EXAMINING THE ROLE OF VENTRAL TEGMENTAL AREA - NUCLEUS ACCUMBENS CIRCUIT IN MEDIATING COCAINE SEEKING BEHAVIORS AFTER MEMORY DEVALUATION

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ABSTRACT

Cocaine addiction remains one of the leading health concerns in the United States. Unlike other substances, the development of therapeutic targets for disrupting cocaine seeking behaviors has been limited and ineffective. In this series of studies, I first describe the development of a novel approach that can attenuate cocaine seeking behaviors by devaluing the memory of cocaine reward. Subsequently, I provide evidence of the brain circuitry that underlies memory devaluation of cocaine reward. To examine memory devaluation of cocaine reward, rats received the pairing of cocaine infusions and a light/sound cue during the self-administration training. Subsequently, they underwent cocaine-paired cue exposure in a different context, which would retrieve memories of cocaine reward. Immediately after cue exposure, rats received either saline or lithium chloride (LiCl) injection, inducing temporary gastric illness, in the absence of cocaine. We found that the pairing of memory retrieval of cocaine with LiCl was sufficient to attenuate cocaine seeking behaviors in extinction session the following day, compared to saline pairing control group, which suggested that the memory of cocaine was successfully devalued by LiCl in this approach. Implications of these results indicated that memory devaluation approach could be an effective way to study addiction and disrupt drug seeking behaviors. In addition, we investigated the fundamental role of ventral tegmental area (VTA), containing dopaminergic (DA), GABAergic, and glutamatergic neurons, to nucleus accumbens (NAc) circuit in memory devaluation. The circuit was inhibited by employing a bilateral viral construct with retrograde-cre within NAc and inhibitory hM4Di within VTA. The results indicated that selective inhibition of Gi signaling in designer receptors within VTA-NAc neurons prevented memory devaluation and the reduction in cocaine-seeking. This finding demonstrates that intact VTA-NAc circuit is necessary for disrupting drug seeking dependent manner.

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INTRODUCTION

Cocaine Addiction

Cocaine is one of the most used psychostimulants worldwide, and contributes to significant medical and societal costs across the world (Cénat et al., 2021). According to the National Institute on Drug Abuse, the cocaine overdose death rate in 2020 increased by more than four times the rate in 2010 (National Institute on Drug Abuse, 2022). In the United States, around 4.8 million people aged 12 years or older reported cocaine use, and around 1.4 million people developed cocaine abuse disorder (Miech et al., 2023; SAMHSA, 2022). Cocaine addiction leads to tremendous adverse health consequences, including neurologic impairment, toxicity and psychiatric dysfunction (Cregler, 1989). In order to restrict the prevalence of drugs and the fatal effects of drugs on society, the U.S. government has spent nearly a trillion dollars on drug control over the past 50 years, and the budget request for drug prevention and treatment alone has grown rapidly to as high as $670 million just in 2021 (White House, 2021). Unlike opioids, which can be treated by an opioid antagonist that blocks opioid effects and prevents intoxication, one of the major problems of cocaine is the lack of useful treatment and medications (Johansson et al., 2006; Kampman, 2019). Therefore, there is an urgent need to develop a new approach to study cocaine addiction and disrupt cocaine-seeking behaviors.

Current Models to Disrupt Cocaine Seeking Behaviors

The rat self-administration (SA) paradigm is a well-established model for mimicking self-administered behaviors in a controlled laboratory setting (Panlilio & Goldberg, 2007). Researchers have taken advantage of the SA model to develop techniques attempting to attenuate drug seeking behaviors (Panlilio & Goldberg, 2007; Mueller et al., 2021). In Miles, Everitt, and Dickinson's early work on the oral ingested cocaine devaluation approach (2003), rats were

trained to respond to levers to consume either cocaine-sucrose solution or lemon-sucrose solution. Both groups were later paired with lithium chloride (LiCl) injection, inducing a temporary gastric illness. The results revealed that, unlike the lemon-sucrose control group, the group exposed to the cocaine-sucrose solution was insensitive to the devaluation, suggesting that rats more readily displayed habit-like behavior that was rigid and difficult to attenuate. It is worth noting that additional caveats with this approach is that, due to the unpleasant flavor of cocaine, direct oral consumption of cocaine (without adulteration with sucrose) was not feasible.

Besides oral cocaine consumption, Zapata and colleagues (2010) used a drug seeking/taking chained schedule approach to disrupt drug seeking behaviors. In this study, the 'drug-seeking' lever is initially provided. After the rat responded on this lever, it would be retracted, and the 'drug-taking' lever was made available, leading to cocaine infusions after each response. Early on in training, the extinction of the drug-taking lever was found to disrupt drug-seeking responses. However, with extended training, rats quickly displayed habit-like cocaine seeking as they continued to respond to the ‘drug-taking’ lever.

In addition, applying an electric shock to the rat after responding for drug infusion has also been used; however, studies have shown the resistance to this approach when the stimulus is paired with the foot shock devaluation model (Vanderschuren & Everitt, 2004). For example, in Deroche-Gamonet and colleagues’ work (2004), rats continued to respond to the nose poke despite presentations of a conditioned stimulus (CS) that predicted the delivery of electric shocks. Other work also suggested a mixture of results by looking at lever presses followed by an electric shock (Holtz et al., 2013).

Cues Paired with Drugs Influence Drug Seeking Behaviors

In addition to the capacity of cocaine to promote compulsive drug seeking-behaviors, cocaine also increases the value of environmental cues that are paired with cocaine. These cues can be described within a context of classical conditioning (Pavlov, 1927), in which a neutral environmental CS, and a motivational reinforcer (unconditioned stimuli, US) alters the value of the CS, such that it becomes capable of motivating drug-seeking behaviors. For instance, the incentive sensitization model of addiction suggests that due to sensitization of DA activities produced by drugs, such as cocaine, drug-paired stimuli (CSs) become more attractive and capable of directing motivational (drug) behaviors (Robinson & Berridge, 1993).

However, CSs paired with motivational consequences, do more than acquire values, they can also contain detailed information about the events that they predict (Holland, 1981). For example, when a CS, such as the shape of pizza boxes, is frequently presented with a US, such as pizza, to people, the paired association can enable the cue (CS) to promote people's food consumption by retrieving a detailed memory of the associations (e.g., the delicious taste of pizza) (Reppucci & Petrovich, 2012; Johnson, 2013). Interestingly, at least with natural rewards like foods, paired cues can evoke the memory of the experience from a reward, which can then substitute for the reward itself and form a new association with the reward. Previous work has used this concept to devalue the memory of a reward that was retrieved by cue exposure to subsequently disrupt reward seeking behaviors. For example, in Holland's earlier studies on mediating learning of food reward (1981, 1998), a group of rats were initially exposed to food that was paired with the presentation of a sound cue (CS). After rats learned the association, they were exposed to the CS without the presence of food for a short period of time. At this time, the CS exposure retrieved a detailed memory of the food, and rats later experienced temporary

gastric discomfort by injections with LiCl. The following day, rats underwent lever response testing to examine whether they had lost their desire to seek out and consume the food reward. The results showed that food consumption was significantly suppressed in rats despite the fact that the food itself had never been directly paired with gastric malaise. To account for these findings, it was suggested that the value of the memory associated with the food reward, as retrieved by the CS, became reduced—this was described as mediated devaluation (Holland, 1981). As of now, it is unknown whether the same approach could be adapted to disrupt cocaine-seeking behaviors.

