EFFECTS OF NEURAL MANIPULATION OF THE NUCLEUS ACCUMBENS IN REWARD LOSS

by

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ABSTRACT

Research has shown that the nucleus accumbens (NAc) released less dopamine following unexpected reward loss. The current study further examined the function of the NAc in the initial and recovery processes of reward loss using the Reward Downshift (RD) task, a behavioral paradigm known to induce frustration in rats. Temporary neural manipulation of the NAc was hypothesized to leave the consummatory suppression accompanying RD postshift Stage 1 intact. However, during postshift Stage 2, NAc excitation and inhibition were predicted to facilitate and suppress recovery, respectively. To produce transient neural manipulation of the NAc, the study utilized a chemogenetic technique known as Designer Receptor Exclusively Activated by Designer Drugs (DREADDs), which involved an intracranial infusion of a viral construct to deliver the DREADD and an intraperitoneal injection of Clozapine N-Oxide (CNO), the activator drug for DREADD. DREADD activation prior to exposure to 32-to-2% sucrose downshift showed that NAc inhibition had no effects on consummatory behavior during both postshift stages. Similarly, NAc excitation did not disrupt the consummatory suppression typical of postshift Stage 1. However, during postshift Stage 2, NAc excitation produced a significantly lower level of sucrose consumption compared to controls. Our study was the first to explore NAc function using DREADDs in the RD situation, contributing to the current literature on the role of NAc in reward devaluation and recovery.

Effects of Neural Manipulation of the Nucleus Accumbens in Reward Loss

Reward loss is defined as a negative discrepancy between one's expectation of a reward and what one receives (Papini et al., 2015). One major instance of reward loss examined in the current study was reward devaluation, a situation in which the incentive value (in quality or quantity) of an appetitive outcome is lower than anticipated from environmental cues (Papini et al., 2015). When significant, reward loss could elicit a state of emotional distress, referred to as psychological pain (Papini et al., 2015) or frustration (Amsel, 1992). Indeed, a large amount of research has linked reward loss to a higher risk of emotional disorders, such as depression and anxiety (Huston et al., 2013). One specific study on a national sample of 3400 people revealed that events involving financial and professional loss made up six of the top ten most common sources of stress (Hobson & Delunas, 2001). Nevertheless, clinical evidence can only point to the correlation between reward loss and stress. Evidence in support of a causal relationship between the two factors would require experimental manipulations, which has stimulated the formation of animal models of reward loss. Moreover, animal models also allow for the study of the neural circuitry underlying reward loss, a clinically relevant research area given the emotional distress often associated with loss-related events. One reliable model of reward loss in rats is Reward Downshift (RD).

RD, spanning a total of 14 days, consists of two phases, preshift and postshift. During preshift, all animals receive access to a highly preferred reward, typically a 32% sucrose solution, for 10 consecutive daily sessions. On the four postshift sessions, however, the reward is downshifted to a lower value, such as a 2% or 4% sucrose solution. The postshift phase involves two distinct stages according to the typical response pattern of the animals. During postshift Stage 1, particularly Session 11, the animals detect the change in reward value and display

suppression of consummatory behavior. From Sessions 12 through 14, or postshift Stage 2, their consumption gradually returns to baseline, indicating recovery from the reward downshift. It is important to note that RD is a modification of the consummatory successive negative contrast (cSNC) model (Flaherty, 1996), which included a group of unshifted controls receiving the low-value sucrose solution throughout 14 sessions. However, as the postshift response of downshifted animals compared to that of the unshifted ones has been well established by previous research, our study was conducted using the RD model to reduce the number of animals assigned to the experiment while still allowing the exploration of reward devaluation and its neural underpinnings.

Behavioral models of reward loss, such as RD and cSNC, combined with neural manipulation techniques, have allowed for neurobiological research to assess the function of brain regions in the hypothesized neural circuitry underlying reward loss (Figure 1). The circuitry is composed of two levels, with Level 1 illustrating the taste-licking modal action pattern and Level 2 the telencephalic modulatory circuit. Specifically, while the former portrays the neural pathways underlying the animals' detection and consumption of sucrose, the latter presents the hypothesized telencephalic circuits that modulate such consummatory behavior. Level 2 further encompasses two circuits, reward comparison and negative emotion. A key area in the reward comparison circuitry is the basolateral amygdala (BLA). Specifically, Kawasaki et al. (2017) revealed that lesions to the basolateral amygdala (BLA) eliminated the cSNC and the anticipatory negative contrast (ANC) effects, both of which involve reward comparison, while leaving the open field task, a measure of negative emotion, intact. Such findings suggest a role for the BLA in reward comparison, but not in the negative emotions resulting from reward devaluation. However, BLA projections to the nucleus accumbens (NAc) might drive the

emotional response typical of RD. Indeed, the BLA-NAc pathway has been implicated in the encoding of positive valence, with research showing the activation of BLA efferents to the NAc in response to a cue for sucrose (Beyeler et al., 2016). This suggests a role of the NAc in mediating positive signals within the RD situation, specifically during the recovery phase.

