FRUSTRATIVE NONREWARD AND THE BASAL GANGLIA:

ROLE OF OUTPUTS FROM THE NUCLEUS ACCUMBENS IN REWARD LOSS

by

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Introduction

Early studies on incentive relativity

From an evolutionary perspective, the identification and evaluation of rewards are two adaptive functions that evolved across vertebrates. Rewards might take the form of food, shelter, sexual or social partners, maternal care, or a number of other resources that ultimately provide some sort of reproductive benefit. Behavioral traits that promote and maintain the ability to seek out and obtain these rewards directly inform an organism's success in a variety of necessary actions for survival and reproduction. Consequently, specific biological and psychological mechanisms have evolved to evaluate qualitative and quantitative information about a specific reward and then direct an organism's behavior according to the reward's value in absolute terms or relative to some other reward (Papini, 2002). This is especially relevant when expected rewards are omitted or devalued. First, expectancies regarding various properties of a specific reward must be established. Then, if these properties are altered in ways that leave the reward devalued or omitted entirely, there is a behavioral adjustment that can involve intense negative emotional responses, including behaviors related to anxiety, conflict, and even pain. Together, these emotions are referred to as *frustration*. Frustration allows for the emotional detachment from a resource or associated site and ultimately redirects behavioral to other sources of reward—a process known as incentive disengagement (Papini, 2003, 2014). In humans, *unexpected reward downshifts* (URDs) can be a major source of emotional distress and conflict. URDs include unemployment, salary reduction, loss of a loved one, and similar situations. Moreover, these downshifts have been shown to increase the risk of anxiety disorders, depression, and substance abuse (Hobson & Delunas, 2001; Hutson et al., 2013; Papini et al., 2015).

The psychological and behavioral outcomes from URDs in humans can be clearly demonstrated when examining the societal impact of the COVID-19 pandemic. Reward loss resulting from the global pandemic has taken a host of forms including confinement, social distancing, lockdowns, limited access to health care, and the loss of jobs as well as the lives of loved ones, all of which have contributed negatively to the mental and physical wellbeing of countless individuals (Brooks et al., 2020). To give further relevance to the relationship between URDs, frustration, and overall mental health, frustrative nonreward was determined to be a key endophenotype in the "negative valence" domain in the Research Domain Criteria (RDoC). This NIMH initiative characterizes mental disorders based on the notion that related behaviors under the same domain share neurobiological circuits (Anderzhanova et al., 2017; Watson et al., 2017). This also indicates that investigating the neurobiology behind one endophenotype can inform the neurobiological patterns of other endophenotypes in the same domain. Consequently, understanding the complex circuits behind frustration and the response to URDs can also provide a unique perspective on other mental health issues like anxiety and depression that are relevant to several mental disorders.

Behavioral disruptions symptomatic of frustration following URDs are observed in several species, including adult and infant humans, monkeys, opossums, dogs, mice, and rats among others (Papini, 2014). In experimental contexts, there have been a variety of paradigms to model different aspects of this phenomenon, most of which involve training an animal on a task with a reward and after several training sessions, omitting or devaluing the reward. Frustration was first characterized in a couple of seminal studies. Tinklepaugh (1928) showed a macaque monkey food being placed under one of two cups. The subject was then allowed to choose between the two cups. Some trials involved showing either a banana

(high-value reward) or lettuce (low-value reward) and the subject would select the appropriate cup to receive the reward. However, there were other trials in which the banana was shown to be placed under a cup, but the reward was covertly substituted for the lettuce. This led to the monkey ultimately rejecting the reward, and on certain trials she even "shrieked at them [the observers] in apparent anger" (Tinklepaugh, 1928, p. 224). This is in contrast to other trials where the subject had seen the lettuce being hidden under the cup, the lettuce was consumed without hesitation. This study was the first to demonstrate how the same reward can elicit different behaviors depending on expectations based on prior experience. In a second study, Elliott (1928) trained rats to reach a goal box with some reward in a complex maze (Figure 1). This reward was either of high value to the rat (in this case a wet mixture of cereal) or low value (sunflower seeds). It was found that rats trained with the high-value reward learned the maze quicker than rats trained with the low-value reward. However, when rats that were trained with the high-value reward were downshifted and rewarded with the low-value reward, they made more errors and had a higher latency to the goal box than rats that were always trained with the low-value reward. These results suggested that reward devaluation, when a higher reward is expected, induces a state that disrupts motivation to complete a trained task.

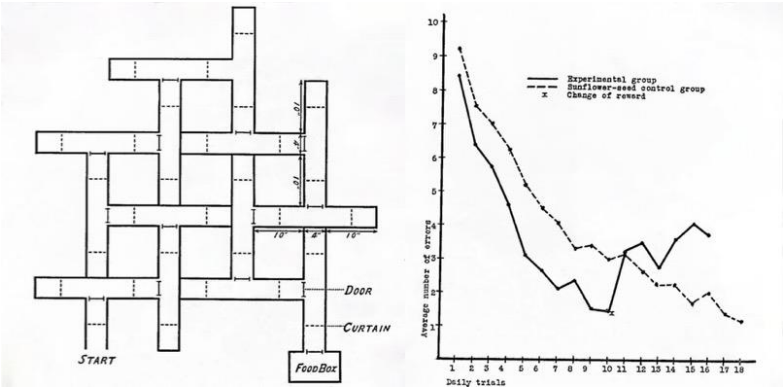

Figure 1. (Left) Complex maze used in the experiment. (Right) Errors made by rats before (sessions 1-9) and after (sessions 10-16) the group switched from wet cereal to sunflower seeds (marked by an "x" in the original figure). Notice the increase in errors after the downshift (from Elliott, 1928).

Early theories on incentive learning failed to fully explain the effects shown by Tinklepaugh and Elliott with regards to the observed rapid changes in behavior after reward shifts. Thorndike (1911) and Hull (1943) argued that incentives served only to strengthen stimulus-response associations. One implication of this view was that information about the incentive was not encoded as part of the acquired knowledge. Under this framework, it would be expected that after a reward shift behavior would slowly adapt to the new incentive value across sessions, an effect that was not demonstrated by either Tinklepaugh or Elliott. Tinklepaugh's monkeys in particular suggested a distinct emotional component that accompanied a violation in reward expectation. This was echoed by Crespi's (1942) runway experiments in which rats were downshifted from 256 to 16 units of food (with one unit defined as 0.02 g of Purina dog biscuit; 256 and 16 units were about 5 and 0.3 g, respectively), which accompanied rapid behavioral changes including, in addition to decreased runway speed, "general frantic peering, general delayed eating, repeated jumping-attempts to escape the food box… and refusal to eat all or part of the incentive" (Crespi, 1942, p. 510). Crespi described this as a "depression" effect caused by frustration, implying a distinct emotional component that drives rapid behavioral adjustments to violations in reward expectancies. Elliott provided an alternative explanation and accounted for the performance decrement after a reward shift in his maze not as an emotional reaction, but as animals searching for the missing reward. Thus, Elliott argued against a purely emotional mechanism given that in his experiments the behavior did not diminish after 6 days, whereas emotional responses are usually seen as transient.

Other experiments with similar instrumental paradigms have further supported both emotional and cognitive frameworks for incentive relativity using biological manipulations.

Inactivation of the central amygdala, an area involved in a wide variety of emotional behaviors, via lidocaine was shown to disrupt contrast on the second postshift session when infusions occurred immediately after the first postshift session (Salinas et al., 1993). Similar effects were shown with manipulations to the hippocampus, an area widely implicated in memory related plasticity (Bliss & Gardner-Medwin, 1973; Squire & Kandel, 2009). Lesions to the hippocampus eliminated signs of contrast after reward downshift in a runway (Franchina & Brown, 1971) and disrupted choice performance in an 8-maze situation after rewards were downshifted (Hagen et al., in prep).

Overall, these experiments demonstrate how animals do in fact represent incentives as having specific value relative to prior experience in various instrumental contexts. Reward downshifts have been shown to rapidly modify behavior, a phenomenon with both emotional and motivational qualities.

Consummatory successive negative contrast

In addition to reward downshifts modifying instrumental behavior, negative contrast has been widely studied in the context of consummatory behavior in response to shifts in sucrose concentration. This paradigm was first developed by Vogel et al. (1968) and is known as consummatory successive negative contrast (cSNC; see Figure 2, left). This procedure consists of two phases: a preshift phase and a postshift phase. During preshift, rats are placed in consummatory boxes with access to a bottle of sucrose solution that is either of high value (high concentration of sucrose) or low value (low concentration of sucrose) for several training sessions. Fluid intake either through volume consumed or number of licks is measured throughout each session to either the high concentration (usually 32% sucrose) or low concentration (usually 2% or 4% sucrose). It is important to note that while the low

sucrose concentration is of lesser relative value to the high sucrose concentration, it is still an acceptable reward to encourage consumption. After completion of the preshift trials, rats who received 32% sucrose during preshift are then given access to 4% sucrose during postshift. On the first trial of postshift, rats are seen to reject the downshifted solution and suppress licking behavior for overall less fluid intake compared to unshifted controls. Over subsequent trials with access to the 4% sucrose, rats are seen to increase their consummatory behavior until they reach equivalence to the unshifted controls always receiving 4% sucrose. Interestingly, this specific behavioral response to an unexpected sucrose downshift was eliminated when animals were unexpectedly upshifted from 4% to 32% sucrose. In this case, animals gradually adjusted their behavior to match unshifted controls (Figure 2, right), a pattern consistent with the Rescorla-Wagner model (1972) and a result that has been replicated in an extensive series of analogous experiments (Annicchiarico et al., 2016).

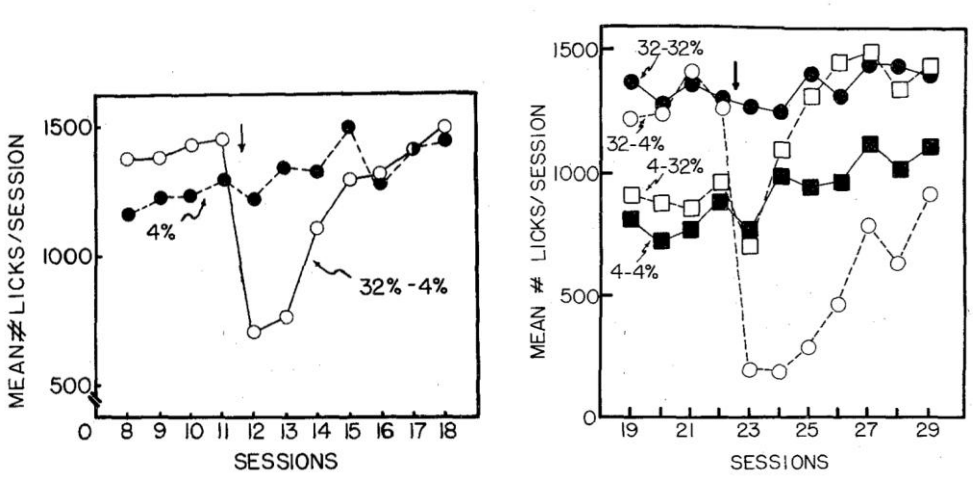

Figure 2. (Left) Effect of 32-4% sucrose downshift on licking. The downshift occurred on session 12. (Right) Symmetrical switches in reward value. A downshift from 32% to 4% sucrose leads to suppression of licking, evidence for successive negative contrast. However, an upshift from 4% to 32% sucrose leads only to an adjustment of licks without evidence of successive positive contrast (from Vogel et al., 1968).

