food predictive cue must be attributed with incentive salience for it to induce c-fos mRNA expression in cortico-striatal-thalamic brain regions. *Neuroscience*, *196*, 80-96.

Flaherty, C. F. (1996). *Incentive Relativity*. Cambridge, UK: Cambridge University Press.

Flaherty, C. F., Grigson, P. S., & Lind, S. (1990). Chlordiazepoxide and the moderation of the initial response to reward reduction. *The Quarterly Journal of Experimental Psychology*, *42*(1), 87-105.

Flaherty, C. F., Grigson, P. S., & Rowan, G. A. (1986). Chlordiazepoxide and the determinants of contrast. *Animal Learning & Behavior, 14,* 315-321.

Genn, R. F., Ahn, S., & Phillips, A. G. (2004). Attenuated dopamine efflux in the rat nucleus accumbens during successive negative contrast. *Behavioral Neuroscience, 118,* 869-873.

Genn, R. F., Barr, A. M., & Phillips, A. G. (2002). Effects of amisulpride on consummatorynegative contrast. *Behavioural Pharmacology, 13,* 659-662.

Genn, R. F., Tucci, S., Parikh, S., & File, S. E. (2004). Effects of nicotine and a cannabinoid receptor agonist on negative contrast: Distinction between anxiety and disappointment?. *Psychopharmacology*, *177*, 93-99.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z. V. I., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science, 250,* 1429-1432.

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience*, *34*, 441-466.

Glueck, A. C., Dennis, T. S., Perrotti, L. I., Torres, C., & Papini, M. R. (2015). Brain expression of pCREB in rats exposed to consummatory successive negative contrast. *Neuroscience Letters*, 587, 93-97.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., ... & Pomper, M. G. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science, 357,* 503-507.

Grigson, P. S., Spector, A. C., Norgren, R. (1994). Lesions of the pontine parabrachial nuclei eliminate successive negative contrast effects in rats. *Behavioral Neuroscience, 108,* 714-723.

Guarino, S., Conrad, S. E., & Papini, M. R. (2020). Frustrative nonreward: Chemogenetic inactivation of the central amygdala abolishes the effect of reward downshift without affecting alcohol intake. *Neurobiology of Learning & Memory, 169,* 1-7.

Holingue, C., Badillo-Goicoechea, E., Riehm, K. E., Veldhuis, C. B., Thrul, J., Johnson, R. M., ... & Kalb, L. G. (2020). Mental distress during the COVID-19 pandemic among US adults without a pre-existing mental health condition: findings from American trend panel survey. *Preventive Medicine, 139,* 106231.

Hsu, D. T., Kirouac, G. J., Zubieta, J. K., & Bhatnagar, S. (2014). Contributions of the paraventricular thalamic nucleus in the regulation of stress, motivation, and mood. *Frontiers in Behavioral Neuroscience, 8,* 73.

Huang, A. C. W., Shyu, B. C., & Hsiao, S. (2010). Dose-dependent dissociable effects of haloperidol on locomotion, appetitive responses, and consummatory behavior in water deprived rats. *Pharmacology Biochemistry & Behavior, 95,* 285-291.

Huston, J. P., de Souza Silva, M. A., Komorowski, M., Schulz, D., & Topic, B. (2013). Animal models of extinction-induced depression: loss of reward and its consequences. *Neuroscience & Biobehavioral Reviews, 37,* 2059-2070.

Igelstrom, K. M., Herbison, A. E., & Hyland, B. I. (2010). Enhanced c-Fos expression in superior colliculus, paraventricular thalamus and septum during learning of cue-reward association. *Neuroscience, 168,* 706-714.

Ilg, A. K., Enkel, T., Bartsch, D., & Bähner, F. (2018). Behavioral effects of acute systemic low dose clozapine in wild-type rats: Implications for the use of DREADDs in behavioral neuroscience. *Frontiers in Behavioral Neuroscience, 12,* 1-10.

Jones, L. M., Fontanini, A., & Katz, D. B. (2006). Gustatory processing: a dynamic systems approach. *Current Opinion in Neurobiology, 16,* 420–428.

Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nature Reviews Neuroscience, 8,* 844-858.

Kamenetzky, G. V., Mustaca, A. E., & Papini, M. R. (2008). An analysis of the anxiolytic effects of ethanol on consummatory successive negative contrast. *Avances En Psicología Latinoamericana, 26,* 135-144.