The Mesolimbic Dopamine Circuit in Drug Addiction

Although cocaine addiction is likely attributed to multiple factors, such as environment and social context, understanding the neurobiological mechanism of cue-reward relationships is always fundamental for developing novel treatments and medications (Robinson et al., 2016). Researchers have investigated brain regions that underlie addiction for decades, and one of the most important regions in the limbic system is the NAc, which receives major DA inputs from the VTA via mesolimbic circuitry (Di Chiara, & Imperato, 1988; Robinson et al., 2016).

In the VTA, 50–70% of neurons express DA, with the remaining 30–35% of γ-amino butyric acid (GABA) neurons and ~5 % of glutamate neurons (Di Chiara, & Imperato, 1988; Cai & Tong., 2022). Previous work indicates that VTA GABA neurons have a strong impact on neuronal signaling through local inhibition, which mediates motivating and addicted behaviors (Van Bockstaele & Pickel, 1995). For example, chemogenetic activation of VTA GABA neurons significantly decreased reward seeking behaviors (Wakabayashi et al., 2021). In addition, DA neurons also have a clear role in motivation and reward processing, including natural stimuli, such as food and sex (Kelly & Berridge., 2002), and drug addiction (Di Chiara, & Imperato,

1988). DA neurons exerts its actions via D1 type (D1R) and D2 type (D2R) DA receptors, which are G protein–coupled receptors (GPCRs) that cause intracellular signaling cascades and lead to different downstream responses. The activity of these receptors is centered on coupling to either Gαs/olf or Gαi/o to respectively activate (i.e., D1R) or suppress (i.e., D2R) the synthesis of cAMP and stimulation of downstream signaling cascades (Pierce et al., 2002). Due to their distinct contribution on neuronal activities, blocking VTA D1R neurons attenuates rewarding, such as cocaine seeking behaviors (Ranaldi & Wise, 2001), while decreasing VTA D2R shows notably increased motivation for both natural stimuli and drugs after reinforcement (de Jong et al., 2015).

As described above, VTA DA neurons send robust projections to the NAc. In the NAc, only around 5%–10% of cells are GABAergic interneurons, and the major type of cells are GABAergic medium-size spiny neurons (MSNs), which comprise about 90-95% of striatal neurons and belong to two distinguishable populations of cells based on their expression of dynorphin, substance P and D1Rs, compared to those expressing enkephalin, adenosine A2a receptors, and D2Rs (Matamales et al., 2009). As previously indicated, DA receptors (D1R and D2R) elicit either stimulation or inhibition downstream effects. Due to their distinct physiological properties, previous work has suggested opposite roles of D1R and D2R MSNs in addiction: MSNs expressing D1R promotes drug-seeking behavior while those expressing D2R relates to aversion; however, more recent work with optogenetics has indicated that inhibiting MSNs expressing D2R also involves promoting drug reward (Matamales et al., 2009; Kravitz et al., 2012; Cole et al., 2018). Nevertheless, through this mesolimbic circuit, DA inputs into the NAc are believed to be responsible for integrating motivation and learning behaviors of drug addiction (Schultz et al., 1997; Saddoris et al., 2015).

Using Mediated Devaluation to Disrupt Cocaine-seeking Behaviors Through Mesolimbic Circuitry

In the current project, we adapted the mediated devaluation approach and extended it to determine whether it could disrupt cocaine seeking behaviors. Rats were trained in lever pressing for intravenous cocaine infusions and both light and auditory cues. After all pairing sessions, rats were exposed to cues alone in a new context to retrieve the memory of cocaine without its presence. Rats were then injected with either saline (controls) or LiCl (memory devaluation) immediately after cue exposure, and their drug seeking behaviors were tested on the following day.

In addition, we examined whether any hypothesized reductions in cocaine-seeking behavior required intact VTA-NAc circuit. Accordingly, to study and manipulate the neuronal activity in NAc projecting from VTA, we employed Designer Receptor Exclusively Activated by Designer Drug (DREADD) techniques. Specifically, we used mutated human muscarinic receptors hM4Di, which is a specially engineered and modified cholinergic receptor, muscarinic 4. When binding with clozapine-N-oxide (CNO), hM4Di leads to pre-synaptic inhibition and silencing (Armbruster et al., 2007). In addition, by taking advantages of adeno-associated virus (AAV) vectors, which can transport the virus retrogradely back to neuronal soma from axon terminals, we injected retrograde AAV-Cre into NAc to target neurons that projected into NAc from other brain regions (Surdyka & Figiel, 2021). To selectively mark neurons that are projected from VTA, we use Cre-dependent hM4Di that only binds with cells that express Cre-recombinase, and allowed us to have restricted expression of VTA-NAc selective cells. We inhibited the VTA-NAc circuit by using this dual viral approach, and investigated its role in

mediated devaluation to cocaine. We expect that disrupted drug seeking behavior under this paradigm will be prevented because of the inactive VTA-NAc circuit.

METHODS

Subjects and Animal Housing

42 male Sprague Dawley rats (weight 250-300g) were used as subjects in the current experiment. All rats were pair housed in the facility upon arrival, and were housed under humidity and temperature control with a 12 hour reversed light cycle. Rats were fed *ad libitum* until 3 days post jugular catheterization, after which food pellet was restricted to 20g per day to stabilize their body weight. Water was always available *ad libitum*. The body weight and movement were checked daily for health monitoring. All procedures were approved by the Michigan State University Institutional Animal Care and Use Committee and were conducted in accordance with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Viral Construct

The animals had 7 days of habitation and handling after they arrived at the facility. The rats later underwent stereotax surgeries for viral infusions. Adult rats were induced with 4% isoflurane in oxygen and mounted into a stereotax station, and maintained 1.5-2% isoflurane during surgery. Breathing, toe-spreading reflex, body temperature, and skin color were carefully monitored throughout the surgery. The hair is removed from surgical sites and the scalp is sanitized with iodine and 70% ethanol. An incision was cut towards the midline of the scalp to expose the skull. Following the incision, injectable meloxicam (1mg/ml/kg) was given subcutaneously as an analgesic medication. The subjects were bilaterally injected with (Gi) DREADD (pAAV-hSyn-DIO-hM4D(Gi)-mCherry) (hM4Di) and mCherry (pAAV-hSyn-DIO-mCherry) in VTA and pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40 (AAV Retrograde) in NAc via the Hamilton syringe in each hemisphere (0.25ul per infusion). The needle was left in the

location for 2-3 minutes for incubation, then slowly withdrawn. For viral microinjection, the coordinates are targeted relatively to bregma as follows: VTA: AP -5.4 mm, ML ±0.7 mm, DV -7.5/-8.5 mm; and AP -6.2 mm, ML ±0.7 mm, DV -7.5/-8.5 mm. NAc: AP 2.2 mm, ML ± 1.6 mm, DV -7 mm; AP 1.8 mm, ML ±1.2 mm, DV -7.5 mm; and AP 1.8 mm, ML ±0.75 mm, DV -7.5 mm. The scalp was carefully stapled after infusion. Following stereotax surgery, all rats were single housed for the rest of the experiment. The first CNO intraperitoneal (IP) injection was administered after at least 25 days of viral incubation, during which time recovery from stereotax surgery, jugular catheterization surgery, and self-administration training occurred.