Recovery after a reward downshift was further described by Flaherty (1996) as involving two stages with the first stage consisting of the initial rejection of the downshifted solution and the second stage defining the recovery of consummatory behaviors. Stage 1 is also associated with an emotional response that has been called primary frustration, which is the emotion associated with the URD event, while Stage 2 is associated with secondary frustration involving the anticipation of reward loss (Papini, 2003).

The response to reward loss in the cSNC paradigm can be described using Amsel's (1992) *frustration theory*, a theoretical connection that includes various stimulus and response elements (Papini, 2003). This behavioral model can be visualized in Figure 3A as it applies to the first postshift session of cSNC. Prior to downshift, a Pavlovian association between the contextual cues of the training environment (S) and the delivery of 32% sucrose is established and strengthened during training sessions so that an expectation of 32% sucrose is generated (e_{32}). These cues promote licking (R_D) to the sipper tube to obtain the sucrose reward. On the first postshift session, there is a discrepancy between the expected 32% solution and the detected 4% solution ($S_{4\%}$). This discrepancy promotes primary frustration (R_F), which both inhibits the licking response and promotes alternative behaviors (R_O). In addition, the context becomes associated with new reward value (Figure 3B) and the internal psychological state associated with primary frustration so that on subsequent postshift trials anticipatory/secondary frustration (r_F) arises as the animal is in conflict between accepting and rejecting the devalued reward. Flaherty (1996) described this process similarly as a series of transitions from detection to rejection to search.

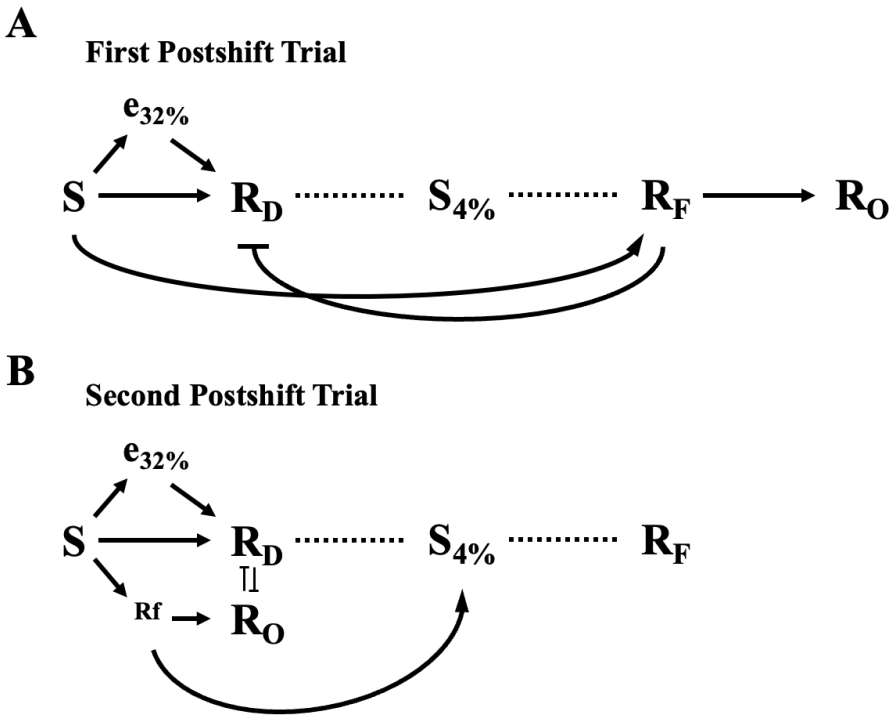

Figure 3. A theoretical learning model for the consummatory response to reward downshift on the first (A) and second (B) postshift session of consummatory successive negative contrast. Arrows represent acquired excitatory associations. The blunted arrow represents response inhibition. Dashed lines represent the passage of time. S: contextual stimuli, e_{32}: expectation of 32% sucrose; R_D: drinking response; $S_{4\%}$: 4% sucrose solution; R_F: primary frustration; R_f: secondary frustration; R_O: other responses. (Modified from Wood et al., 2005).

Frustration can be described as an emotional reaction to an unexpected reward omission and as having an aversive hedonic value (Amsel, 1992; Papini & Dudley, 1997). While these negative emotions are transient as animals recover from the reward downshift, they have been shown to be major components in both Stage 1 and Stage 2 of recovery (Flaherty, 1996; Gray, 1987). These emotional states can also be considered a form of "psychological pain" akin to shock delivery in fear conditioning (Papini et al., 2006, 2015). However, as in fear conditioning, a threshold must be reached to trigger a state of psychological pain. In the case of cSNC, a reward comparison alone is not sufficient to elicit

negative emotion. There needs to be an ample disparity in reward value (i.e., difference between obtained and expected sucrose concentration) to see the effect (Papini & Pellegrini, 2006; Ruetti et al. 2009).

Several lines of evidence support the assertion that reward loss involves a negative emotional state from both a behavioral and biological perspective. For a brief list of some of this evidence, first, reward omission and downshift tend to elicit escape responses that guide animals away from the situation. Traditionally, this phenomenon was called "escape from frustration" and it occurs whether the downshift is to a smaller reward or to no reward (Daly, 1974; Norris et al., 2009). Second, rats trained with light-food pairings in an autoshaping procedure increased lever pressing after light-alone trials. This increase was also significantly greater than in control animals that had the same number and distribution of food presentations, but without a signal, suggesting that bursts in behavior from unexpected reward omission are characterized by emotional arousal, rather than reward frequency alone (Dudley & Papini 1995). A third line of evidence is shown hormonally as cSNC is associated with activation of the hypothalamic-pituitary-adrenal axis resulting in the release of corticosterone (Mitchell & Flaherty, 1998), the predominate stress hormone in rodents and homologous to cortisol in human and nonhuman primates. The release of corticosterone is associated with a variety of observable biological and behavioral changes including activating central and peripheral immune cells, altering dendritic spine density, and attenuating the fear response to novel situations (Campos et al., 2013; Grippo et al., 2013). Rats having undergone adrenalectomy, a surgery that eliminates corticosterone production, have also been shown to have diminished frustration effects in both consummatory contrast situations and appetitive extinction situations (Pecoraro et al., 2005; Pecoraro et al., 2007;

Thomas & Papini 2001). Additional evidence for a role of frustration in URDs comes from pharmacological and neurobiological manipulations.

Pharmacology of cSNC

Various anxiolytics have been shown to have dramatic effects at reducing the cSNC effect at certain timepoints. Both (CDP), a benzodiazepine anxiolytic, and alcohol reduce anxiety by binding to GABAergic neurons in various mesolimbic areas of the brain (Davies, 2003; Vellucci & Webster, 1984). However, research has shown a stark session specificity for their effect in cSNC. Administration of either CDP or alcohol prior to the second session of downshift, but not the first, showed attenuation of consummatory suppression (Becker & Flaherty, 1982, 1983; Flaherty et al., 1986, 1990; Kamenetzky et al., 2008). This suggests that the animal needs previous exposure to the downshift to experience any effects from the compounds. Thus, anxiolytics and the GABAergic neurons they influence play an important role in the recovery of behavior after reward loss.

Opioids and opioid receptors have also been experimentally shown to play a major role in modulating certain aspects of reward loss during cSNC (Ortega et al., 2017). Opioids are a class of drugs that bind to specific receptors present throughout the brain and largely characterized by their ability to regulate pain and promote analgesia when activated (Stein et al., 2003). The role of opioids during the recovery of reward loss can first be explained conceptually using the frustration = fear hypothesis proposed by Gray (1987). Using this perspective, one can equate the function of opioids in the reduction of pain in response to harmful stimuli to the reduction of negative emotions related to frustration during reward loss. Gray suggested that similar neural mechanisms operate under anticipation of both fear and frustration. During fear conditioning, a conditioned stimulus is paired with an electric

foot shock, the unconditioned stimulus. Early in training the shocks caused a startle response, whereas later in training the conditioned stimulus produced a fear response in anticipation of pain, such as freezing in rodents. Using this logic, pain can be compared to primary frustration during reward loss and fear to secondary frustration, which is also defined by the anticipation of a negative event.

This theory has been supported by a variety of studies investigating the impact of various opioid receptor agonists and antagonists on reward loss during cSNC. Morphine, a nonselective opioid-receptor agonist, has been shown to reduce consummatory suppression in downshifted groups during cSNC compared to unshifted controls, suggesting that the analgesic effects of morphine reduced the negative emotion associated with reward loss (Rowan & Flaherty, 1987). cSNC is also attenuated when reward downshift occurs in a context previously paired with morphine (Ruiz-Salas et al., 2022). Further experiments using naloxone, an opioid-receptor antagonist, showed that when administered at high doses before each session of downshift, consummatory suppression was enhanced and prolonged (Pellegrini et al., 2005). When administered together, naloxone prevented the reduction of consummatory suppression observed with morphine treatment, further supporting the hypothesis that opioid receptors play an important role in the neural pathway of reward contrast (Rowan & Flaherty, 1987). Additionally, these effects may be under control of specific subtypes of opioid-receptors, which can have divergent consequences on consummatory behavior. For example, the delta-opioid-receptor agonist DPDPE has been shown to reduce the cSNC effect on the first session of downshift (Stage 1), but not the second (Stage 2). Moreover, the kappa opioid-receptor agonist U50, 488H was shown to reduce the cSNC effect during Stage 2, but not Stage 1 of reward loss (Wood et al., 2005).

Together, these experiments suggest different mechanisms for the initial response versus the recovery from URDs, with delta opioid-receptors being crucial during the onset of response suppression, but kappa opioid-receptors playing a larger role during the recovery from reward downshift.

Neurobiology of cSNC

A variety of studies have begun to investigate the neural circuitry that governs the behavioral outcomes of frustration. This proposed circuit incorporates several key brain areas associated with such processes as the detection of the reward disparity, reward seeking, negative emotion, and action (Ortega et al., 2017; see Figure 4). These circuits are located in both the brainstem, which governs the taste-licking modal action pattern, and the diencephalon-telencephalon, which processes inputs and modulates outputs. Taste information enters from sensory nerve endings in the tongue and is sent to brain stem nuclei through the trigeminal complex and can directly induce the taste-licking modal action pattern (Flynn & Grill, 1988). Information enters the telencephalon through various structures that further process and integrate information to influence behavior. The specific brain areas involved in these circuits were determined largely by lesion studies investigating how individual brain areas regulate specific behaviors. Integrating the results of these studies can provide a hypothetical pathway of information through the telencephalon. For example, Kawasaki et al. (2017) hypothesized that the basolateral amygdala (BLA) integrates information about the current sucrose reward from the gustatory thalamus (GT; Reilly & Tribunovic, 2003) and the expected reward stored in the insular cortex (IC; Lin et al., 2009). When a significant disparity is detected, information is sent to the central amygdala (CeA), an area implicated in the behavioral response to stressful stimuli and propagation of negative

emotional states (Davis & Whalen, 2001; Gilpin et al., 2015). Consistent with this interpretation, chemogenetic inhibition and pharmacological inactivation of the CeA eliminates the suppression of behavior shown in the response to sucrose downshift in the cSNC task (Guarino et al., 2020; Kawasaki et al., 2015).