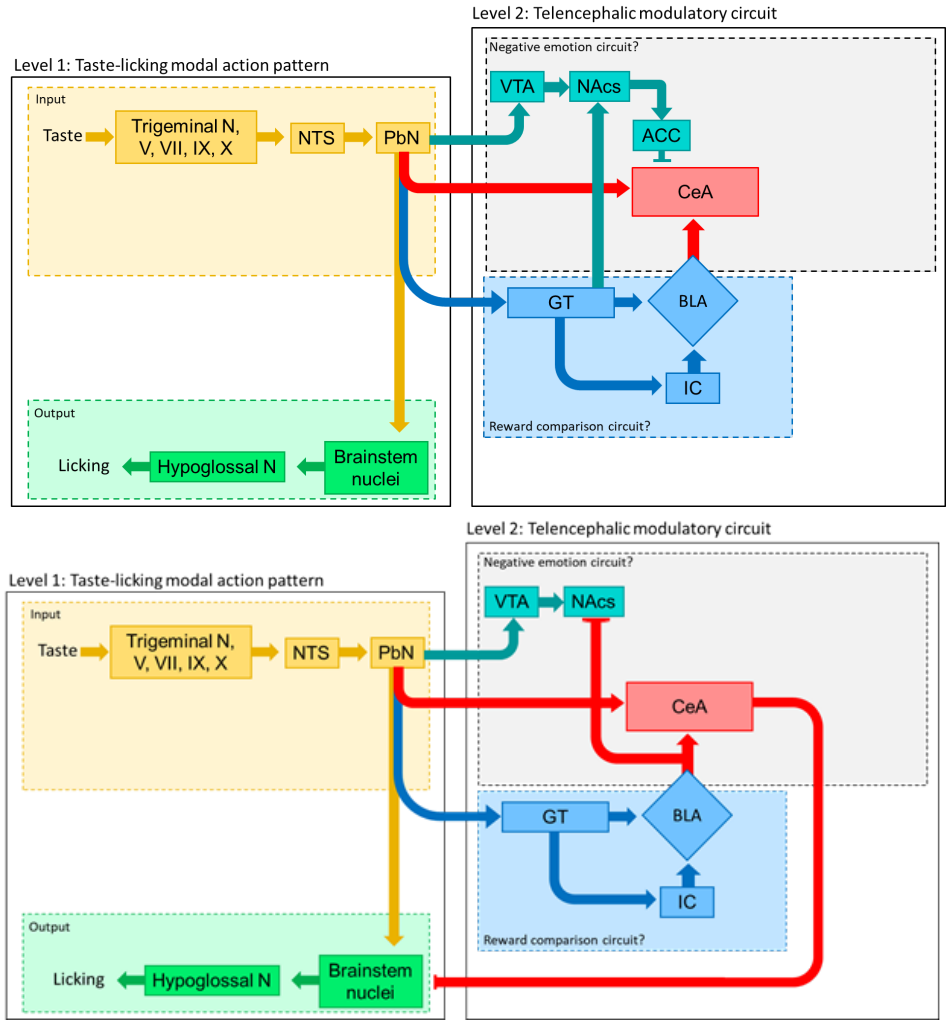


Figure 1. Hypothesized neural circuitry underlying postshift Stage 1 (top) and postshift Stage 2 (bottom). Figure from Ortega et al. (2017) and adapted here for the research.

Nucleus Accumbens

The NAc, a subcortical region in the basal forebrain, represents an important area of the reward system. Within the context of RD, however, research has revealed mixed findings regarding the role of the NAc. According to Leszczuk and Flaherty (2000), electrolytic lesions of the NAc exerted no effects on rats' sensitivity to reward devaluation or their recovery from it. Similarly, rats with NAc ibotenic acid lesions did not exhibit any differences in their response to a downshift in sucrose concentration compared to sham controls (Balleine & Killcross, 1994). Consistent with these findings, Eagle et al. (1999) found that lesion of the ventral striatum, which included the NAc, did not modify rats' sucrose consumption in a within-session successive negative and positive contrast paradigm.