Catheterization

After 5-7 days of recovery from stereotax surgery, intrajugular catheters were implanted. The rats were anesthetized with a mixture of ketamine and xylazine cocktail based on their body weight (1ml/kg). The surgical area was shaved and disinfected with hibiclens. All catheters were tested for leaking and blockage by 70 unit heparin (70U; 7.0 ml 1,000 unit heparin dissolved in 93 ml sterile saline) before and during surgery, as well as after placement. The catheter was passed through the skin of the shoulder and inserted into an opening in the right jugular vein about 3.5 cm above the heart and secured by suturing knots. All incisions were carefully sutured after catheter placement and treated with vetbond. Following jugular catheterization, 0.05ml oral meloxicam and gentamycin spray were given daily for three days to prevent pain and infection. 10U heparin (0.1 ml) and anticoagulant cefazolin (0.1 ml) were used to flush catheters daily to disinfect and prevent bacterial infection until the rats finished self-administering cocaine via catheters. To test the patency of catheters, 0.1 ml propofol was injected into the cannula after a week or two weeks of the surgery. Rats were carefully monitored during propofol testing because temporary immobility would be initiated immediately after injection. We purposefully injected

propofol a few hours before the behavior session, so the immobility won't affect their lever responding to cocaine.

Drugs

CNO was dissolved in 10% (2-Hydroxypropyl)-β-cyclodextrin in 0.2M Phosphate-buffered saline (PBS), then heated in the water bath at 45°C. After the solution was fully dissolved, the CNO stock solution was aliquoted into 62.5µl. CNO working solution was prepared fresh daily from aliquots at a concentration of 7.5 mg/ml by adding 437.5µl 0.2M PBS. Each CNO injection was administered into the intraperitoneal cavity in a volume of 0.3mg/ml/kg.

Cocaine hydrochloride was dissolved in sterile 0.9% saline at 6.0 mg/ml, and subsequently diluted into desired mg/kg/infusion for operant self-administration training based on rats' body weight (3.0 mg/ml for 275-325g; 3.5 mg/ml for 325-375g). For cocaine-primed cue-induced reinstatement we initially injected rats with 10mg/ml/kg cocaine, but we observed a ceiling effect during behavior testing. The dose of cocaine was decreased to 5 mg/ml was injected intraperitoneally in a volume of 1 ml/kg during reinstatement testing.

0.6M LiCl was dissolved in deionized water and was injected intraperitoneally in a volume of 1 ml/kg in this experiment for memory devaluation manipulation.

Self-Administration, Memory Devaluation, and Extinction

Self-administration (SA) The basic behavioral protocol used in these experiments is shown in *Figure 1*. All training and testing sessions were conducted during the dark phase of the 12-hour light/dark cycle at approximately the same time each day, typically within a one-hour window. All rats underwent operant testing in operant chambers (29.5 × 24 × 28 cm; Med Associates Inc., St. Albans, NY), which were equipped with two retractable levers, and each rat

was always exposed to the same chamber. To facilitate the acquisition of cocaine SA, 5 days after catheterization surgery, rats were placed into context A, which was characterized by constant housing light during session (0.4 fc brightness), pine tree order, and a layer of wire mesh (26cm x 27 cm) at the bottom of the chamber. In context A, Rats were trained to press the active lever for cocaine infusions on a fixed ratio (FR1) schedule during initial 120 minutes of training, and was conducted daily until they mastered the training (more than 10 infusions/daily within each 2 hour session for 10 days). Pressing the active lever would deliver 0.05 ml cocaine intravenously through the catheter, with a maximum of 70 infusions to prevent accidental overdose. Each cocaine infusion was accompanied by a concurrent tone (80 dB, 1kHz, 2s on/off) and a light cue (1.2fc brightness, 2s on/off), followed by a 20 s timeout period, while pressing the inactive lever resulted in no programmed consequences. Additionally, rats received mock injections with saline (1.5ml) 30 minutes before the last two SA sessions to eliminate the potential effect from needle poke.

Memory devaluation Following 10 days of SA training, rats underwent memory devaluation session. All rats were injected with 0.3mg/ml/kg CNO 30 minutes prior to the session, and they were placed into the context B for the mediated devaluation test, which was differentiated by no housing light, placing a slanted tile plexiglass, bar flooring (19cm x 27 cm), and vanilla roma order, on day 11. Rats were habituated in the new context for 6 minutes in the dark, and later received the same light-tone cue that was paired with cocaine infusions 10 times in the next 6 minutes at the same time interval, but no cocaine was presented in this session. After 12 minutes of cue exposure, rats received either saline or LiCl IP injection immediately and returned to the housing room. Water and food was also removed for 3 hours to eliminate the potential association between food, water and gastric illness.

Extinction 24 hours after memory devaluation testing, rats underwent an extinction session. During this test, lever presses resulted in neither light/tone cues nor cocaine delivery. This procedure was repeated daily for a minimum of eight 2-hour extinction sessions. The process continued until the criterion was met, defined as fewer than 25 active lever presses for the last two consecutive sessions.

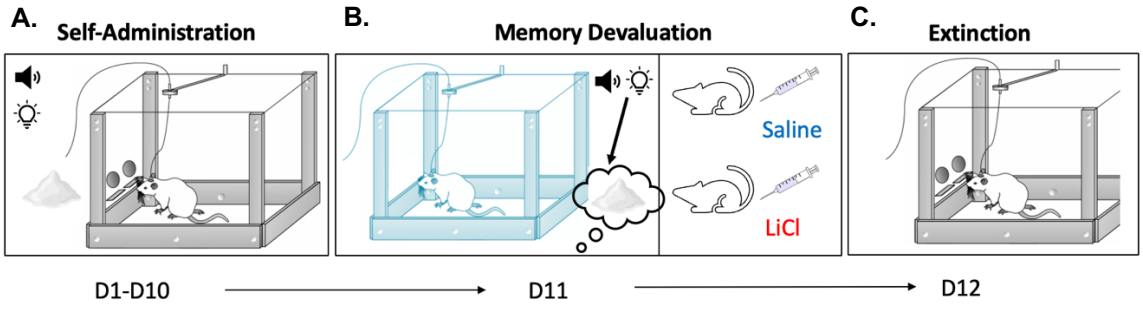

***Figure 1*: Behavioral testing timeline.** A) Following surgery and recovery, hM4D(Gi)-DREADD rats and mCherry controls were trained to self-administer cocaine infusions with a tone/light cue by pressing the active lever. B) All rats were placed into a different context for light-tone cue exposure. Rats later received either saline or LiCl IP injection. 3) After memory devaluation testing, rats underwent at least 8 extinction sessions, when both active and inactive lever pressing resulted in no consequences.