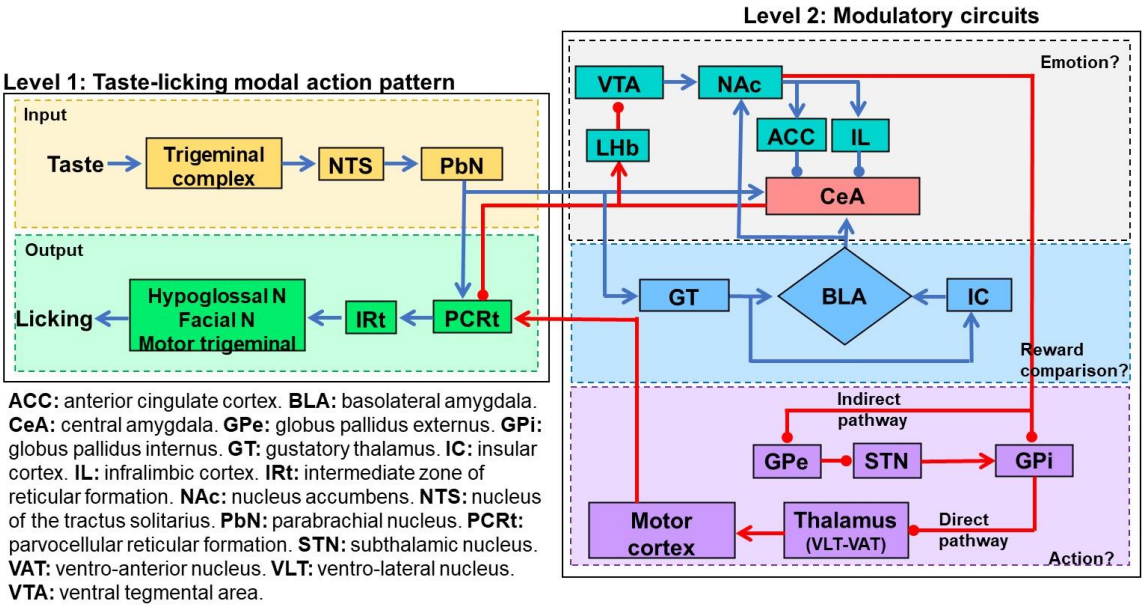

Figure 4. Hypothesized neural circuit underlying the cSNC effect. This circuit involves two main levels. Level 1 contains the sensory input and motor output under the taste-licking modal action pattern. Level 2 contains structures that modulate the taste-licking modal action pattern and are functionally grouped in terms of reward comparison, emotion, and action. The BLA serves as the reward comparison unit which detects the discrepancy between information about the actual reward held in the GT and the reactivated memory of the expected reward held in the IC. The CeA regulates the modal action pattern as a result of this discrepancy and is regulated itself by a loop involving the LHb, NAc, ACC, and IL. The NAc also sends information to the striatum (GPe and GPi) via indirect and direct pathways involving the STN, thalamus, and motor cortex that further modulate behavior (modified from Ortega et al., 2017).

Basal ganglia

Despite steady progress in its development, the circuit shown in Figure 4 is still largely theoretical. The role of several brain regions and their connections in reward loss still remain to be fully explored. One cluster of brain areas that has been suggested to play an

important role in cSNC is collectively known as the basal ganglia (BG), which have been implicated in a variety of emotional disorders (e.g., Gray 1995; Macpherson & Hikida, 2019; Stathis et al., 2007). The BG (Figure 5) consists of the striatum (which further includes the caudate nucleus, putamen, and nucleus accumbens), the globus pallidus, ventral pallidum, the substantia nigra, and the subthalamic nucleus (Lanciego et al., 2012). The BG lies within a larger cortico-basal ganglia-thalamic circuit that regulates a variety of behaviors and thus has multiple inputs and outputs. The striatum specifically receives dopaminergic inputs from the ventral tegmental area (VTA) and substantia nigra pars reticulata (SNr), and glutamatergic inputs from the cortex, hippocampus, amygdala, and thalamus (Britt et al., 2012; Finch, 1996; Philipson & Griffiths, 1985). These inputs are in addition to cholinergic and GABAergic interneurons within the striatum (Kita, 1993; Dutan et al., 2014). Likewise, more than 90% of neurons within the striatum are GABAergic medium spiny neurons (MSNs) that generate inhibitory signals throughout the circuit (Anderson & Hearing, 2019; Kauer & Malenka, 2007; Kemp & Powell, 1971). Notably, glutamatergic inputs to MSNs directly innervate the head of dendritic spines, whereas dopaminergic inputs innervate the spine neck. The resulting interaction between these two inputs allows for the complex modulation of MSN activity (Freund et al., 1984; Xu et al., 1989).

Under the classical understanding of the intrinsic BG circuit, MSNs can further be divided into two major subgroups based on their molecular properties and their anatomical projections within the BG. These two classes constitute the direct and indirect pathways, which modulate downstream thalamic activity in opposite directions. MSNs of the direct pathway project monosynaptically to the output nuclei of the BG: the globus pallidus internus (GPi, also known as entopeduncular nucleus in rodents) and SNr. These MSNs

express dopamine D1 receptors along with dynorphin and substance P receptors. MSNs of the indirect pathway first synapse onto neurons of the globus pallidus externus (GPe), which sends axons to the subthalamic nucleus (STN), and from there to output nuclei. These MSNs also express dopamine D2 and enkephalin receptors. Functionally, the direct pathway tends to facilitate thalamic activity via the inhibition of inhibitory signals of the GPi and SNr, whereas the indirect pathway tends to inhibit activity via disinhibition of glutamatergic neurons of the STN, thus resulting in excitation of inhibitory signals of the GPi and SNr (Albin et al., 1989; DeLong, 1990; Yager et al., 2015). The relationship between the direct and indirect pathway is modulated through endogenous dopamine release from the substantia nigra which then act upon the D1 and D2 receptors and thus balance inhibitory and excitatory signals from these two pathways (Simonyan, 2019).

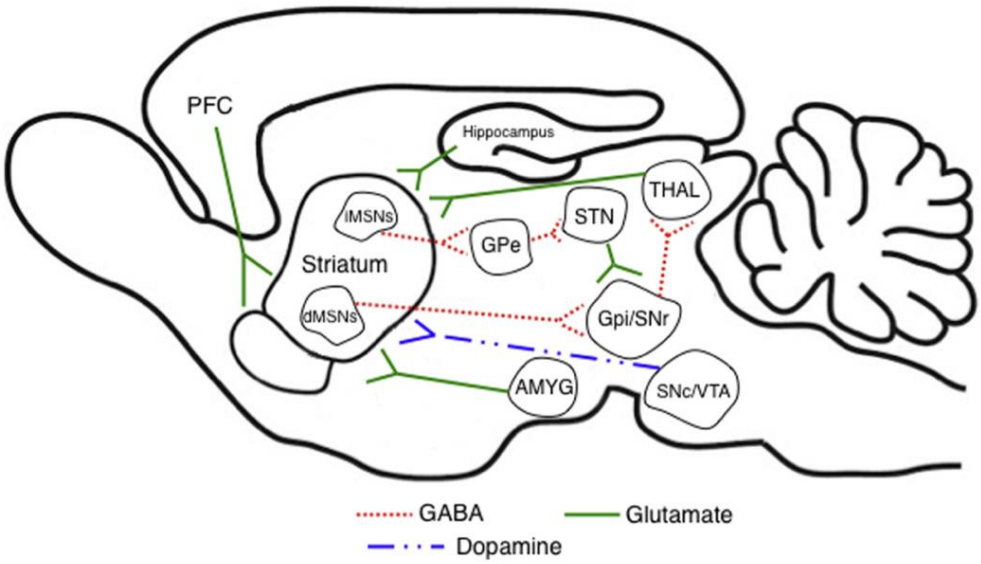

Figure 5. Main striatal inputs and outputs (from Yager et al., 2015). Not shown in this figure is the excitatory influence from thalamic neuros to the motor and premotor cortex that controls behavior.

In addition to the direct and indirect pathway circuit, the NAc sends inhibitory GABAergic fibers to the ventral pallidum (VP), a connection that runs parallel and converges with the pathways to the globus pallidus. The VP also sends inhibitory fibers to the thalamus and projects onto a variety of other structures including the VTA, STN, hypothalamus, and lateral habenula (LHb), which in turn has reciprocal projections to the VP (Jhou et al., 2009; Root et al., 2015). Both the GP and VP send reciprocal inhibitory fibers back to the NAc (Bevan et al., 1998; Haber et al., 1985).

Traditionally, these areas have been studied in the context of selecting wanted versus unwanted movements with its dysfunction contributing to Parkinsonian symptoms (e.g., tremors, muscular rigidity, and hypertonicity). This is largely due to a deficiency in dopamine from the death of neurons in the substantia nigra that then affects various downstream nuclei within the BG (Calebresi et al., 2014; Kravitz et al., 2010). More recently, the BG have been characterized as relay center within different functional loops that carry information regarding movement, cognition, and emotion. These loops (motor, associative, and limbic) are only partially segregated in their extrinsic anatomical connections and thus signals from different functional inputs integrated within the BG influence behavior (Joel & Ruppin, 2002; Simonyan 2019). This is especially important when modulating behaviors related to rewards that require integration of motor, cognitive, and emotional information.

In terms of the potential role of the BG in the response to URDs specifically, there have been several studies that have suggested a major role of these areas in this behavioral context. For example, a decrease in dopamine efflux in the NAc following a URD along with an increase in the expression of c-Fos, a protein used as a marker for neural activity (Genn et al., 2004; Pecoraro & Dallman, 2005). Furthermore, experiments using chemogenetic

inactivation and excitation of the ventral (NAc) and dorsomedial (GPe) striatum demonstrated how these areas can influence consummatory suppression after a sucrose downshift (Guarino et al., 2023). These experiments revealed a complicated interaction between these striatal areas and reward downshift. While chemogenic inhibition of the NAc failed to disrupt consummatory suppression after downshift, excitation significantly enhanced suppression. It was hypothesized that excitation of MSNs of the NAc causing suppression of behavior was facilitated via activation of the inhibitory pathway and neurons in the GPe. This hypothesis was supported by excitation of the GPe causing a reduction in consummatory suppression following sucrose downshift (Figure 6). These results were also obtained in the absence of any motor effects in the open field task. Taken together, these experiments gave the first insight into how the BG can influence consummatory downshift.

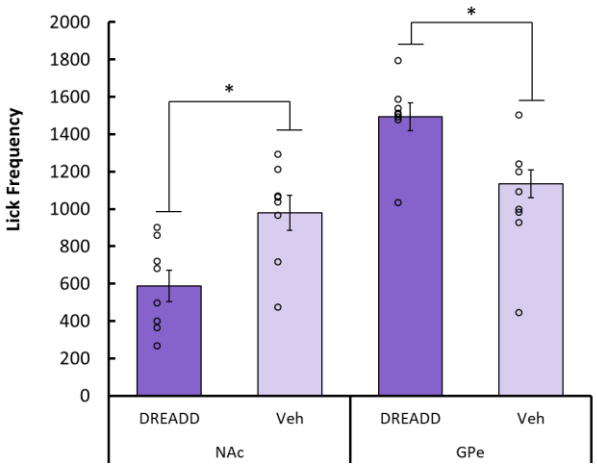

Figure 6. NAc and GPe excitation during consummatory reward downshift (from: Guarino et al., 2023). Notice that whereas NAc excitation enhances suppression of consummatory behavior, GPe excitation eliminates such suppression after reward downshift.