On the other hand, research evidence also points to an involvement of the NAc in reward loss. Specifically, in a study by Judice-Daher and Bueno (2013), water-deprived rats were trained to press a lever for water according to a fixed-interval reinforcement schedule for 10 consecutive sessions. During the ensuing test phase, in which the animals did not receive reinforcement in 50% of the trials following a correct response, rats with lesioned NAc exhibited significantly more behavioral suppression compared to shams, suggesting a role of the NAc in the response to the omission of expected reward. Biesdorf et al. (2015) presented similar findings. In this study, rats were trained to receive food reward in association with cue-lights. In the subsequent extinction phase, during which the expected reward did not follow the appearance of cue-lights, the animals displayed a significant decline in dopamine (DA) release from the NAc. Likewise, NAc neurons also secreted less DA in rats exposed to the 32-to-4% sucrose downshift relative to 4-to-4% controls during postshift Stage 1 (Genn et al., 2004).

The Current Study

In light of the equivocal pattern of findings within the current literature, the present study aimed to further investigate the role of the NAc in reward devaluation through both inhibition and excitation of the area. Specifically, we have successfully manipulated different neural areas using a chemogenetic technique known as designer receptors exclusively activated by designer drugs (DREADDs), which entails the intracranial infusions of a viral vector construct carrying a designer receptor. DREADDs are later activated by the intraperitoneal injection of clozapine N-oxide (CNO), the activator drug for DREADDs (Roth, 2016). Particularly, the DREADD approach could be employed to either inhibit (e.g., Guarino et al., 2020) or excite (e.g., Arico et al., 2017; Nation et al., 2016; Sweeney & Yang, 2015) neural activity. The current study aimed to validate the findings of previous studies involving temporary inhibition and permanent lesions of the NAc in the context of reward loss using inhibitory DREADDs, and extend the current literature and shed more light on the function of this neural structure using excitatory DREADDs. Moreover, since most research studies have focused on the emotional response to reward devaluation in postshift Stage 1, as demonstrated above, a second aim of the current study was to explore the role of the NAc in the recovery from reward devaluation during postshift Stage 2.

In view of our hypothesized model regarding the BLA-NAc pathway and previous findings on its specialization in encoding positive valence (Beyeler et al., 2016), we predicted that the inhibition and excitation of the NAc would not exert any effects on RD during postshift Stage 1. However, during postshift Stage 2, NAc excitation would reduce behavioral suppression and NAc inhibition would enhance behavioral suppression.

Method

Subjects

The subjects included 48 experimentally naïve male Wistar rats, all of which were individually housed in wire-bottom cages equipped with rodent enrichment retreat and free access to water. All the cages were kept in a colony room with constant temperature (22 – 23^{o}C), humidity (45-65%), and a set light schedule of 12 h light /12 h dark, with the lights on at 7:00 h. The animals had readily available water from their cages throughout life. They were free-fed standard laboratory chow until roughly 90 days of age, at which point, in preparation for surgery, they were gradually food restricted to 90% of their free-food weights, estimated as the mean weight from two consecutive days.

Surgical Procedure and DREADDs Infusion

To induce anesthesia, the animals were placed in a chamber filled with a mixture of breathing air and isoflurane vapor, 5% for induction and 1-2% for maintenance. When their breathing grew deep and slow, 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 the 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, and protective layers were 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 (2007) 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: +1.70; medial/lateral, ML: 1.00; dorsal/ventral, DV: -7.60). The Hamilton syringe was left 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 were housed individually in polycarbonate cages, where they were kept at a 90% food-restriction level with 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.

Design

Based on their neural manipulation condition, animals were assigned to three major groups, excitation (EXC), inhibition (INH), and control (CON). More specifically, the EXC group received a bilateral infusion of excitatory DREADD, whereas the INH group received a bilateral infusion of inhibitory DREADD into the NAc. The control group received a bilateral infusion of an adeno-associated control virus, which did not contain 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). In total, we included six groups of animals in our study – EXC/CNO, EXC/Veh, INH/CNO, INH/Veh, CON/CNO, and CON/Veh.