Kawasaki, K., Glueck, A. C., Annicchiarico, I., & Papini, M. R. (2015). Function of the centromedial amygdala in reward devaluation and open-field activity. *Neuroscience, 303,* 73–81.

Kawasaki, K., Annicchiarico, I., Glueck, A. C., Moron, I., & Papini, M. R. (2017). Reward loss and the basolateral amygdala: A function in reward comparisons. *Behavioural Brain Research, 331,* 205-213.

Kita, H. (2007). Globus pallidus external segment. *Progress in Brain Research, 160,* 111-133.

Kravitz, A. V., Tye, L. D., & Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nature neuroscience*, *15*, 816-818.

Lafferty, C. K., Yang, A. K., Mendoza, J. A., & Britt, J. P. (2020). Nucleus accumbens cell type- and input-specific suppression of unproductive reward seeking. *Cell Reports, 30,* 3729-3742.

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor Perspectives in Medicine*, *2,* a009621.

Leisman, G., Braun-Benjamin, O., & Melillo, R. (2014). Cognitive-motor interactions of the basal ganglia in development. *Frontiers in systems neuroscience*, *8*, 16.

Leszczuk, M. H., & Flaherty, C. F. (2000). Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats. *Behavioural Brain Research, 116*(1), 61-79.

Li, S., & Kirouac, G. J. (2008). Projections from the paraventricular nucleus of the thalamus to the forebrain, with special emphasis on the extended amygdala. *Journal of Comparative Neurology, 506*, 263-287.

Liao, R. M., & Chuang, F. J. (2003). Differential effects of diazepam infused into the amygdala and hippocampus on negative contrast. *Pharmacology, Biochemistry, & Behavior, 74*, 953-960.

Lin, J. Y., Roman, C., & Reilly, S. (2009). Insular cortex and consummatory successive negative contrast in the rat. *Behavioral Neuroscience, 123*, 810-814.

Liu, C. H., Zhang, E., Wong, G. T. F., & Hyun, S. (2020). Factors associated with depression, anxiety, and PTSD symptomatology during the COVID-19 pandemic: Clinical implications for US young adult mental health. *Psychiatry Research*, *290*, 113172.

Lundy, R. F., & Norgren, R. (2004). Activity in the hypothalamus, amygdala, and cortex generates bilateral and convergent modulation of pontine gustatory neurons. *Journal of Neurophysiology, 91,* 1143-1157.

MacLaren, D. A., Browne, R. W., Shaw, J. K., Radhakrishnan, S. K., Khare, P., España, R. A., & Clark, S. D. (2016). Clozapine N-oxide administration produces behavioral effects in Long–Evans rats: implications for designing DREADD experiments. *Eneuro*, *3*, 1-14.

Madarasz, T. J., Diaz-Mataix, L., Akhand, O., Ycu, E. A., LeDoux, J. E., & Johansen, J. P. (2016). Evaluation of ambiguous associations in the amygdala by learning the structure of the environment. *Nature neuroscience*, *19*, 965-972.

Mahler, S. V., & Aston-Jones, G. (2018). CNO Evil? Considerations for the use of DREADDs in behavioral neuroscience. *Neuropsychopharmacology*, *43*, 934-936.

Manvich, D. F., Webster, K. A., Foster, S. L., Farrell, M. S., Ritchie, J. C., Porter, J. H., & Weinshenker, D. (2018). The DREADD agonist clozapine N-oxide (CNO) is reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. *Scientific Reports*, *8*(1), 1-10.

Moga, M. M., Weis, R. P., & Moore, R. Y. (1995). Efferent projections of the paraventricular thalamic nucleus in the rat. *Journal of Comparative Neurology*, *359*, 221-238.

Nambu, A., Tokuno, H., and Takada, M. (2002). Functional significance of the cortico-subthalamo–pallidal “hyperdirect” pathway. *Neuroscience Research, 43,* 111–117.

Nambu, A. (2008). Seven problems on the basal ganglia. *Current Opinion in Neurobiology, 18,* 595-604.

Namburi, P., Beyeler, A., Yorozu, S., Calhoon, G. G., Halbert, S. A., Wichmann, R., ... & Tye, K. M. (2015). A circuit mechanism for differentiating positive and negative associations. *Nature, 520,* 675-678.

Norgren, R. (1995). Gustatory system. In G. Paxinos (Ed.) *The rat nervous system, Second Edition,* San Diego, CA: Academic Press.