Chemogenetic manipulation

To investigate the role of specific brain areas during key moments of behavior, the current experiments utilized a neuromodulatory technique known as DREADDs (designer receptors exclusively activated by designer drugs). DREADDs are engineered G protein-

coupled receptors modified to respond to the synthetic compound clozapine N-oxide (CNO), but not to the endogenous ligand acetylcholine (Urban & Roth, 2015). This allows for the direct manipulation of these receptors regardless of basal neural activity. DREADDs are delivered into tissues via an adeno-associated virus (AAV) which acts as a vector for in vivo expression of the engineered receptor. These receptors are able to be incorporated into various cell types using a promoter sequence that is specific to a certain class of cells (e.g., neurons, microglia, other glial cells). Furthermore, the two promoters that are specific to neurons, CaMKIIa and hSyn1, have additional neuronal biases. Studies have shown that the CaMKIIa promoter exhibits a bias towards cortical glutamatergic neurons, whereas hSyn1 exhibits as bias towards inhibitory neurons (Bucci & Mahler, 2016; Radhiyanti et al., 2021).

A common DREADD variant is the engineered muscarinic receptor M3 designed for neural excitation. Allosteric CNO binding to the M3 receptor stimulates phospholipase C, which causes the release of intracellular calcium stores and thus stimulates neuronal burst firing (Ambruster et al., 2007). Across all its variants, DREADDs are considered nontoxic, affecting neuronal activity while keeping neurons healthy (Roth, 2016). The engineered receptor is incorporated into neurons via a viral vector, which is infused intracranially into target brain regions. After a set amount of time to allow for neuronal expression of the receptor, a peripheral injection of CNO is used to activate the DREADD and thus excite neurons which have expressed the designer receptor. CNO is a metabolite of the antipsychotic drug clozapine, but it appears to be pharmacologically inert in rodents. Further, CNO itself is unable to cross the blood brain barrier and requires back metabolism into clozapine to enter the central nervous system. This function of CNO metabolism requires

experimenters to control for the potential off-target effects of clozapine during behavioral testing.

Given the complex nature of the DREADD procedure and the number of experimental components required when used in conjunction with consummatory reward downshift (cRD), a variety of control conditions have been previously implemented to verify the specificity of the technique. These have involved conditions that control for the effects of DREADDs in the absence of CNO, CNO in the absence of DREADDs, the viral vector in the absence of the engineered receptor, as well as their specific interaction with different elements of the behavioral task. It is particularly important to include controls that test the use of CNO in cRD specifically, given its metabolism into clozapine, which at high enough doses can result in sedation-like side effects (Roth, 2016) and could affect licking behavior. In the case of cRD, experiments (Guarino et al., 2020, 2023) have included the following controls:

(1) In non-DREADD, intact animals, CNO (1 and 3 mg/kg, ip) administration 30 min prior to a 32-to-2% sucrose downshift led to similar behavioral suppression to that observed in vehicle-treated animals;

(2) CNO administration (3 mg/kg, ip) in animals expressing DREADDs in the CeA did not disrupt the behavior of unshifted controls always exposed to 2% sucrose;

(3) CNO administration (3 mg/kg, ip) in animals expressing DREADDs in the CeA alleviated the behavioral effects of a 32-to-2% sucrose downshift. Thus, the dosage of CNO resulted in levels of back-metabolized clozapine which was within the levels of specificity needed to activate DREADDs, but below the threshold for potentially altering behavior independent of DREADDs; and

(4) A virus vector control (VVC) subjected to the same surgery and infusion in the NAc or GPe as in DREADD animals, except that it does not include the engineered receptor, failed to demonstrate any alterations in consummatory suppression after a 32-to-2% sucrose downshift, whether after CNO or vehicle administration.

Together, data from these control conditions support the consensus that alterations in the behavioral response to reward downshift are a result of the unique interactions between the DREADD receptor and CNO, rather than either component alone.

The present experiment utilized a double-infection chemogenetic procedure used to activate a specific pathway during selected sessions (Figure 7; Oguchi et al., 2015). Two types of viral vector constructs were infused intracranially. Excitatory Cre-dependent DREADDs were delivered bilaterally into the departure area (always the NAc) for each pair of pathways. This DREADD included a viral vector construct (pAAV5-hSyn-DIO-hM3D-mCherry) containing a red fluorescent reporter (mCherry) and a DNA fragment for an engineered muscarinic receptor (M3 receptor). However, the genetic material encoding the engineered receptor is inverted and unreadable by endogenous polymerases and thus cannot be expressed in its initial state. A Cre recombinase enzyme is required to reverse the orientation of the genetic material for the neuron to be able to express the protein. Vector constructs (pENN-AAV9-hSyn-HI-eGFP-Cre-WPRE-SV40) carrying this Cre protein and containing a green fluorescent reporter (eGFP) were delivered bilaterally into the destination areas (GPe, GPi, and VP in different groups). These constructs are retrogradely transported to the departure area, which allows the enzyme to interact with the DREADD construct and the receptor to be expressed and incorporated into the cellular membrane of the neuron. The

dependence of the excitatory DREADD on Cre ensures that, although all neurons exposed to the virus are infected, only projection neurons containing the Cre protein that originated in the departure area express the M3 excitatory DREADDs. Therefore, CNO only excited neurons projecting to the destination areas thus activating the pathway. The red and green fluorescence allows for an accurate determination of the expression location of the M3 excitatory DREADDs (red) and Cre protein (green), both present in individual cells located in the departure area.

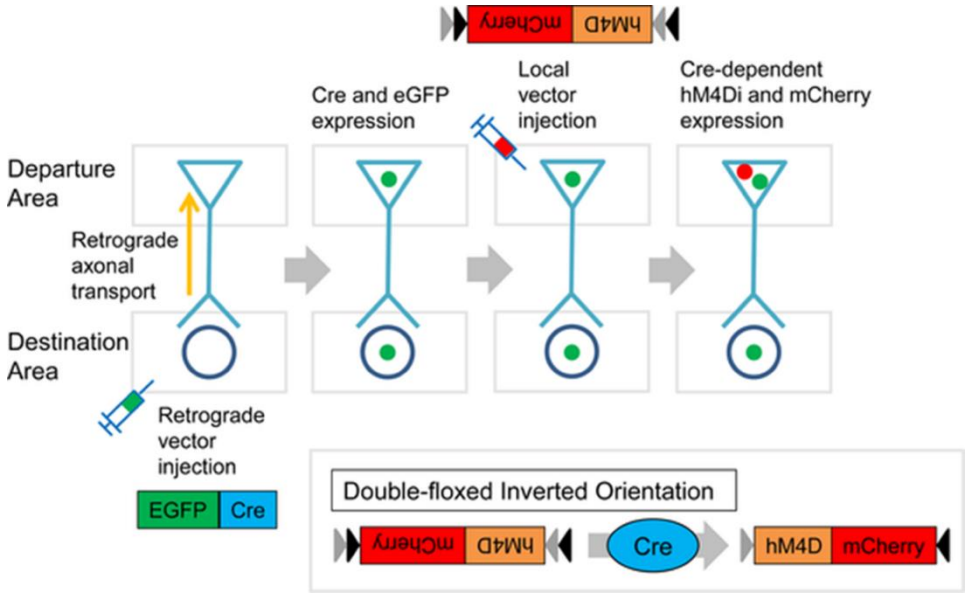

Figure 7. Double infection procedure for DREADDs. A viral vector containing genetic material for the Cre enzyme as well as an eGFP fluorescent tag is infused in the destination area and is expressed locally as well transported retrogradely to the cell bodies of all neurons which synapse onto the destination area, including neurons originating in the departure area. A second viral vector containing double-floxed genetic material for the engineered receptor and a mCherry fluorescent tag is infused in the departure area. Neurons which originate in the depature area and synapse onto the destination area thus contain both the DREADD and Cre enzyme. The Cre enzyme flips the DNA fragment and allows the neuron to read and express the engineered receptor (from Oguchi et al., 2015).

Open field task

When using various biological manipulations to investigate different components of the frustration response to URDs, the open field (OF) test provides information about the one specific component of the system the agent is acting upon: gross motor function. The OF task was first introduced to measure individual differences in emotionality as a response to a novel environment (Hall, 1934). In rodents, there is usually a strong fear component to this task as the animals are placed in an arena with bright lights and loud white noise. This procedure is therefore often used to measure the animal's unconditioned fear responses to stressful stimuli with rats exhibiting higher levels of anxiety or negative emotion exploring and moving less than animals with lower levels anxiety or negative emotion. A more specific analysis of behavior in an OF shows that rodents naturally adhere to the edges of the chamber in response to being in a novel environment. Therefore, rats that are less anxious not only have increased locomotor activity, but this activity has more dramatic increase in the center of the field compared to the periphery (Prut & Belzung, 2003). This is important when distinguishing between changes in the anxiety/fear response versus changes in overall motor capability independent of emotionality.

Kawasaki et al. (2015, 2017) provided examples of using OF to delineate the roles of specific brain areas in URD tasks. The first of these studies (Kawasaki et al., 2015) was designed to investigate the role of the CeA in consummatory suppression after sucrose downshift using lidocaine microinfusions to inactivate neurons in a reversible manner. Functional inhibition of the CeA was shown to both blunt consummatory suppression induced by a sucrose downshift and increase locomotion in the center of the OF (see Figure 8). The following study took a similar approach to investigating the BLA and found that

while consummatory suppression was eliminated, there was no change in OF behavior (see Figure 9). With both areas disrupting cSNC, it can be clearly suggested that they play an important role in the circuit. However, their specific actions in consummatory suppression must be distinct given the OF results. These data suggest that the CeA plays more of a general emotional role leading to response suppression and also enhancing activity in the central area of an OF, while the BLA plays a more cognitive role in terms of reward comparisons, since it disrupts cSNC, but has no effects on OF activity (Kawasaki et al., 2017). Thus, including OF as an additional behavioral task can aid in separating the different components of frustrative nonreward. This is especially important to the present set of experiments given the well-established connection between the BG and the motor system. Not only can the OF task isolate emotional effects, especially in terms of activity in the central area of the OF arena, but it can also provide information on how these specific areas affect gross motor function, mainly in terms of activity in the peripheral area.

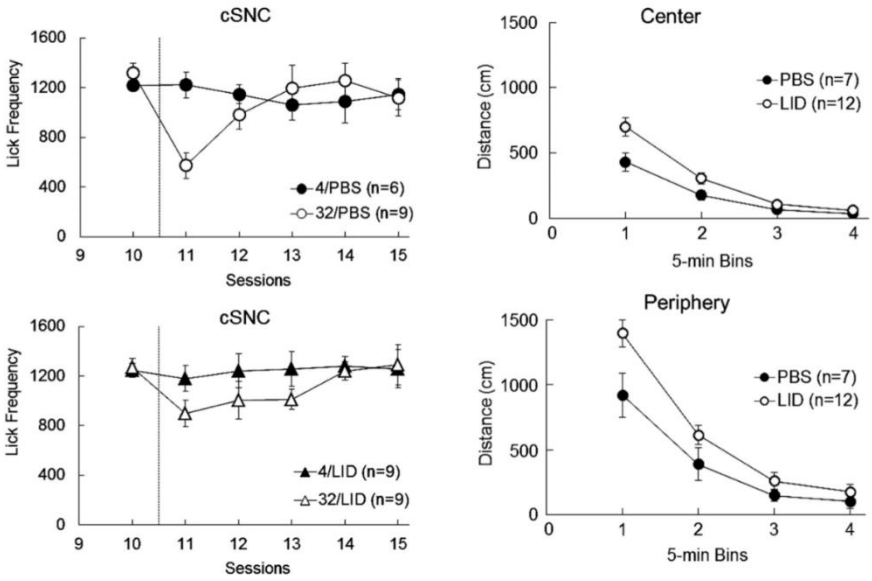

Figure 8. cSNC (left panels) and OF data (right panels) after lidocaine inactivation of CeA neurons versus PBS vehicle infusions (from Kawasaki et al., 2015).