Post-surgery Food Deprivation Procedure

In preparation for behavioral testing, animals were further food-restricted, receiving a regulated daily amount of rat chow to maintain their weight at 81-84% of their average free-food weight for the entirety of the experimental phases. During the period of behavioral testing, animals were fed at about the same time every day, at least thirty min after behavioral testing. To allow time for DREADD expression, behavioral training began 15 days following viral infusion, which was also when the rats' weights were within the target range.

Behavioral Testing Apparatus

The animals were trained in the RD task in eight operant chambers (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 RD 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, where three holes measuring 1 cm wide, 2 cm long, and 4 cm from the floor, were 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 RD 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 in an adjacent room controlled the 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.

Behavioral Testing Procedures

Behavioral testing started 15 days after surgery and when all animals were within 81-84% of their free-food weight. 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 throughout the four sessions.

Preparation of the sucrose solutions (32% and 2%) involved mixing 68 g (98 g) of deionized water for every 32 g (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 RD sessions.

CNO Preparation and Injection Procedure

CNO preparation involved dissolving CNO (NIDA Drug Supply Program) in 5% dimethyl sulfoxide (DMSO) and 95% sterile saline. Injection delivery occurred thirty minutes prior to behavioral testing in a room different from those where the tests were performed.

Histology and Virus Localization

Rats were transcardially perfused and the brains were immediately extracted and embedded in 4% paraformaldehyde for at least 3 days. Brains were then embedded in 30% sucrose for at least 2 days. Once fixed, brains were sectioned in 40 μm sections using a cryostat. Sections were placed onto slides, Fluoromount-G mounting medium was applied to preserve the

fluorescent tag mCherry and be able to locate the virus at a later time, and cover slips were applied on the slides. 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 maximum 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.

Statistical Analyses

Data analysis was performed using IBM SPSS (Version 26) mixed-model analyses of variance (ANOVA) with an alpha value set at 0.05 level.

Results

Preshift Behavior

A one-way between-subject analysis of variance (ANOVA) examined the effects of the 10 preshift sessions on the animals' average number of licks. The results were not significant, $F(5, 33) = .71$, $p = .621$, $\eta^2_p = .10$ (see Figure 2 for descriptive statistics of all sessions). Overall, these results suggest that the consummatory behavior of the rats did not differ prior to the downshifted sessions.

Postshift Behavior

Stage 1

NAc Inhibition. A one-way between-subject analysis of variance (ANOVA) found no significant effect of NAc inhibition on sucrose consumption on Session 11, $F(3, 15) = 1.34$, $p =$

.299, $\eta^2_p = .21$, which corresponded with our prediction that NAc manipulation would not affect the behavioral suppression typical of postshift Stage 1.

NAc Excitation. Analyses presented no overall effect of NAc excitation on Stage 1 behavior, $F(3, 25) = 2.65$, $p = .070$, $\eta^2_p = .24$. Follow-up tests using LSD showed that animals in the EXC/CNO, CON/CNO, and CON/Veh groups were not significantly different in behavior, *p*s ≥ .465. However, EXC/Veh animals displayed significantly higher licking than the CON/CNO and EXC/CNO ones, *p*s ≤ .034.

Stage 2

For postshift Stage 2, we examined the effects of NAc inhibition and excitation individually on Sessions 12, 13, and 14.

NAc Inhibition. We found no effects of NAc inhibition on consummatory behavior on Session 12 ($F(3, 15) = .28$, $p = .838$, $\eta^2_p = .05$), Session 13 ($F(3, 15) = .50$, $p = .691$, $\eta^2_p = .09$), or Session 14 ($F(3, 15) = .39$, $p = .760$, $\eta^2_p = .07$).

NAc Excitation. There was a significant effect of NAc excitation on Session 12, $F(3, 25) = 4.56$, $p = .011$, $\eta^2_p = .35$. Follow-up tests using LSD revealed no differences between the control groups CON/CNO, CON/Veh, and EXC/Veh groups, *p*s ≥ .409. However, animals in the EXC/CNO group consumed significantly less sucrose than the EXC/Veh and CON/CNO ones, *p*s ≤ .048, and marginally less than CON/Veh animals, $p = .052$.