Ortega, L. A., Daniel, A. M., Davis, J. B., Fuchs, P. N., & Papini, M. R. (2011). Peripheral pain enhances the effects of incentive downshifts. *Learning & Motivation, 42,* 203-209.

Ortega, L. A., Glueck, A. C., Daniel, A. M., Prado-Rivera, M. A., White, M. M., Papini, M. R. (2014). Memory interfering effects of chlordiazepoxide on consummatory successive negative contrast. *Pharmacology Biochemistry & Behavior, 116,* 96-106.

Ortega, L. A., Glueck, A. C., Uhelski, M., Fuchs, P. N., & Papini, M. R. (2013). Role of the ventrolateral orbital cortex and medial prefrontal cortex in incentive downshift situations. *Behavioural Brain Research, 244,* 120-129.

Ortega, L. A., Solano, J. L., Torres, C., & Papini, M. R. (2017). Reward loss and addiction: Opportunities for cross-pollination. *Pharmacology Biochemistry & Behavior, 154,* 39-52.

Ortega, L. A., Uhelski, M., Fuchs, P. N., & Papini, M. R. (2011). Impairment of recovery from incentive downshift after lesions of the anterior cingulate cortex: Emotional or cognitive deficits? *Behavioral Neuroscience, 125,* 988-995.

Padovan-Hernandez, Y., & Knackstedt, L. A. (2018). Dose-dependent reduction in cocaine induced locomotion by clozapine-N-oxide in rats with a history of cocaine self-administration. *Neuroscience Letters, 674,* 132-135.

Papini, M. R. (2006). Role of surprising nonreward in associative learning. *Japanese Journal of Animal Psychology, 56,* 35-54.

Papini, M. R. (2009). Role of opioid receptors in incentive contrast. *International Journal of Comparative Psychology, 22,* 170-187.

Papini, M. R., Fuchs, P. N., & Torres, C. (2015). Behavioral neuroscience of psychological pain. *Neuroscience & Biobehavioral Reviews, 48,* 53-69.

Papini, M. R., & Ortega, L. A. (2011). *Endogenous opioids, opioid receptors, and incentive processes.* In V. R. Preedy, R. R. Watson, & C. R. Martin (Eds.), Handbook of behavior, food, and nutrition (pp. 1011-1019). New York: Springer.

Papini, M. R., & Pellegrini, S. (2006). Scaling relative incentive value in consummatory behavior. *Learning & Motivation, 37,* 357-378.

Paxinos, J., & Watson, C. (2013). *The rat brain in stereotaxic coordinates.* 7th Edition. Elsevier.

Peck, C. J., & Salzman, C. D. (2014). Amygdala neural activity reflects spatial attention towards stimuli promising reward or threatening punishment. e*Life, 3,* e04478.

Pecoraro, N., & Dallman, M. F. (2005). c-Fos after incentive shifts: Expectancy, incredulity, and recovery. *Behavioral Neuroscience, 119,* 366-387.

Pellegrini, S., & Papini, M. R. (2007). Scaling relative incentive value in anticipatory behavior. *Learning & Motivation, 38,* 128-154.

Pellegrini, S., Wood, M., Daniel, A. M., & Papini, M. R. (2005). Opioid receptors modulate recovery from consummatory successive negative contrast. *Behavioural Brain Research, 164,* 239-249.

Penzo, M. A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., ... & Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature, 519*, 455-459.

Ransing, R., Ramalho, R., Orsolini, L., Adiukwu, F., Gonzalez-Diaz, J. M., Larnaout, A., ... & Kilic, O. (2020). Can COVID-19 related mental health issues be measured?. *Brain, Behavior, and Immunity, 88*, 32-34.

Reilly, S. (1998). The role of the gustatory thalamus in taste-guided behavior. *Neuroscience & Biobehavioral Reviews, 22,* 883-901.

Reilly, S., & Trifunovic, R. (2003). Gustatory thalamus lesions eliminate successive negative contrast in rats: Evidence against a memory deficit. *Behavioral Neuroscience, 117,* 606-615.

Roth, B. L. (2016). DREADDs for neuroscientists. *Neuron, 89,* 683-694.

Rogan, S. C., & Roth, B. L. (2011). Remote control of neuronal signaling. *Pharmacological Reviews, 63*, 291-315.

Rossi, P. J., Gunduz, A., & Okun, M. S. (2015). The subthalamic nucleus, limbic function, and impulse control. *Neuropsychology review, 25*, 398-410.