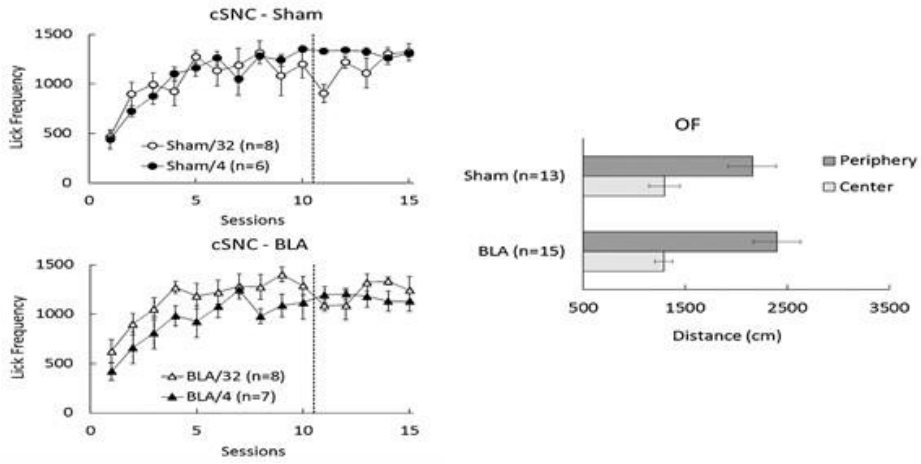

Figure 9. cSNC (left panels) and OF (right panel) data from animals with BLA excitotoxic lesions and shams (from Kawasaki et al., 2017).

Present Experiments

The current set of experiments aimed to investigate three pathways within the BG to determine how their activation affects consummatory behavior in a cRD paradigm. This was performed in conjunction with several other behavioral tasks to determine what specific behavioral element these pathways influence when activated. The OF task was also chosen to test gross motor function. Thus, Experiments 1-3 was designed to test how chemogenetic activation of these BG circuits affect consummatory suppression after a 32-2% sucrose downshift and in OF. Each experiment is distinguished based on the specific neural pathway that they manipulate. Experiment 1 tested the NAc-to-GPe pathway, Experiment 2 tested the NAc-to-GPi pathway, and Experiment 3 tested the NAc-to-VP pathway. Given previous rsearch (Guarino et al., 2023), it was initially hypothesized that activation of the NAc-to-GPe pathway in Experiment 1 would exacerbate consummatory suppression. This would be in contrast to Experiment 2 in which activation of the NAc-to-GPi pathway would be hypothesized to yield the opposite results given the opposing actions of the direct and indirect BG circuits. No specific hypotheses were made regarding the NAc-to-VP pathway in Experiment 3 given the relatively unknown function of the VP in relation to reward

downshift. All together, these experiments aimed to determine the role of the NAc and which of its output structures can influence the behavioral response to frustration.

Experiments 1-3

Method

Experimental design. These three experiments each investigated one specific pathway in the BG circuitry. Experiment 1 involved the NAc-to-GPe pathway, Experiment 2 involved the NAc-to-GPi pathway, and Experiment 3 involved the NAc-to-VP pathway. All these experiments utilized chemogenetic excitation via the double infection DREADD procedure described previously. Animals were randomly assigned to either CNO or Veh conditions as well as either DREADD or virus vector control (VVC) conditions. In total for each experiment, there were three experimental conditions: one experimental condition with both the DREADD construct and CNO injection (Group CNO), and two control conditions one of which had the DREADD construct and vehicle injections (Group Veh) and the other with VVC construct and CNO injections (Group VVC). Including both controls allowed for further confidence in the validity of any behavioral effects. All animals were tested in the cRD task, a modified version of the cSNC in which unshifted control groups are omitted to emphasize the suppression of consummatory behavior after a drastic disparity in sucrose concentrations. Essentially, all animals experienced a 32-2% sucrose downshift. Further, this cRD task involved a downshift to a 2%, as opposed to the 4% sucrose solution more frequently used in these experiments (e.g., Flaherty, 1996), to provide more robust consummatory suppression. Table 1 describes the different experiments, pathways, and injection conditions

.

Table 1

Experimental conditions

Experiment	*DREADD*	Departure	Destination	Injection	Downshift
1	Excitatory	NAc	GPe	CNO	32-2%
	Excitatory	NAc	GPe	Veh	32-2%
	VVC	NAc	GPe	CNO	32-2%
2	Excitatory	NAc	GPi	CNO	32-2%
	Excitatory	NAc	GPi	Veh	32-2%
	VVC	NAc	GPi	CNO	32-2%
3	Excitatory	NAc	VP	CNO	32-2%
	Excitatory	NAc	VP	Veh	32-2%
	VVC	NAc	VP	CNO	32-2%

Note. CNO: clozapine N-oxide, the DREADD activator. DREADD: designer receptors exclusively activated by designer drugs. GPe: globus pallidus externus. GPi: globus pallidus internus. NAc: nucleus accumbens. Veh: vehicle injection. VP: ventral pallidum. VVC: virus vector control that contains all the elements of the regular DREADD infusion, except for the engineered receptor.

Table 2

Brain areas and coordinates

Area	A/P	M/L	D/V
NAc	1.7	+/- 1.0	-7.6
VP	0.12	+/- 2.4	-8.2
GPe	-2.2	+/- 4.3	-7.2
GPi	-2.3	+/- 2.8	-7.8

Note. All values are in millimeters and calculated relative to bregma. Coordinates from Paxinos and Watson (2013). A/P: anterior/posterior. M/L: medial/lateral. D/V: dorsal/ventral. Acronyms for areas in Table 2.

Subjects. Across Experiments 1-3, subjects were 73 male Wistar rats bred from parents purchased at Charles River Labs in accordance with approved IACUC breeding protocol # 2022-4. Animals were weaned at 21-24 days of age, group housed until around 40 days of age, and individually housed thereafter with an enrichment retreat device. Animals had ad libitum access to standard rat chow until they were 90 days old. The mean (±SEM) of all surgery animals was 463.9 g (±4.6 g). Rats had a 12-h light-dark cycle (lights on at 07:00 h), constant temperature (22-23 °C) and humidity (50-64%), and ad libitum access to water throughout their lives.

Surgery. Animals were anesthetized using a mixture of breathing air and isoflurane vapor, 5% for induction and 1-2% for maintenance. Once breathing was deepened and slowed, the area of the incisions was shaved and the animal positioned in a stereotaxic frame (Angle Two, program version 3.0.0, Leica Biosystems, Deer Park, IL) equipped with blunt-tipped ear bars, a bite bar, and a mask to allow for continual delivery of isoflurane vapor to

maintain anesthesia during surgery. Prior to incision, the shaved area was wiped with Betadine (povidone-iodine topical solution, 10%) and eyes were covered with Vaseline to protect them from the microscope light and prevent eye dryness. Then, a midline incision was made in the scalp, the skull was cleaned, carefully peeling back the protective layers from the surface. Blunted hooks were used to pull each side of the incision apart to expose the skull. The position of the skull was verified and adjusted to ensure flatness. Coordinates for the target brain regions were located using a rat atlas (Paxinos & Watson, 2013) and marked on the skull (see Table 2). Once marked, four holes were drilled in the skull for each infusion site. The viral constructs were delivered bilaterally using a 10-µl Hamilton syringe mounted on a stereotaxic injector (Quintessential Stereotaxic Injector, Stoelting, Wood Dale, IL) programmed to deliver 1 µL of virus per side at a rate of 0.2 µL/min, followed by a 10-min waiting period to allow the fluid to diffuse into the brain tissue. After this period, the Hamilton syringe was slowly withdrawn. Once each infusion was complete, the scalp was stapled back together to promote healing. After surgery and again 24 h later, animals were injected with buprenorphine hydrochloride (0.05 mg/kg, sc) to alleviate pain induced by surgery. Animals were housed individually during a 5-day recovery period. Supplementary recovery gel added to typical lab rodent chow was provided immediately after the surgery. Animals were then housed in their home cage and food was gradually restricted to an 81-84% of their average ad libitum weight. This weight level was maintained throughout the duration of the experiment by feeding a controlled amount of rat chow every day at about the same time, at least 30 min following behavioral testing. Behavioral training began at least 15 days after viral infusion to ensure maximal DREADD expression and when the weight of all

rats was within the target range. Training was administered between 10:00 and 13:00, 7 days/week.

CNO preparation and injection procedure. CNO (3 mg/kg, ip; NIDA Drug Supply Program) was dissolved in 5% dimethyl sulfoxide (DMSO) and 95% sterile saline. CNO and vehicle (Veh) injections were both administered using the same dosage volume (1 mL/kg) and content, except CNO was not included in Veh preparation. Injections were administered 30 min prior to behavioral testing in a room different from that where the tests were performed. Injections were given before cRD sessions 11 through 14 and before the OF session. Repeated CNO injections have raised some concerns on the possibility that clozapine accumulates in the nervous system causing effects independently of DREADDs (Claes et al., 2022). However, previous experiments in the absence of the DREADD receptor have shown no effect of CNO administration at this dosage on consummatory behavior in the cRD task (Guarino et al., 2020).

Consummatory reward downshift (cRD). Training was carried out in 8 conditioning boxes (MED Associates, St. Albans, VT) made of aluminum and Plexiglas (29.3×21.3×26.8 cm, L×H×W). Each box was inside a sound-attenuating chamber containing a speaker (white noise) and a fan (ventilation). The speaker and fan produced masking noise with an intensity of 80.1 dB (SPL, scale C). A diffuse light (GE 1820) was located in the center of the box's ceiling. The floor consisted of steel rods running parallel to the feeder wall. A tray with corncob bedding was placed below the floor to collect feces and urine. In the feeder wall were three holes, each 1 cm wide, 2 cm long, and 4 cm from the floor, equidistant from each other and from the edge of the wall. Sipper tubes 1 cm in diameter and equipped with a ball bearing to minimize leakage were inserted through these holes flush to

the outside of the right wall to deliver sucrose solutions from attached bottles. These experiments only utilized the central sipper to deliver the sucrose solutions. Animals received 10 preshift sessions of access to 32% sucrose and 4 postshift sessions of access to 2% sucrose. Each session was 5-min long starting from the first recorded contact with the sipper. Licking responses were detected by means of a circuit involving the rods in the floor and the sipper tube, closed by the animal standing on the floor when it licked the sipper. All events were controlled by a computer located in an adjacent room. CNO or vehicle was administered 30 min prior to the start of target sessions. Experimental events during cRD sessions were programed using MED Notation (MED Associates, St. Albans, VT). Sucrose concentrations were prepared by weight by mixing 32 g (or 2 g) of sucrose for every 68 g (or 98 g) of water.

Open Field (OF). Rats were placed in an open arena with black vinyl flooring and Plexiglas walls. Arenas were arranged in a 2×2 grid and placed in a room that was brightly-lit. Each of the four OFs was visually isolated by opaque cardboards. Sessions lasted 15 min with the behavior of each rat being tested captured by a camera mounted above the arenas. Videos from each session were analyzed to measure the overall ambulatory time of the rat using EthoVision XT Version 11 Software (Noldus, Leesburg, VA). Arenas were cleaned at the end of each session in preparation for the next squad.