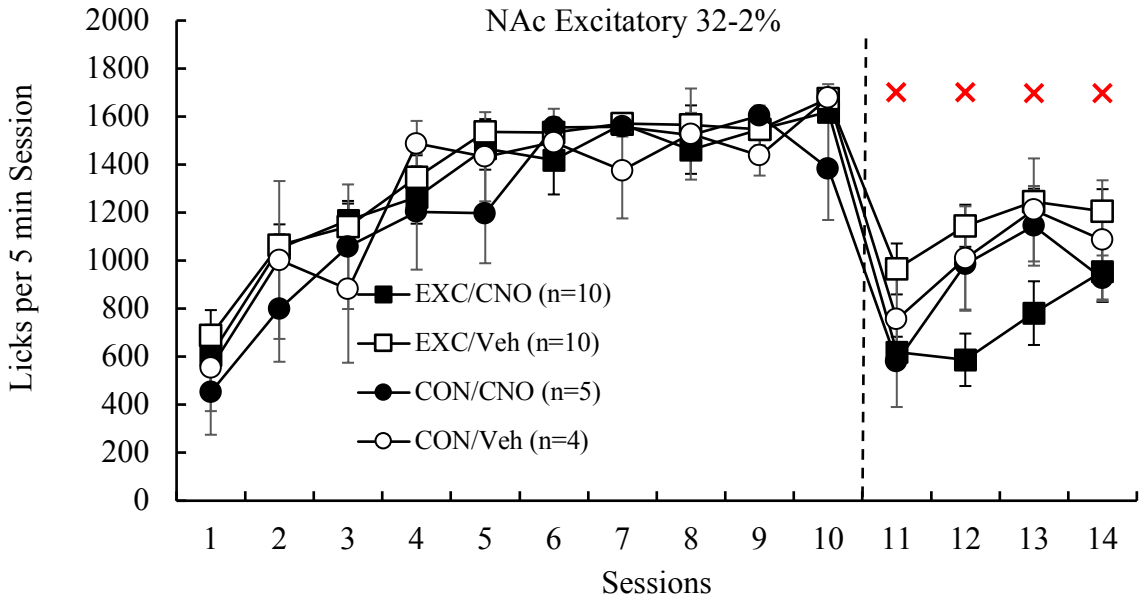
NAc excitation also exerted significant effects on sucrose consumption on Session 13, $F(3, 25) = 3.54$, $p = .029$, $\eta^2_p = .30$. Specifically, follow-up tests using LSD showed that animals in the EXC/CNO condition exhibited significantly lower consummatory behavior than those in the CON/Veh and EXC/Veh groups, *p*s ≤ .044, and marginally lower than CON/CNO animals,

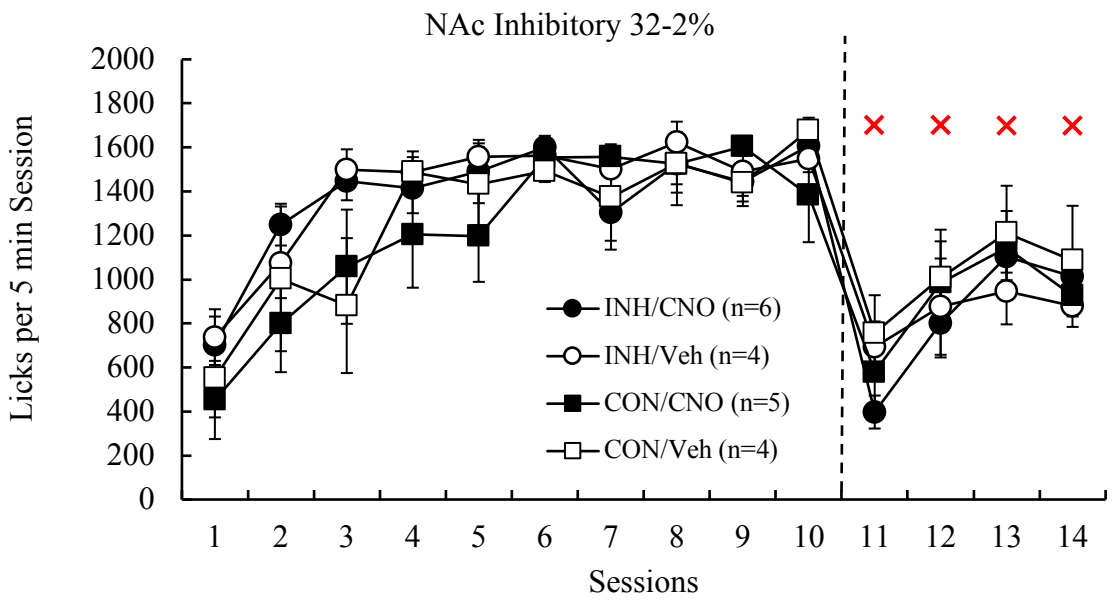
p = .064. However, animals in the CON/CNO, CON/Veh, and EXC/Veh groups did not differ in their behavioral response, ps ≥ .595. There were no differences between the groups on Session 14, $F(3, 25) = 1.09$, $p = .370$, $\eta^2_p = .12$.