Rowan, G. A., & Flaherty, C. F. (1987). The effects of morphine in the consummatory contrast paradigm. *Psychopharmacology, 93,* 51-58.

Rowan, G. A., & Flaherty, C. F. (1991). Behavior of Maudsley reactive and nonreactive rats (*Rattus norvegicus*) in three consummatory contrast paradigms. *Journal of Comparative Psychology*, *105*, 115-124.

Russo, S. J., & Nestler, E. J. (2013). The brain reward circuitry in mood disorders. *Nature Reviews Neuroscience*, *14*(9), 609-625.

Saint-Cyr, J. A. (2003). Frontal-striatal circuit functions: context, sequence, and consequence. *Journal of the International Neuropsychological Society, 9,* 103–127.

Saunders, A., Huang, K. W., & Sabatini, B. L. (2016). Globus pallidus externus neurons expressing parvalbumin interconnect the subthalamic nucleus and striatal interneurons. *PLoS ONE, 11*, e0149798.

Shammah-Lagnado, S. J., Alheid, G. F., & Heimer, L. (1996). Efferent connections of the caudal part of the globus pallidus in the rat. *Journal of Comparative Neurology, 376*, 489-507.

Simonyan, K. (2019). Recent advances in understanding the role of the basal ganglia. *F1000 Research, 8*, 122.

Smith, Y., Bevan, M. D., Shink, E., & Bolam, J. P. (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience*, *86*, 353-387.

Smith, K. S., Bucci, D. J., Luikart, B. W., & Mahler, S. V. (2016). DREADDS: Use and application in behavioral neuroscience. *Behavioral Neuroscience, 130*, 137-155.

Smith, L., Jacob, L., Yakkundi, A., McDermott, D., Armstrong, N. C., Barnett, Y., ... & Tully, M. A. (2020). Correlates of symptoms of anxiety and depression and

mental wellbeing associated with COVID-19: a cross-sectional study of UK based respondents. *Psychiatry Research, 291,* 113138.

Soares-Cunha, C., de Vasconcelos, N. A., Coimbra, B., Domingues, A. V., Silva, J. M., Loureiro-Campos, E., ... & Rodrigues, A. J. (2020). Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion. *Molecular Psychiatry*, *25*, 3241-3255.

Spear, N. E., & Hill, W. F. (1965). Adjustment to new reward: Simultaneous- and successive contrast effects. *Journal of Experimental Psychology*, *70,* 510-519.

Stephenson-Jones, M., Yu, K., Ahrens, S., Tucciarone, J. M., van Huijstee, A. N., Mejia, L. A., Penzo, M. A., Tai, L.-H., Wilbrecht, L., & Li, B. (2016). A basal ganglia circuit for evaluating action outcomes. *Nature, 539*, 289-293.

Tai, L. H., Lee, A. M., Benavidez, N., Bonci, A., & Wilbrecht, L. (2012). Transient stimulation of distinct subpopulations of striatal neurons mimics changes in action value. *Nature Neuroscience, 15*, 1281-1289.

Tan, K. R., Rudolph, U., & Lüscher, C. (2011). Hooked on benzodiazepines: $GABA_A$ receptor subtypes and addiction. *Trends in Neurosciences*, *34*, 188-197.

Thorndike, E. L. (1911). *Animal intelligence*. New York: Lemcke & Buechner.

Tinklepaugh, O. L. (1928). An experimental study of representative factors in monkeys. *Journal of Comparative Psychology, 8,* 197-236.

Torres, C., Glueck, A. C., Conrad, S. E., Moron, I., & Papini, M. R. (2016). Dorsomedial striatum lesions affect adjustment to reward uncertainty, but not to reward devaluation or omission. *Neuroscience,* 332, 13-25.

Torres, C., & Papini, M. R. (2017). Incentive relativity. In J. Vonk & T. K. Schackelford (Eds.), *Encyclopedia of animal cognition and behavior*. New York: Springer.

Tye, K. M., Stuber, G. D., de Ridder, B., Bonci, A., & Janak, P. H. (2008). Rapid strengthening of thalamo-amygdala synapses mediates cue–reward learning. *Nature*, *453*, 1253-1257.

Twenge, J. M., & Joiner, T. E. (2020). Mental distress among US adults during the COVID-19 pandemic. *Journal of Clinical Psychology*, *76*, 2170-2182.