Histology. After the last behavioral session, rats were transcardially perfused, and brains were immediately extracted and embedded in 4% paraformaldehyde for at least 3 days. Brains were then embedded in 30% sucrose in 1x Phosphate Buffered Saline for at least 2 days. Once fixed, brains were sliced in 40-μm sections using a cryostat (Leica Biosystems, Buffalo Grove, IL). Sections were placed onto slides, Fluoromount-G mounting medium and

cover slips were applied to preserve the fluorescent tags (mCherry, eGFP) and localize the virus. DREADD location was assessed via fluorescence microscopy.

Statistical analyses. The primary dependent variable for the cRD task was the total number of licks per 5-min sessions and the primary dependent variable for the OF task was distance traveled measured in centimeters per 15-min sessions. For cRD, data were analyzed with a mixed-design analysis of variance (ANOVA) with experimental condition as a between subject factor and session as a within subject factor. For OF, data were analyzed using a mixed-design ANOVA with experimental condition as a between subject factor and zone (Center vs Periphery) as a within subject factor. Significant differences were assessed using an alpha value of $p < 0.05$ and comparisons between groups were conducted using a Bonferroni pairwise correction. All analyses were performed using the IBM SPSS Statistics 27 package.

Results

DREADD expression. For animals infused with excitatory DREADDs, to validate expression, visualization of both an mCherry tag (indicating presence of the local viral infusion into the NAc) and a GFP tag (indicating the presence of the retrograde virus infused in the destination area) must be present within the boundaries of the NAc as well as demonstrate overlap in their expression. Due to the spread of the virus, no distinction was made between the core and shell of the NAc. Thus, animals that lacked either mCherry or GFP fluorescent markers in the boundaries of the NAc ($n = 5$) were eliminated from all analyses. For animals infused with the viral vector control, only the GFP marker was needed to confirm expression. Figure 10 shows representative images from each experiment as well as indicators of single neurons that expressed both mCherry and GFP, thus demonstrating

that the neuron was infected with both viruses and therefore expressed the excitatory DREADD.

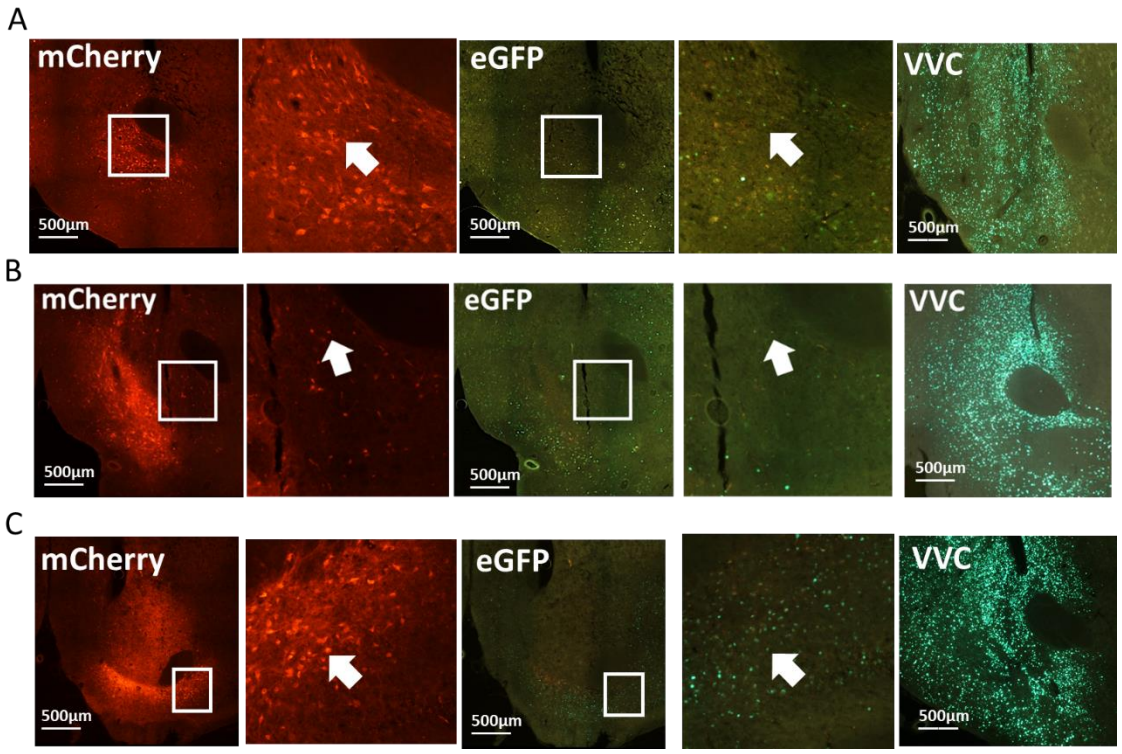

Figure 10. Representative sample of brain sections in the NAc from the NAc-to-GPe pathway (A) and the NAc-to-GPi pathway (B) and NAc-to-VP pathway (C). mCherry (left) and eGFP (center) fluorescence can be seen within the same section demonstrating DREADD expression. Arrows are pointing to neurons that express both fluorescent tags. eGFP fluorescence (right) can be seen for viral vector controls.

cRD. Given that the primary objective of these experiments was to investigate how these neural pathways affect consummatory suppression after reward downshift, it was necessary that animals exhibit robust licking to the 32% sucrose by the end of preshift. High licking at the time of the terminal preshift session minimizes the possibility that a floor effect of licking is not responsible for the response suppression observed when animals were downshifted. Such floor effect would potentially obscure signs of frustration. Thus, due to some animals showing erratic and/or low licking behavior during training, a selection

criterion was implemented based on preshift licking data. Animals that did not reach at least 1000 licks for at least three (out of 10) different preshift sessions (n = 30) were eliminated from analysis. It is most likely that the neurosurgical procedure itself disrupted motivation to lick during training. In fact, almost half of these excluded animals (n = 14) almost never reached above 10 licks in a session. Table 3 shows final sample numbers distributed between experiments and conditions after all exclusion criteria.

Table 3

Final subject numbers

Experiment	CNO	Veh	VVC
GPe	n = 7	n = 5	n = 5
GPi	n = 8	n = 8	n = 6
VP	n = 8	n = 8	n = 6

Note. The experiment column refers to the destination area in which the Cre virus was infused. Both CNO and Veh columns consist of animals infused with excitatory DREADDs and the VVC column consists of animals infused with the viral vector and injected with CNO.

Experiment 1. To assess preshift performance in animals with excitatory NAc-to-GPe DREADDs, a Group (CNO, Veh, VVC) by Session (1-10) analysis was conducted. Licking data for all sessions are summarized in Figure 11. Results revealed a significant main effect of Session, $F(9, 126) = 13.15$, $p < 0.001$, $\eta_p^2 = 0.48$, such that licking increased across sessions. The main effect of Group as well as the Group by Session interaction were both nonsignificant, Fs < 1.15. To assess the response to the 32%-to-2% downshift, the last two

sessions were averaged together to create an average preshift performance value, which was compared to the first downshift session in a Group by Session analysis. Results revealed a significant main effect of Session, $F(1, 14) = 19.67$, $p < 0.001$, $\eta_p^2 = 0.58$, such that licking decreased from preshift to the first postshift session. However, both the main effect of Group and the Group by Session interaction were nonsignificant, Fs < 1. To assess recovery performance, a Group by Session (12-14) analysis was conducted. Results revealed no significant effects, Fs < 1. Overall, results indicated no group differences across preshift or downshift indicating that all groups behaved similarly during preshift and that activation of the NAc-to-GPe pathway had no effect on either the initial response to the downshift on Session 11 or during the recovery on Sessions 12-14.

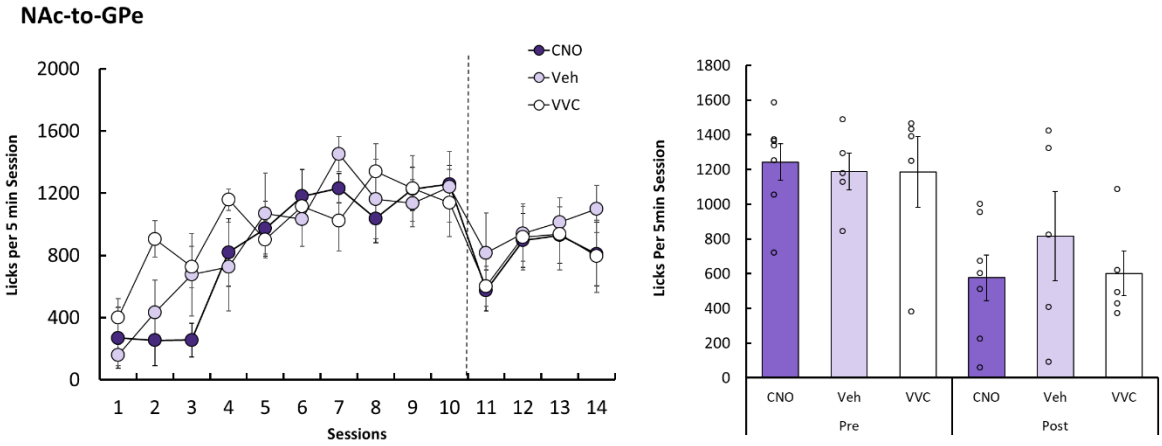

Figure 11. cRD lick frequency from DREADDs infused in the NAc and GPe. Injections of CNO or Veh were administered 30 min before Sessions 11-14 (left). cRD lick frequency for average preshift (Session 9 and 10) and first downshift (Session 11) (right).

Experiment 2. Similar analyses to Experiment 1 were conducted for Experiment 2 for animals with excitatory NAc-to-GPi DREADDs. Licking data for all sessions are summarized in Figure 12. A Group by Session analysis for preshift performance revealed a significant effect of Session, $F(9, 171) = 19.27$, $p < 0.001$, $\eta_p^2 = 0.50$, such that licking

increased across session. The main effect of Group as well as the Group by Session interaction were both nonsignificant, *F*s < 1. Then, a Group by Session analysis was conducted to assess differences between preshift and downshift. Results revealed a significant main effects of Session, $F(1, 19) = 95.41$, $p < 0.001$, $\eta_p^2 = 0.83$, Group, $F(9, 171) = 3.95$, $p < 0.05$, $\eta_p^2 = 0.29$, as well as a significant interaction, $F(2, 19) = 6.44$, $p < 0.01$, $\eta_p^2 = 0.40$. Pairwise Bonferroni comparisons revealed no significant differences in preshift, *p*s > 0.05, but Group CNO was significantly different from both control groups on the first downshift session, *p*s < 0.05. Animals in the CNO group had significantly less licking behavior on downshift compared to injection and virus controls indicating that excitation of the NAc-to-GPi pathway enhanced consummatory suppression after a 32%-to-2% sucrose downshift. Finally, a Group by Sessions analysis for recovery sessions revealed an approaching significant effect of Session, $F(2, 38) = 2.89$, $p = 0.08$, $\eta_p^2 = 0.24$, a nonsignificant main effect of Group, and a nonsignificant interaction, *F*s < 1. The approaching significant effect of Session appears to suggest animals increasing their licking across downshift sessions and the lack of any Group effects shows that effects of the NAc-to-GPi activation were restricted to the first session of downshift and animals recovered to levels of controls by Session 12.