Reinstatement

Following extinction training, rats underwent a series of reinstatement tests, and one rat was dropped due to an inability to pass the extinction criteria (< 25 lever press within the last two consecutive sessions). In cue-induced reinstatement test, rats (n=41) were placed into the same context A, and active lever presses delivered both light and sound cues, but no cocaine was provided, followed by a 20 second timeout period. Then, all rats underwent at least 2 sessions of re-extinction (< 25 presses in 2 consecutive sessions). After meeting the extinction criteria, rats

(subject = 37; four rats were excluded due to an inability to pass the extinction criteria) completed a cocaine-primed cue-induced induced reinstatements. They were IP injected with cocaine solution (5mg/ml/kg) before the session. In the cocaine-primed cue-induced reinstatement session, pressing the active lever resulted in both sound and light cues, but no cocaine infusion was delivered. After both reinstatement sessions, rats were returned to their housing room and fed *ad libitum*.

Tissue Fixation, Immunohistochemistry, Fluorescence Microscopy, and Cell Quantification

Rats were sedated with sodium pentobarbital (6 mg/kg), and perfused intracardially with 4% paraformaldehyde (PFA). After the removal of the dorsal skull cap, the brain was extracted from the skull and subsequently fixed in 4% PFA and 12% sucrose overnight at 4C refrigerators. Brains were later frozen and stored at -80°C. Brains were sectioned in the coronal plane at 30 μm by using a Spencer Lens Co. AO 860 microtome, and immediately stored in the antifreeze solution, consisting of 30% ethylene glycol, 30% glycerol, 40% 0.05 M Phosphate Buffer (PB), at −20°C.

To label tyrosine hydroxylase (TH)-positive neurons, we performed immunohistochemistry (IHC). Region of interest was washed in 0.01M phosphate-buffered saline (PBS) for 6x8 minutes at room temperature (RT). Brain sections were blocked with blocking solution (3% normal donkey serum in PBS containing 0.2% Triton X-100) for 1 hour at RT. Sections were then transferred and incubated in the primary antibody solution with rabbit anti-TH (1:1000; Invitrogen, #P21962) overnight at RT. On the following day, sections were then washed three times in 0.01M PBS for 10 minutes each. Then, sections were washed with 0.1M PBS 6x8 minutes, and incubated in secondary antibody solution with AlexaFluor donkey-anti-rabbit-488 (1:200; Invitrogen, #A21206) for 2 hours at RT. Sections were washed 2x8 minutes

with PBS and mounted onto glass slides and coverslipped with ProLong Antifade Mounting Media (Invitrogen) and DAPI (4′,6-diamidino-2-phenylindole). Finally, the slides were cured and stored in the dark at −20°C.

Images were acquired using Olympus BX51 epifluorescence microscopy. For verifying successful mCherry viral targeting, NAc and VTA slices were systematically selected and mounted on slices, and was carefully checked per depth per animal. Subjects (n=10) and data were excluded from the experiment because no mCherry viral expression was shown. For quantifying the colocalization of TH+ expression with mCherry, images of VTA were acquired by using 4x and 10x objectives with 0.13 and 0.3 N.A respectively. For the presentation of viral and TH expression within the VTA, we conducted a 4x6 tile scan using the Leica Stellaris 5 laser confocal microscope with a 20x objective featuring a 0.75 N.A. (*Figure 2B*). Two channels were scanned separately using the following wavelengths: 488nm for green (TH-GFP), and 561 nm for red (mCherry) label. In order to characterize the extent of the mCherry expression within the VTA, we carefully examined brain slices across the rostral-caudal axis of the VTA from rostral VTA (bregma -4.68, -4.8, -5.0), mid VTA (bregma -5.2, -5.4), caudal VTA (bregma -5.6, -5.8) (Yamaguchi et al., 2007). The number of neurons with TH+ and hM4Di-mCherry colocalization in VTA was quantified bilaterally within each slice. A total of 9 rats from hM4Di groups were used for colocalization quantification. Some slices were missing or damaged during slicing and staining, so we replaced the number with group means. For cell quantification of the colocalization between TH+ expression and mCherry targeting, all subjects were assigned to a team and each slice of individual subject was manually quantified by raters. We used 90% of accuracy for all variance across raters and each subject.

Statistical Analysis

All statistical analyses were conducted by Microsoft Excel and SPSS. For TH+ and hM4Di-mCherry quantification analysis, the mean and standard error of the mean (S.E.M.) of each bregma depth and each animal was calculated, and a two-way ANOVA with repeated measure was used with a between group comparison of condition and a repeated measure with bregma depth for VTA presentation (bregma -4.68 to -5.8, interval 0.2). The number of active, inactive lever presses, and cocaine infusions were recorded for each session, as well as the time elapsing between each lever response, regardless of the infusion time out. For behavioral data analysis, first, we ran a three-way ANOVA with repeated measures for self-administration active lever presses, and followed by post-hoc Bonferroni comparisons when statistical interaction significance was reported. The two factors taken into consideration were 1) virus (mCherry vs. hM4Di), and 2) upcoming condition (Saline vs. LiCl). We examined all 10 SA sessions. At this time, some rats displayed outlier performance in the SA sessions, so we replaced it with the group means. We calculated the outliers by using the interquartile range, and upper and lower fence for all data. The same analysis was employed for inactive lever presses and cocaine infusions. Following mediated devaluation to cocaine, data of both active and inactive lever responses across each 20 min bin for the first extinction session was examined. This measure was used to determine whether mediated devaluation would subsequently disrupt cocaine-seeking, and if so whether this effect required intact VTA-NAc circuit. A three-way ANOVA with between subject variables of virus (mCherry, hM4Di) and mediated devaluation condition (saline, LiCl), and within-subject variable of time bin (1-6) during the cocaine-seeking test was used. Significant interactions were followed-up by separate two-way virus X time and condition X time ANOVAs to examine differences between viral groups and conditions. For

reinstatements, we first examined whether both cue-induced and cocaine-primed cue-induced reinstatement could elicit cocaine seeking with a three-way ANOVA analysis (phase (previous extinction session vs. reinstatement) X condition X virus). In addition, a three-way condition X virus X time repeated measure ANOVA was used to analyze both active and inactive lever responding during both reinstatement sessions. An alpha level on $p<0.05$ was employed for all the analyses.