Urban, D. J., & Roth, B. L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annual Review of Pharmacology & Toxicology*, *55*, 399-417.

Vogel, J. R., Mikulka, P. J., & Spear, N. E. (1968). Effects of shifts in sucrose and saccharine concentrations on licking behavior in the rat. *Journal of Comparative & Physiological Psychology*, *66*, 661-666.

Wei, W., & Wang, X. J. (2016). Inhibitory control in the Cortico-Basal Ganglia-Thalamocortical loop: Complex regulation and interplay with memory and decision processes. *Neuron*, *92*, 1093-1105.

Yager, L. M., Garcia, A. F., Wunsch, A. M., & Ferguson, S. M. (2015). The ins and outs of the striatum: role in drug addiction. *Neuroscience*, *301*, 529-541.

Yasoshima, Y., Scott, T. R., & Yamamoto, T. (2007). Differential activation of anterior and midline thalamic nuclei following retrieval of aversively motivated learning tasks. *Neuroscience*, *146*, 922-930.

Weintraub, D. B., and Zaghloul, K. A. (2013). The role of the subthalamic nucleus in cognition. *Reviews in the Neurosciences*, *24*, 125–128.

Wirtshafter, D., & Stratford, T. R. (2016). Chemogenetic inhibition of cells in the paramedian midbrain tegmentum increases locomotor activity in rats. *Brain Research, 1632*, 98-106.

Wood, M., Daniel, A. M., & Papini, M. R. (2005). Selective effects of the δ-opioid receptor agonist DPDPE on consummatory successive negative contrast. *Behavioral Neuroscience, 119*, 446-454.

Wood, M., Norris, J. N., Daniel, A. M., & Papini, M. R. (2008). Trial-selective effects of U50,488H, a kappa-opioid receptor agonist, on consummatory successive negative contrast. *Behavioural Brain Research, 193,* 28-36.

Zahm, D. S., & Root, D. H. (2017). Review of the cytology and connections of the lateral habenula, an avatar of adaptive behaving. *Pharmacology, Biochemistry and Behavior, 162*, 3-21.

Zeaman, D. (1949). Response latency as a function of the amount of reinforcement. *Journal of Experimental Psychology, 39*, 466.

Zhu, H., & Roth, B. L. (2014). Silencing synapses with DREADDs. *Neuron, 82*, 723-725.

VITA

PERSONAL Sara Guarino

EDUCATION B.A., Psychology, St. Mary's University, 2016

M.S., Experimental Psychology, Texas Christian University, 2018

Ph.D., Experimental Psychology (Minor: Quantitative Psychology), Texas Christian University, 2018-2021.

SELECTED PUBLICATIONS

Guarino, S., Conrad, S. E., & Papini, M. R. (2020). Frustrative nonreward: Chemogenetic inactivation of the central amygdala abolishes the effect of reward downshift without affecting alcohol intake. *Neurobiology of Learning & Memory, 169,* 1-7.

Guarino, S., Conrad, S. E., & Papini, M. R. (2020). Control of free-choice consummatory behavior by absolute reward value. *Learning & Motivation, 72,* 101682.

Conrad, S. E, **Guarino, S.,** & Papini, M. R. (2020). Surprising nonreward and response effort: Extinction after progressive ratio training in rats and pigeons. *Learning & Motivation, 72,* 101676.

Hill, H. M., Dietrich, S., **Guarino, S.,** Banda, M., & Lacy, K. (2019). Preliminary observations of an unusual mouth interaction between beluga calves (*Delphinapterus leucas*). *Zoo biology, 38,* 149-156.

Yeater, D., **Guarino, S.,** Lacy, S., Dees, T., Hill, H. (2017). Do belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) display lateralized eye preference when presented with familiar or novel objects?. *International Journal of Comparative Psychology, 30,* 1-13.

Guarino, S., Yeater, D., Lacy, S., Dees, T., & Hill, H. M. (2017). Responses to familiar and unfamiliar objects by belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *Animal Cognition, 20,* 823-827.

Hill, H. M., **Guarino, S.,** Geraci, C., Sigman, J., & Noonan, M. (2017). Developmental changes in the resting strategies of killer whale (*Orcinus orca*) calves and their mothers from birth to 36 months. *Behaviour, 154,* 435-466.