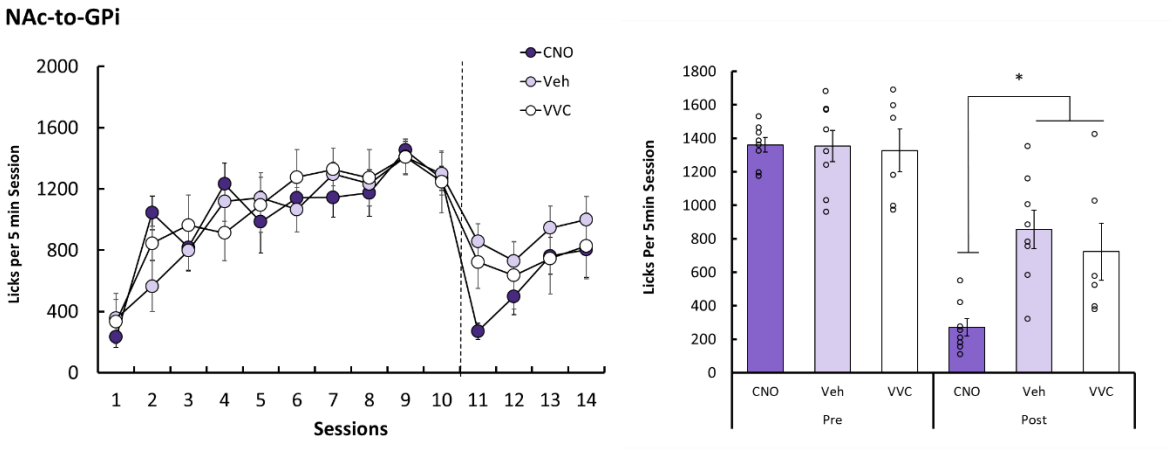

Figure 12. cRD lick frequency from DREADDs infused in the NAc and GPi. Injections of CNO or Veh were administered 30 min before Sessions 11-14 (left). cRD lick frequency for average preshift (Session 9 and 10) and first downshift (Session 11) (right). *: significant differences by Bonferroni pairwise comparisons, $p < 0.05$

Experient 3. As with Experiments 1 and 2, separate analyses were conducted to assess preshift, downshift, and recovery performance for animals with excitatory NAc-to-VP DREADDs. Licking data for all sessions are summarized in Figure 13. A Group by Session analysis for preshift performance revealed a significant effect of Session, $F(9, 171) = 9.96$, $p < 0.001$, $\eta_p^2 = 0.34$, such that licking increased across session. The main effect of Group as well as the Group by Session interaction were both nonsignificant, Fs < 1.12. Then, a Group by Session analysis was conducted to assess differences between preshift and downshift. Results revealed a significant main effects of Session, $F(1, 19) = 63.29$, $p < 0.001$, $\eta_p^2 = 0.77$, a nonsignificant Group effect, $F = 1.19$, but there was a significant interaction, $F(2, 19) = 8.71$, $p < 0.01$, $\eta_p^2 = 0.48$. Pairwise Bonferroni comparisons revealed no significant differences in preshift, ps > 0.05, but Group CNO was significantly different from both control groups on the first downshift session, ps < 0.01. Animals in the CNO group had significantly less licking behavior on downshift compared to injection and virus controls indicating that like in Experiment 2 with the NAc-to-GPi pathway, excitation of the NAc-to-

VP pathway also enhanced consummatory suppression after a 32%-to-2% sucrose downshift. Finally, a Group by Sessions analysis for recovery sessions revealed nonsignificant main effect of Session, and Group, *F*s < 1, and an approaching significant interaction, $F(4, 36) = 2.31$, $p = 0.08$, $\eta_p^2 = 0.20$. The approaching significant interaction appears to suggest a higher rate of recovery for animals in the CNO condition as they reach the levels of control animals. Overall, these data suggest similar behavioral effects of activation of the NAc-to-GPi and the NAc-to-VP pathways in the cRD paradigm.

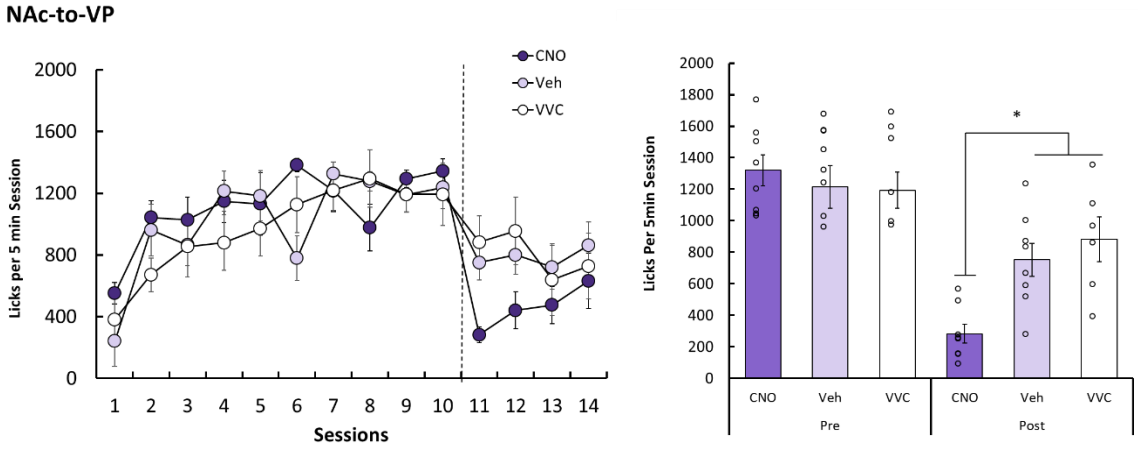

Figure 13. cRD lick frequency from DREADDs infused in the NAc and VP. Injections of CNO or Veh were administered 30 min before Sessions 11-14 (left). cRD lick frequency for average preshift (Session 9 and 10) and first downshift (Session 11) (right). *: significant differences by Bonferroni pairwise comparisons, $p < 0.05$

OF. Open field testing was implemented as a control for gross motor movement administered to animals the day after the last session of cRD. All animals received CNO or Veh injections 30 min prior to testing. Only animals that were included in cRD analyses were part of the OF analyses. For each experiment, OF data were analyzed using a mixed ANOVA with Group (CNO, Veh, VVC) as a between subject factor and Area (Center, Periphery) as a within subject factor.

Experiment 1. Results from a Group by Area analysis revealed a significant main effect of area, $F(1, 14) = 28.65$, $p < 0.001$, $\eta_p^2 = 0.67$, such that all animals traveled more in the periphery than in the center. The main effect of Group as well as the interaction were nonsignificant, Fs < 1.7. The effect of area was expected given that the peripheral zone counts for a larger area than the center and animals tend to adhere to the edges of novel environments in a behavior known as thigmotaxis. Moreover, these data indicate that activation of the NAc-to-GPe pathway had no effect on overall gross motor function and did not alter the distribution of movement between center and peripheral zones.

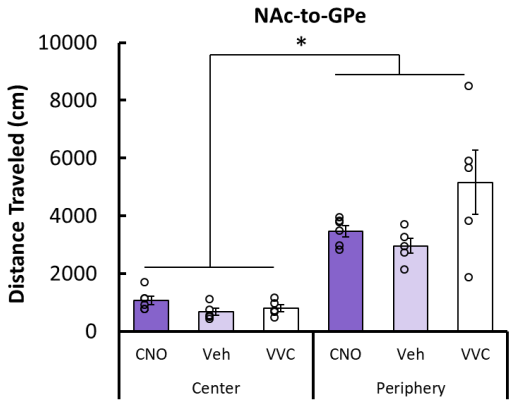

Figure 14. OF activity of animals infused with DREADDs in the NAc and GPe. Distance traveled was measured in both the central (left) and peripheral (right) areas of the arena during a 15-min session. Injections of either CNO or Veh were administered 30 min before testing.

Experiment 2. Results from a Group by Area analysis revealed a significant main effect of area, $F(1, 19) = 37.38$, $p < 0.001$, $\eta_p^2 = 0.65$, such that all animals traveled more in the periphery than in the center. The main effect of Group as well as the interaction were nonsignificant, Fs < 1.7. These results mimic Experiment 1 and also indicate that activation of the NAc-to-GPi pathway had no effect on overall gross motor function and did not alter the distribution of movement between center and peripheral zones.

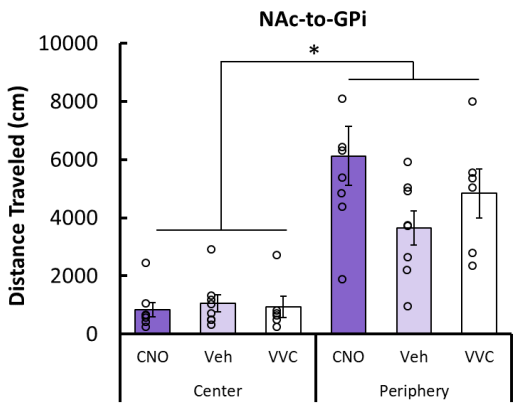

Figure 15. OF activity of animals infused with DREADDs in the NAc and GPi (see legend to Figure 14 for more details).

Experiment 3. Results from a Group by Area analysis revealed a significant main effect of area , $F(1, 19) = 181.40$, $p < 0.001$, $\eta_p^2 = 0.91$, such that all animals traveled more in the periphery than in the center. The main effect of Group as well as the interaction were nonsignificant, Fs < 1. These results also mimic Experiments 1 and 2, again indicating that activation of the NAc-to-VP pathway had no effect on overall gross motor function and did not alter the distribution of movement between center and peripheral zones.

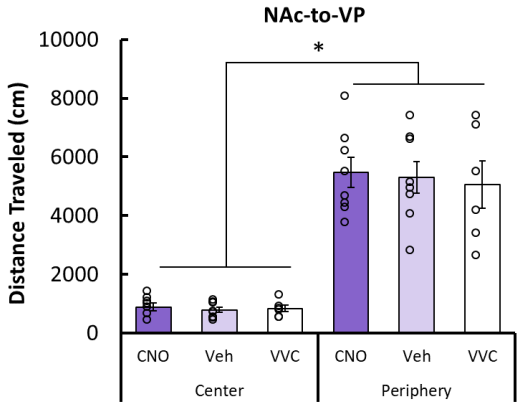

Figure 16. OF activity of animals infused with DREADDs in the NAc and VP (see legend to Figure 14 for details).

General discussion

This set of experiments was the first to investigate the role of specific pathways within the BG in a consummatory reward downshift paradigm. The BG are a complex system of interconnected nuclei involved in a wide variety of behaviors including voluntary motor control, language, decision making, procedural learning, and working memory (Simonyan 2019). More specifically, these areas most prominently aid in the balance between the facilitation of wanted movements while simultaneously inhibiting competing unwanted movements through two major circuits, known as the direct and indirect pathways. The direct pathway generally promotes movement and consists of inhibitory GABAergic MSNs which are categorized by their expression of D1 receptors. These neurons synapse onto the GPi thereby inhibiting the GPi's own inhibitory signals to the thalamus. This causes rebound excitation and overall increases thalamus signaling. This is contrasted to the indirect pathway which instead consists of GABAerigic MSNs expressing D2 receptors. These neurons synapse first onto the GPe suppressing activity of its own inhibitory neurons. This causes increased activity of its output, the STN, through disinhibition and rebound excitation. STN neurons then send glutamatergic excitatory signals onto the GPi which, in turn, inhibits the GPi's output to the thalamus. MSNs of the ventral striatum also project onto the ventral pallidum, in their own set of reciprocal circuits that also project to the thalamus as well as other regions including the lateral habenula, amygdala, and other regions within the BG (Root et al., 2015). The present experiments aimed to investigate how chemogenetic activation of these different outputs from the ventral striatum affect the response to a 32-to-2% sucrose downshift.