RESULTS

Quantification of Colocalized TH+ and hM4Di-mCherry Expression

After excluding rats with no viral expression due to the deficient quality of the virus (n=10), data from a total of 32 rats were collected for further data analysis. The overall colocalization rate of TH+ and mCherry viral expression across nine subjects with IHC staining was approximately 57% (*Figure 2*). Among a total count of 4,351 cells expressing mCherry, 2,403 cells also exhibited TH+ expression. This composition of dopaminergic neurons is consistent with findings from previous research (Di Chiara & Imperato, 1988). We did not observe significant interaction between depth and condition ($F(6,48) = 0.5$; $p=0.8$). However, we found a significant differences in colocalization rate across depth ($F(6,48) = 16.974$; $p<0.0001$), and a subsequent pairwise comparison between each bregma depth was conducted. We found a significantly lower colocalization expression within the rostral VTA (bregma -4.68, M= 28.05% ± 2.2%; bregma -4.8, M=41% ± 4.5%; bregma -5.0, M=50.18% ± 6%;) compared to mid VTA and caudal VTA. The colocalization expression increased as the depth moved to mid VTA (bregma -5.2, M=68.74% ± 2.3%; bregma -5.4, M=81.2% ± 4.48%), and slightly dropped again within caudal VTA (bregma -5.6, M=71.17% ± 4.8%; bregma -5.8, M=63% ± 7.3%). Noticeably, we found a significant higher colocalization rate in mid VTA, bregma -5.4 (*Figure 2*), compared to bregma -4.8 to -5.2 ($p<0.05$).

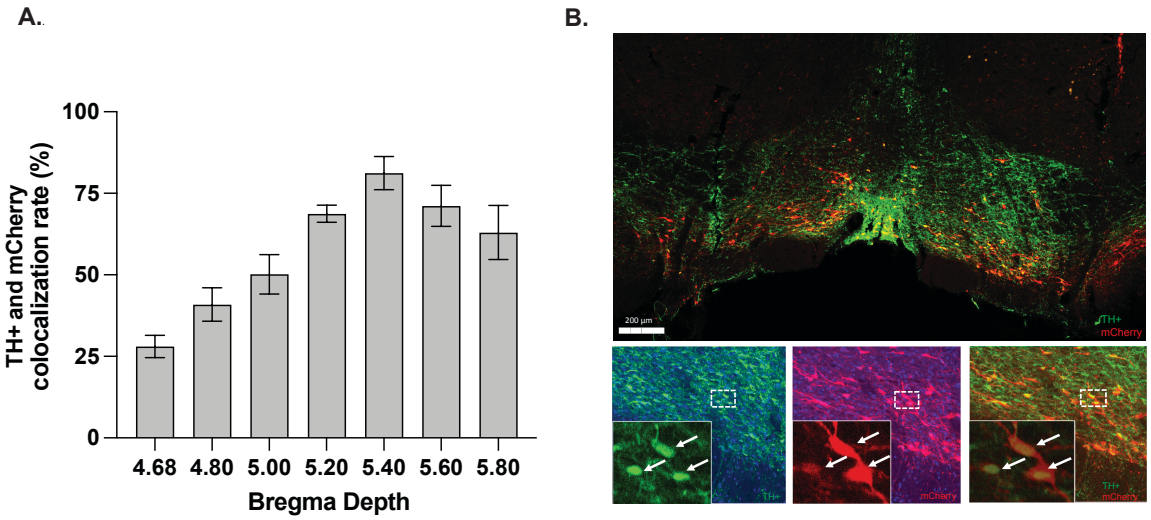

Figure 2: **TH+ and mCherry colocalization quantification.** A) Quantification of cells that express both TH+ and mCherry expression within the VTA (bregma -4.68, -4.8, -5.0, -5.2, -5.4, -5.6, -5.8). B) 20x confocal images of VTA showing the neuronal density of TH+ (green) and hM4Di-mCherry (red) expression. The scale bar is 200 µm.

Self-administration

All rats similarly acquired self-administration, irrespective of viral condition (mCherry vs. hM4Di) or upcoming treatment during memory devaluation (saline vs. LiCl) (*Figure 3*). The three-way ANOVA revealed no effect of virus, condition or interaction among any of the variables (F's<0.8; p's>0.38). However, a main effect of session was revealed (*Figure 3A*; $F(9, 252) = 23.95$; $p < 0.0001$), and post-hoc Bonferroni comparisons indicated significant increase in active lever responding from session 1 and session 2 relative to all other sessions (p's < 0.001), which suggested that rats showed increased active lever responses to cocaine over SA sessions. In addition, we observed a main effect of session on inactive lever responding, indicating that rats gradually responded less to the inactive lever (*Figure 3B*; $F(9, 252) = 3.59$, $p < 0.001$). This low level of inactive lever responding remained consistent throughout training, with no effects

observed for block or condition (*Figure 3B*; $F < 2.9$; $p > 0.09$). The subsequent post hoc comparisons revealed a significant increase in cocaine infusions from session 1 and session 2 compared to all other sessions (p's <0.03), besides session 6 (p's > 0.1). The sessions 7 to 10 remained stable, with no significant differences found between them (p's >0.18). Similar to active lever responding, the three way ANOVA also revealed a significant increase across sessions (*Figure 3C*; $F(9, 252) = 47.773$; $p < 0.0001$) with no main effects of virus, condition or interaction among any of the variables (F's<0.8; p's>0.39). The post-hoc comparisons indicated a significant increase in cocaine infusions from the first half SA training sessions (sessions 1-5) relative to all remaining sessions (p's < 0.03), and no significant increase was observed during sessions 6-10 (p's > 0.1). Finally, to confirm that self-administration was comparable before mediated devaluation, we examined average active lever responding (F's <1.5; p's >0.2), inactive lever responding (F's<2.9, p's>0.09), and cocaine infusions (F's<1.79, p's>0.2) across the final 3 self-administration sessions, which revealed comparable responding to cocaine in all rats. These findings suggested that prior to mediated devaluation, all rats from both viral conditions did not display differences in lever responses to cocaine (*Figure 3*).