Hill, H. M., **Guarino, S.,** Calvillo, A., Gonzalez, A., Zuniga, K., Bellows, C., Polasek, L., & Sims, C. (2017). Lateralized swim positions are conserved across environments for beluga (*Delphinapterus leucas*) mother-calf pairs. *Behavioural Processes, 138,* 22-28.

Hill, H., **Guarino, S.,** Dietrich, S. (2017). Field of comparative psychology. In T.K. Shackelford, & V.A. Weekes-Shackelford (Eds.), *Encyclopedia of Evolutionary Psychological Science*. Springer International Publishing AG.

Guarino, S., Hill, H., & Sigman, J. (2016). Development of sociality and emergence of independence in a killer whale (*Orcinus orca*) calf from birth to 36 months. *Zoo Biology*, *36*(1), 11-20.

Guarino, S., & Hill, H. (2016, September). Marine mammal behavior: Captivity vs. natural habitats. *Eye on Psi Chi*, *21*(1), 12-15. http://www.psichi.org/resource/resmgr/eye_pdf/21

Hill, H. M., **Guarino, S.,** Dietrich, S., & St Leger, J. (2016). An inventory of peer-reviewed articles on killer whales (*Orcinus orca*) with a comparison to bottlenose dolphins (*Tursiops truncatus*). *Animal Behavior and Cognition, 3,* 135-149.

Hill, H., Yeater, D., Gallup, S., **Guarino, S.,** Lacy, S., Dees, T., & Kuczaj, S. (2016). Responses to familiar and unfamiliar humans by belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*): A replication and extension. *International Journal of Comparative Psychology, 29*(1), 1-21.

Hill, H. M., **Guarino, S.,** Crandall, S., Lenhart, E., & Dietrich, S. (2015). Young belugas diversify adult beluga (*Delphinapterus leucas*) behavior. *Animal Behavior and Cognition, 2,* 267-284.

ABSTRACT

FRUSTRATIVE NONREWARD AND THE BASAL GANGLIA: FUNCTION OF THE INDIRECT PATHWAY DURING CONSUMMATORY REWARD DOWNSHIFT

By Sara Guarino, M.S., 2018
Department of Psychology
Texas Christian University

Keywords: Nucleus Accumbens, Globus Pallidus Externus, Consummatory Reward Downshift, Open Field, Chemogenetics

Dissertation Advisor: Mauricio R. Papini, Professor of Psychology

The role of the nucleus accumbens (NAc) and globus pallidus externus (GPe) in the adjustment to the consummatory reward downshift (cRD) and in open field (OF) activity was studied using designer receptors exclusively activated by designer drugs (DREADDs). Rats exposed to unexpected sucrose downshifts exhibit significant suppression of licking response to the downshifted sucrose solution relative to unshifted controls always given access to the same sucrose solution. This effect was replicated in Experiment 1. A 32-to-2% sucrose downshift induced significant suppression of licking relative to an unshifted 2% sucrose controls. Groups of rats received bilateral infusion of the inhibitory or excitatory designer receptors into the NAc (Experiments 2a-b) and GPe (Experiment 3). NAc excitation, using the DREADD activator clozapine N-oxide (CNO) to activate receptors, led to reduced licking in early 32-to-2% downshift sessions, whereas NAc inhibition, using CNO to activate receptors, did not have significant effects on 32-to-2% and 8-to-2% sucrose downshifts in the RD situation. NAc chemogenetic manipulations did not produce detectable effects in the OF task. GPe enhanced consummatory suppression in early 32-to-2% sucrose downshift sessions, without affecting late downshift session. By

contrast, GPe excitation led to reduced consummatory suppression in early 8-to-2% sucrose downshift sessions, and enhanced suppression in late downshift sessions. These chemogenetic manipulations of GPe neurons also had no detectable effects on OF activity. First, NAc experiments are the first to show effects of chemogenetic manipulations of NAc in the RD task. Results of NAc chemogenetic inhibition corroborated findings from NAc lesion studies. NAc chemogenetic excitation experiment is the first to identify a function for NAc neurons in reward loss. These effects occurred in absence of any evidence of motor alterations after either inhibitory or excitatory DREADD activation in the OF task. Second, GPe experiments are the first to show an effect of chemogenetic manipulation of GPe neurons on behavior during sucrose downshift events. This research is the first to demonstrate an inhibitory role of the NAc in response to reward loss, and a role of the GPe in frustrative nonreward, a pattern of findings that encourages further investigation of basal ganglia functions in frustration.