Experiment 1 showed that activation of the NAc to GPe pathway had no detectable effect on the response to the downshift compared to control animals who received the excitatory virus, but vehicle injection, or who received only the viral vector, but with the CNO injection. In fact, animals behaved similarly throughout all four downshifted sessions and all exhibited similar open field activity. This finding was in opposition to the initial hypothesis that this connection would produce an exacerbated response given the results of previous experiments looking at activating these areas separately (Guarino et al., 2023). It was found in the Guarino study that chemogenetic excitation of the NAc enhanced consummatory suppression (specifically concentrated in the recovery from downshift) whereas excitation of the GPe nearly eliminated suppression after a 32-to-2% downshift. These distinct effects led to the conclusion that exciting inhibiting signals from the NAc would inhibit neurons that lead to the GPe and thus exciting neurons directly in the GPe would lead to the opposite behavioral outcome. However, due to the null results from excitation of the NAc to GPe pathway, it appears that the exacerbation of consummatory suppression after exciting solely the NAc were most likely a result of neurons other than those that synapse onto the GPe. Whereas activation of the NAc to GPe pathway had no significant effect on cRD, Experiment 2 showed that activation of the NAc to GPi pathway led to enhanced suppression after downshift compared to both control conditions. Further, this effect was concentrated on the first day of downshift with no significant differences by the second downshift session despite repeated activation of this pathway across all four postshift sessions. This suggests that of NAc-GPi neurons have the ability to affect behavior when animals are experiencing a negative emotional response as with the initial session of cRD. These results were mimicked by Experiment 3 with NAc-to-VP activation which

showed similar behavioral functions in that consummatory suppression was exacerbated on the first day of downshift and recovered to the level of controls by the second downshift session. Activation of either of these pathways also led to no behavioral changes in OF either in terms of overall locomotion or in the distribution of behavior between the center and periphery of the field.

Given the opposing actions of the direct and indirect pathways of the basal ganglia with the direct pathway via D1 MSNs generally promoting behavior and the indirect pathway via D2 MSNs generally inhibiting behavior, it was initially hypothesized that chemogenetic activation of these two pathways would result in opposite behavioral effects in the cRD paradigm. This hypothesis was based on the "go/no-go" model of selecting wanted and unwanted movements where D1 neurons would be active during actions whereas D2 neurons would be inactive (for a review see Cox & Witten, 2019). This model has been further extended to relate to reward processing with D1 MSNs shown to increase their activity during reward presentation whereas D2 MSNs increase activity during unrewarded outcomes (Nonomura et al., 2018; Zalocusky et al., 2016). It is also important to note that NAc-to-VP neurons traditionally lie outside this dichotomy with neurons within the VP expressing both D1 and D2 receptors as well as receiving input from accumbal D1 and D2 MSNs (Robertson & Jian, 1995). However, this "go/no-go" framework is more than likely too simplistic to account for behavioral changes in the cRD paradigm as shown in the present experiments. Activation of the NAc-to-GPe indirect pathway failed to produce behavioral changes while activation of the NAc-to-GPi direct pathway decreased licking immediately after downshift. This discrepancy is likely due to the complex and reciprocal connections between pathways as well as with the VP. For example, D1 and D2 MSNs have been shown to encode both

positive and negative reward outcomes with brief optogenetic stimulation of these neurons facilitating place preference and enhancing cocaine conditioning whereas prolonged stimulation reduced place preference and cocaine conditioning (Soares-Cunha et al., 2020). However, despite the complex circuitry involved between these and other brain areas, activation of specific pathways do induce clear behavioral effects in the cRD task demonstrating that specific connections between areas alone are sufficient to modulate consummatory behavior.

The effects shown after activation of the NAc-to-GPi or the NAc-to-VP pathway strongly suggest the requirement of an emotional component in order to modulate behavior given the lack of alterations in locomotor activity in the OF as well as the demonstration of recovery of licking behavior across downshifted sessions. Nevertheless, considering the strong and well-documented role of the BG in motor control, the possibility that these effects on cRD largely reflect motor components should not be ignored. The OF task does provide key insight into gross motor function in addition to being a common test of the innate fear response to in novel environments (Prut & Belzung, 2003). However, there are large discrepancies into the motor control of the forelimbs and hindlimbs in general locomotion versus the fine motor control of licking to a sipper tube. This can somewhat be addressed by examining behavior across downshifted sessions. Because negative emotion is concentrated on the first session of downshift and dissipates across subsequent sessions, differences seen early in downshift can be more attributed to emotional effects, whereas differences seen later in downshift can be more attributed to non-emotional motor function. This is consistent with data from NAc-to-GPi and NAc-to-VP activation as their effects are largely concentrated on the first downshift session. Still, it can be argued that the performance increase for these

animals across sessions could be due to a practice effect and that their motivation to receive the reward eventually overcomes any motor-dependent obstacles. However, it is less likely that practice effects would be seen in so few sessions, so that these effects cannot be simply explained as purely motor.

Ongoing pilot experiments are beginning to address the problem of potential motor effects by utilizing a behavioral paradigm nearly identical to cRD except without any downshift in reward value. Essentially, animals are trained with the same concentration of sucrose across all 14 sessions and chemogenetic activation of either the NAc-to-GPi or NAc-to-VP pathway is implemented during sessions 11-14. This provides insight into how activation of these pathways impacts consumption in the absence of any reward relativity. This procedure also minimizes much emotional components that come along with frustration associated with reward downshift. However, there is a possibility that frustration cannot be avoided whenever training involves food-deprived animals. Crespi (1942) suggested that a food-restricted animal receiving an insufficient amount of food is in a constant state of frustration. He claimed that "eating the small incentive serves to stimulate and increase desire or tension in the rat without, however, improving the chances for obtaining more food. […] This state of heightened tension is unpleasant. Therefore, though food is present and the animal has had no food for some twenty-two hours, the situation is labelled 'frustrating'" (p. 498). Thus, food-restricted animals who are receiving 2% sucrose regardless of prior experience could always be in a mild state of frustration. As a result, investigating how the activation of these pathways affects licking to 32% sucrose provides more clear insight into potential motor effects than licking to 2% given that a higher value of sucrose would be more satisfying to a food-restricted animal reducing basal frustration, as suggested by Crespi

(1942). In fact, preliminary data suggest that activation of the NAc-to-GPi pathway has no effect on licking to 32% sucrose, whereas activation of the NAc-to-VP pathway causes a reduction in licking. While more data need to be collected, this does seem to indicate that these pathways modulate behavior differently under specific circumstances with the NAc-to-VP pathway potentially having a larger motor component than the NAc-to-GPi, which could require negative emotion to cause behavioral changes.

The notion that the NAc-to-GPi pathway encodes emotional information is supported by several other lines of research that have investigate the role of the BG in various emotional disorders (Gray, 1995; Hikida et al., 2016; Macpherson & Hikida, 2019; Stathis et al., 2007). In fact, the GPi makes both glutamatergic and GABAergic downstream connections with the lateral habenula (LHb; Zahm & Root, 2017), a structure that plays a role in establishing negative values to rewards (Friedman et al., 2011; Proulx et al., 2015). LHb dysfunction has been linked to mood disorders, such as major depression (Hu et al., 2020). The LHb has also been widely implicated in contributing to negative emotions associated with reward loss (Donaire et al., 2019). Thus, activation of the NAc-GPi pathway done in Experiment 2 may have exacerbated activity in this downstream connection to enhance consummatory suppression. Future experiments investigating different components into this circuitry are needed to test this hypothesis.

Conclusions

Ultimately, these experiments aimed to further expand the literature on the neural circuit of reward loss adding new insight into how specific pathways out of the NAc differentially affect the response to a sucrose downshift. This work is a departure from many existing studies in that it takes a wider circuit approach rather than only investigating single

brain areas. Moreover, data from these experiments inform future directions and suggest novel neural mechanisms of frustration. On a grander scale, this work has significance in a variety of fields involving both mental and physical health. Frustrative nonreward can influence mental disorders either directly or as a contributing factor, and its systematic study is consistent with NIMH's RDoC initiative. A vivid illustration of the connection between frustration and mental health is provided by the consequences of the COVID-19 outbreak: loss of family members, social distancing, lockdowns, limited access to health care, and loss of jobs. These consequences have contributed to increased stress, anxiety, and uncertainty (Brooks et al., 2020). URDs have also been shown to specifically increase the risk of anxiety disorders, depression, and substance abuse (Hobson et al., 2001; Huston et al., 2013; Papini et al., 2015). Pre-clinical research into identifying potential neurologic targets and brain areas has been a useful tool for testing new treatments for individuals suffering from both physical and mental ailments. Thus, continued research into the overall mechanism behind frustration, as performed in the present experiments, can have vast implications for the treatment and management of a variety of mental health and other stress-related disorders.

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EDUCATION

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SELECTED PUBLICATIONS

Hagen, C., Ogallar, P., & Papini, M. R. (2023) Open field activity is linked to, but is not affected by, the rate of recovery from reward downshift in female Wistar rats *Behavioural Processes, 213,* 104966

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Guarino, S., **Hagen, C.,** Nguyen, Q., & Papini, M. (2023) Frustrative nonreward and the basal ganglia: Chemogenetic inhibition and excitation of the nucleus accumbens and globus pallidus externus during reward downshift. *Neurobiology of Learning & Memory, 200*, 107736

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Brice, K., **Hagen, C**., Peterman, J., Figg, J., Braden, P., Chumley, M., & Boehm, G. (2020) Chronic sleep restriction increases soluble hippocampal AB42 and impairs cognitive performance *Physiology & Behavior, 226,* 113-128

SELECTED PRESENTATIONS

Hagen, C., Ogallar, P., Papini, M.R., (November 2023) Frustrative nonreward: Role of the accumbens-to-globus pallidus pathway in reward downshift. Society for Neuroscience Annual Meeting, Washington D.C.

Hagen, C. (September, 2022) Reevaluating the Role of the Hippocampus in Frustrative Nonreward. International Conference of the Spanish Society for Comparative Psychology, Almeria, Spain

ABSTRACT

FRUSTRATIVE NONREWARD AND THE BASAL GANGLIA:
ROLE OF OUTPUTS FROM THE NUCLEUS ACCUMBENS IN REWARD LOSS

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Mammals in general experience bouts of negative emotion when they unexpectedly experience a reduction in expected reward, known as unexpected reward downshifts (URDs). The specific biological and psychological mechanisms which have evolved to respond to downshifted rewards with behaviors related to anxiety, conflict, and even pain, are known collectively as *frustration*. The present set of experiments examined three neural pathways in and their role in the frustration response to URDs. Using a double-infection chemogenetic manipulation procedure, neurons originating in the nucelus accumbens (NAc) and synapsing onto the globus pallidus externus (GPe), globus pallidus internus (GPe), or ventral pallidum (VP), were activated during key moments in a reward loss paradigm. Animals were trained with 32% sucrose and downshifted to 2% sucrose. It was found that exciting the pathway between the NAc and GPe had no effect on their response to being downshifted, whereas activating either the pathway between the NAc to GPi or the pathway from the NAc to VP caused a significant increase in consummatory suppression and exacerbation of the frustration response. All these effects were in the absence of any gross motor effects shown in an open field. Overall, these findings provide new insights into how animals process the emotional value of rewards when their expectations are violated.