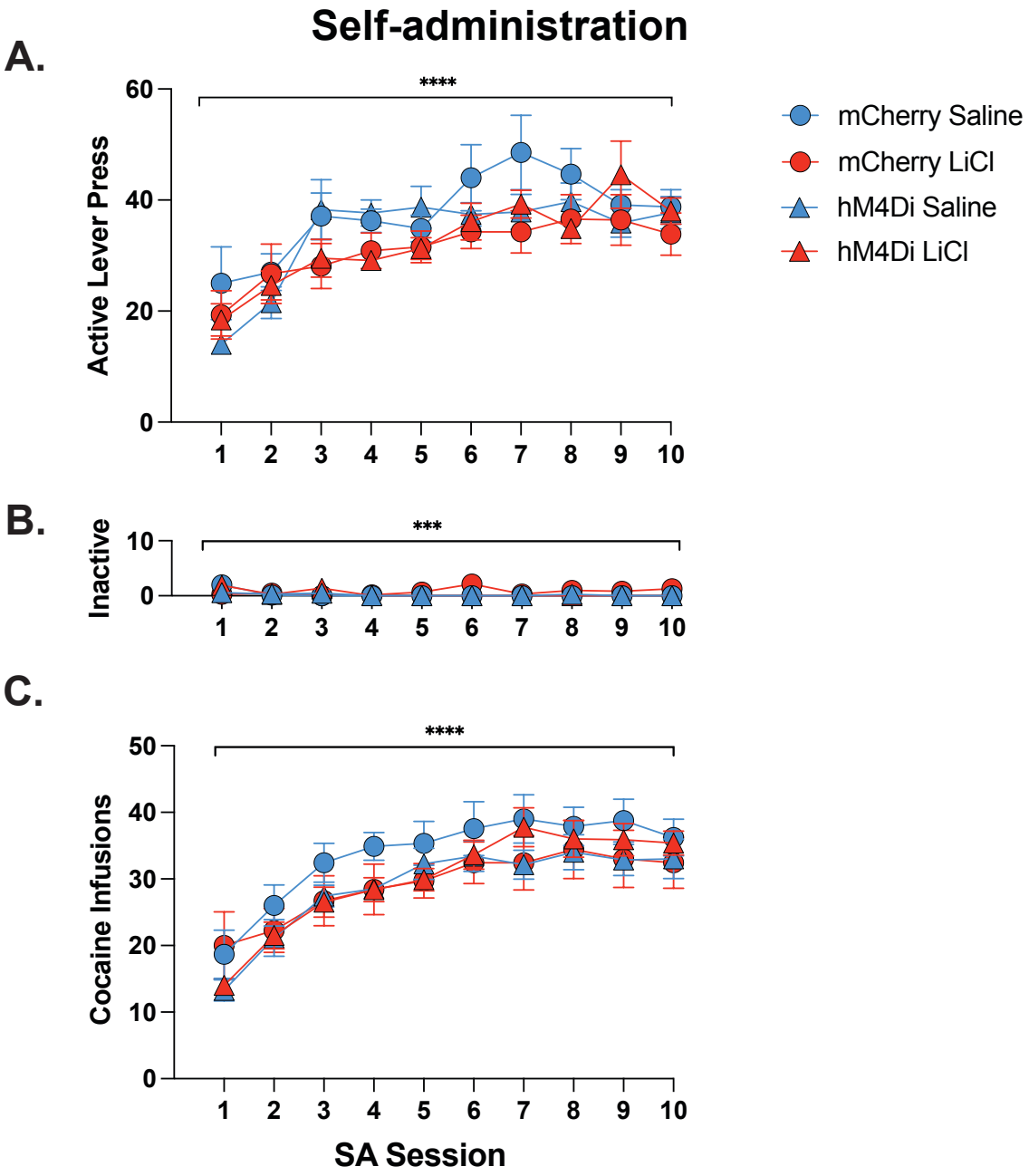

Figure 3*: Lever responding and cocaine infusions (mean ± S.E.M.) during cocaine SA.** All animals received both light and sound cues throughout cocaine SA. Rats showed a significant increase in active lever responding of cocaine. * indicates significant main effect of session in active lever responding and cocaine infusions, $p<0.0001$. *** indicates significant main effect of session in inactive lever, $p<0.001$.

Extinction

The day following mediated devaluation to cocaine, the pattern of lever responding during the first extinction test differed drastically as a function of viral group and memory devaluation condition (*Figure 4*). The three-way ANOVA revealed a main effect of condition ($F(1,28) = 9.037$, $p < 0.01$), time ($F(5, 140) = 53.09$, $p < 0.0001$), but no effect of virus ($F < 1.4$; $p > 0.2$). Importantly, a significant three-way interaction was revealed (*Figure 4B*; $F(5, 140) = 2.37$, $p < 0.05$). In order to examine the nature of this interaction, we conducted separate two-way ANOVAs for each viral group. In mCherry rats, ANOVA revealed a main effect of condition ($F(1,14) = 13.05$, $p < 0.01$), time ($F(5, 70) = 29.18$, $p <0.001$), and a tendency for an interaction between the two variables ($F(5, 70) = 2.00$, $p = 0.08$). These differences reflected a suppression of active lever responding in mCherry LiCl rats compared to mCherry saline at 40, 60 and 80 mins (*Figure 4B*; F's > 5.18; p's <0.05) and a tendency for suppression during the first 20 mins ($F(1,14) = 4.15$; $p = 0.06$). On the contrary, hM4Di groups revealed a main effect of time ($F(5,70)= 24.485$; $p<0.001$), but no main effect of condition (*Figure 4A*; $F(1,14) = 1.182$, $p=0.295$), or time X condition interaction (*Figure 4B*; $F(5,70) = 1.057$; $p > 0.3$), which suggested that inhibiting VTA-NAc circuit prevented mediated devaluation to cocaine and the subsequent reduction on cocaine-seeking behavior. Additionally, when we examined responding between viral groups, for rats that received saline, ANOVA revealed a main effect of time only ($F(5,70)= 25.833$; $p<0.001$), suggesting that silencing VTA-NAc circuit alone did not disrupt cocaine seeking behaviors ($F(1,14) = 0.002$, $p=0.966$). However, for rats in the LiCl condition, we found an effect of time ($F(5,70)= 28.126$; $p<0.001$) and a tendency for an effect of virus ($F(1,14) =3.922$, $p=0.068$) consistent with an attenuation of mediated devaluation of cocaine reward following VTA-NAc inactivation, which prevented the subsequent reduction in cocaine-

seeking in hM4Di rats. In addition, the three way ANOVA revealed a significant main effect of session in inactive lever responding ($F(5,140) = 118.44$; $p<0.0001$), but no main effect of virus or condition was observed (F's < 0.9; p's > 0.3). The post hoc comparisons showed a significant decrease in inactive lever from session 1 compared to all remaining sessions (p's<0.001). In conclusion, the study demonstrated that mediated devaluation approach resulted in decreased drug seeking behaviors, and inhibiting the VTA-NAc circuit prevented mediated devaluation to cocaine and subsequent reduction in cocaine-seeking behavior.