Overall, statistical analyses revealed that NAc inhibition did not interfere with recovery behavior, countering our prediction that it would further suppress consumption and thus decelerating recovery rate. Meanwhile, NAc excitation enhanced behavioral suppression during this stage, particularly on Sessions 12 and 13, which was the opposite of our prediction.

Figure 2

Sucrose Consumption as a Function of Neural Manipulation





Discussion

As a significant source of emotional distress in daily life, reward loss has increasingly become clinically relevant, stimulating a large amount of research on its neurobiological mechanisms. Collectively, these studies have been contributing to a hypothesized neural circuitry underlying reward loss, which includes the taste-licking modal action pattern and the telencephalic modulatory circuit (Figure 1). However, research on the role of the NAc during postshift Stage 1 remains inconclusive, with some studies showing no effects of NAc neural manipulation on reward devaluation (Leszczuk & Flaherty, 2000; Balleine & Killcross, 1994; Eagle et al., 1999), while others point to reduced activity of the area in response to reward omission (Judice-Daher & Bueno, 2013; Biesdorf et al., 2015; Genn et al., 2004). Therefore, one aim of the current study was to address these mixed findings using both excitatory and inhibitory DREADDs. Additionally, in light of the scant attention to the NAc in the recovery from reward devaluation, we also explored the role of this area during postshift Stage 2.

Results revealed that chemogenetic inhibition of the NAc exerted no effects on RD consummatory behavior during both postshift Stages 1 and 2, a behavioral pattern inconsistent with previous findings demonstrating diminished NAc activity induced by reward devaluation (Judice-Daher & Bueno, 2013; Biesdorf et al., 2015; Genn et al., 2004). Nonetheless, our findings aligned with research showing no effects of permanent NAc lesions on rats' response to and recovery from incentive devaluation (Leszczuk & Flaherty, 2000; Balleine & Killcross, 1994; Eagle et al., 1999), therefore lending support to this segment of research.

With regards to NAc excitation, we also found no effects during postshift Stage 1, which corroborated our prediction and provided further evidence for a lack of NAc involvement in the behavioral suppression characteristic of RD. Interestingly, however, analyses showed that NAc excitation enhanced the suppression of consummatory behavior during postshift Stage 2, rather than facilitating the recovery process as we had predicted. Specifically, on Sessions 12 and 13, animals with activated excitatory DREADDs consumed significantly less sucrose compared to the three control groups. One possible explanation for our findings was that the excitatory DREADDs might have activated GABA-ergic neurons within the NAc, which have been implicated in mediating aversive signals. Indeed, stimulation of NAc GABA-ergic afferents has been shown to produce avoidance behavior in a real-time place preference task (Lee et al., 2014), while injection of muscimol, a $GABA_A$ receptor agonist, in the caudal shell of the NAc evoked fearful defensive behavior and aversive reactions to sucrose (Reynolds & Berridge, 2002). Therefore, in the context of our study, chemogenetic excitation of NAc GABA-ergic cells might have elicited aversion and enhanced behavioral suppression in the animals.

Nonetheless, we were not able to verify the sub-populations of neurons within the NAc that were activated due to a constraint of the DREADD technique, which only allowed the

targeting of a particular area, rather than a specific group of neurons. Therefore, one future direction is to employ a pathway-specific variation of the DREADD approach that will enable the manipulation of NAc-projecting BLA neurons specifically, which will also facilitate the study of BLA-NAc communication in the context of RD. Another limitation of the study was a small sample size, with each group consisting of 10 or fewer animals. Accordingly, we will be adding animals to each condition in future experiments. However, we do not expect changes in the pattern of results, except the elimination of the differences between the sucrose consumption of EXC/Veh animals and that of the CON/CNO and EXC/CNO ones in postshift Stage 1. Despite these limitations, this was the first study to our knowledge that explored the NAc using excitatory DREADDs during both stages of RD, thereby contributing to the current literature on the role of NAc in behavioral suppression and especially in recovery, which has received little attention.

To sum up, we found no effects of NAc inhibition or excitation during Stage 1 of RD. During Stage 2, while NAc inhibition did not disrupt consummatory behavior, transient excitation of the NAc enhanced behavioral suppression, decelerating the recovery rate.

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