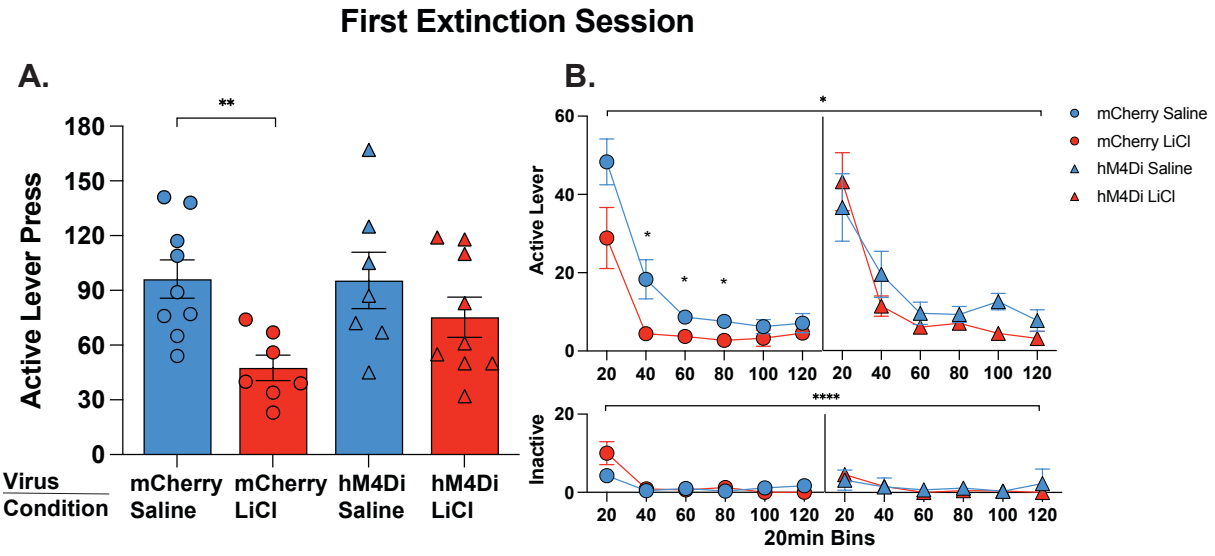

Figure 4*: Data analysis of active lever presses from the first extinction session**. A) lever responding (mean ± S.E.M.) in the first extinction session across all groups. ** indicates a significant main effect of condition in mCherry groups, $p<0.01$. B) Plots of average active and inactive lever presses in the first extinction session for four groups within 20 minute bins. * above all fours groups indicates a significant time X condition X virus interaction in active lever press, $p<0.05$. * indicates a significant time X condition interaction between mCherry groups, $p<0.05$. * indicates a significant time X condition X virus interaction in inactive lever press, $p<0.0001$

Reinstatements

To investigate if the effect of memory devaluation can extend to relapse in drug intake after extinction sessions, data from reinstatement sessions were analyzed. For cue-induced reinstatement analysis, one rat did not meet the extinction criteria prior to cue-induced reinstatement testing, and its data was excluded. A three-way repeated-measures ANOVA was used to examine whether rats reinstated drug intake (*Figure 5A*). The analysis revealed a significant main effect of the phase ($n=31$; $F(1, 28) = 155.56$; $p<0.001$), indicating that rats escalated their cocaine-seeking during cue-induced reinstatement. We also observed a main effect of the virus ($F(1, 28) = 5.96$; $p<0.05$), but no effect of the condition was found ($F(1,28) =0.94$; $p>0.3$), suggesting in general hM4Di groups had more active lever responses during cue-induced reinstatement, compared to mCherry groups. Next, the three-way repeated-measures ANOVA with 20-min bins as repeated measures showed a significant main effect of time (*Figure 5C*; $F(5,140) =55.57$; $p<0.001$), and virus ($F(1, 28) = 6.62$; $p < 0.05$) in active lever responding, but no effect of conditions was observed ($F(1,28)=2.06$; $p>0.1$). In addition, no significant time X virus X condition interaction was found across groups ($F(5,140) = 0.168$; $p>0.9$), suggesting that the effect of memory devaluation could not extend to cue-induced reinstatement.

For cocaine-primed cue-induced reinstatement, four rats were excluded due to an inability to meet extinction criteria. A significantly increased cocaine intake was found during cocaine-primed cue-induced reinstatement compared to the last extinction session ($n=27$; $F(1, 28) = 145.29$; $p<0.001$), which indicated that rats were actively seeking for cocaine (*Figure 5B*). Unlike cue-induced reinstatement, no main effect of virus or conditions was found in cocaine-primed cue-induced reinstatement (F's <0.34; $p>0.5$). A three-way ANOVA with 20-minute time bins as repeated measures was employed. Similar to previous results, no time X condition X

virus interaction was found in cocaine-primed cue-induced reinstatement testing across all groups (*Figure 5D*; F (1,140) = 0.452; p>0.8), which suggested that the effect of mediated devaluation could not extend to cocaine-primed cue-induced reinstatement either.

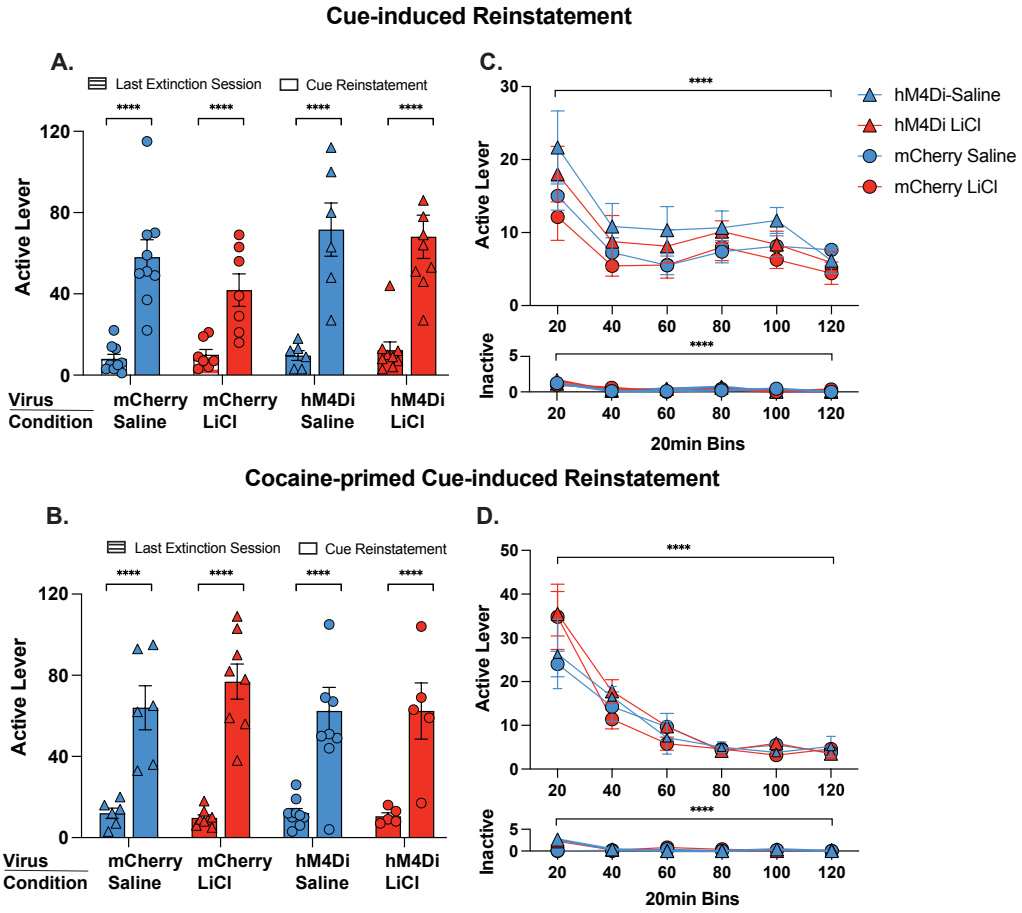

Figure 5*: The number of active and inactive lever presses (mean ± S.E.M.) during reinstatements.** A) and B) showing the active lever responding (mean ± S.E.M.) in the cue-induced reinstatement and cocaine-primed cue-induced reinstatement testing across all groups respectively. The left bar in each group represents the last extinction session and the right bar is proceeding reinstatement testing. * indicates significant main effect of phase, p<0.0001. C) and D) showing plots of average active and inactive lever presses within 20 minute bins in both reinstatement testings across all four groups. **** indicates a significant time X condition X virus interaction between all groups, p<0.0001.

DISCUSSION

In the current project, we examined two questions. Firstly, is it possible to disrupt cocaine-seeking behaviors using mediated devaluation, and secondly, does this require the VTA-NAc circuit. We first trained rats to learn the association between a light-tone cue and cocaine infusions following lever responding. Then, rats were exposed to the cocaine-associated cues in a new context leading to the retrieval of a representation (or memory) of cocaine reward. As such, when rats received LiCl injections immediately after, we examined whether the memory of cocaine reward would be devalued. To test this, rats were placed back into the self-administration context and tested on their active lever presses without the presence of either cues or cocaine. We observed that active lever presses in the LiCl group were significantly lower than the number in the Saline control group. These results indicate that mediated devaluation of cocaine can attenuate cocaine-seeking behaviors during the extinction session and could provide a novel approach for researchers interested in attenuating drug seeking behaviors. Furthermore, I examined the neurobiology of this phenomenon through the use of dual viral infusions to manipulate the VTA-NAc circuit, and found that this circuit was necessary for mediated devaluation to disrupt cocaine-seeking. Specifically, chemogenetic inhibition of neurons projecting from the VTA-NAc blocked the ability of LiCl to devalue the cocaine memory during mediated devaluation. As such, these rats displayed high rates of cocaine-seeking during testing.

Notably, the current study revealed a new approach to effectively attenuate drug seeking behaviors that could benefit the field of addiction. Unlike previous approaches, such as oral consumption devaluation (Miles et al., 2003), electric shock (Vanderschuren & Everitt, 2004), or extinction via a drug seeking/taking chained schedule (Zapata et al., 2010), our mediated devaluation approach had a profound impact in attenuating lever responding to cocaine in a

previous cue-associated environment during the initial extinction session. If we apply Holland's theory (1981; 1998) on mediated aversion to food rewards to the current findings, the devaluation of cocaine reward occurred due to the capacity of cues associated with cocaine reward to retrieve a memory of cocaine. When this was followed by intragastric malaise produced by LiCl, the memory of cocaine reward became devalued, which negatively influenced the value of cocaine and subsequently led to a reduction in cocaine-seeking.

In addition to demonstrating for the first time that mediated devaluation can be an effective tool for reducing cocaine-seeking, the current study also showed that intact signaling of the mesolimbic reward circuit is necessary for this effect. In the VTA, the majority of cells express DA, and the subpopulation of these DA-expressing cells that project to the NAc is known to play a crucial role in the behavioral effects of cocaine (Mahler et al., 2019; Wakabayashi et al., 2021). We found that the majority of cells infected with hM4Di were DA expressing, indicating a critical role for these VTA-NAc cells in mediated devaluation of cocaine reward. Interestingly, the role of DA in mediated devaluation is difficult to reconcile with traditional models of DA function. For instance, when it comes to reinforcement learning, DA is thought to influence, model-free learning, which involves the acquisition of outcomes and their associated values through training, without explicitly constructing a representation of the environment—this is reflected in the reward prediction error theory (Schultz et al., 1997). When the rat receives sound/light cue for the first time, which is paired with cocaine infusions, there is a positive prediction error, as cocaine is unexpected, and DA neurons exhibit a phasic increase in activity (Mirenowicz & Schultz, 1994). However, with additional training the actual outcome becomes predicted by the preceding cues, and the DA activity now transfer to the cues but not the reward itself. The important component about this form of learning is that it reflects changes

in the value of rewards at the time of learning; animals, however, cannot use this information to retrieve memories associated with the drug-paired stimuli. In addition, according to this theory, the absence of cocaine during memory devaluation would diminish DA activity due to a negative prediction error. Conversely, in mediated devaluation, the cues associated with cocaine are believed to trigger a detailed memory of cocaine reward, which is then reshaped through LiCl injections. This requires a more complex computational architecture than that provided by reward prediction error theory (Gardner et al., 2018). Overall, reward prediction error theory lacks the capacity to describe how detailed memories of cocaine reward are retrieved, represented, and updated during cue exposure; therefore, it cannot explain how new learning about the sound/light cue is attributed to the cocaine reward such that future cocaine-seeking is reduced.

In addition, drug-seeking behaviors can be described within a context of the incentive sensitization theory of drug addiction (Berridge & Robinson, 1998). However, it is important to note that this theory does not fully explain the results of the current experiment either. According to the incentive salience theory, responses to a reward can be interpreted as motivation driven by "wanting" behavior, which requires the activation of DA for the "incentive salience" to drug-related cues (Berridge & Robinson, 2016). During SA sessions, rats were repeatedly exposed to the light/sound cue and simultaneous cocaine infusions. The sound/light cue successfully transforms into a "wanted" incentive stimulus, providing motivational inputs to engage in cocaine-seeking behavior, thereby triggering a significant release of midbrain DA to various regions of the brain, including the VTA-NAc circuit. According to incentive salience theory, the sound/light cues should have become associated with gastric malaise during memory devaluation, leading to the devaluation of these cues and subsequently reduce the motivation of

the associated cocaine reward. However, when we examined the ability of sound/light cues to evoke reinstatement behavior, we found no differences during testing irrespective of whether these cues were paired with saline or LiCl during mediated devaluation. This indicates that the cues retained their status as incentive stimuli capable of invigorating motivated responding for drugs.

As mentioned above, approximately 60% of cells within the VTA express DA. DA exerts its biological effects through D1-like and D2-like DA receptors. The activity of these receptors is centered on coupling to either Gαs/olf or Gαi/o to respectively activate (i.e., D1R) or suppress (i.e., D2R) the synthesis of cAMP. Cells in the NAc are composed mainly of MSNs, which belong to two distinguishable populations of cells based on their expression of dynorphin, substance P and D1Rs, compared to those expressing enkephalin, adenosine A2a receptors, and D2Rs. Generally, D1R-expressing MSNs are suggested to encode positive reward value, while D2R-expressing MSNs encode negative reward value (Ranaldi & Wise, 2001; Kravitz et al., 2012). In the context of cocaine-seeking, acute optogenetic stimulation of D1R-expressing MSNs enhances cocaine-seeking, while stimulation of D2R-expressing MSNs suppresses it (Self et al., 1996; Lobo et al., 2010). Since mediated devaluation relies on an aversive component for the relearning of cocaine reward, resulting in a reduction in cocaine-seeking behaviors, it is possible that our effects might be mediated by the actions of D2R-expressing MSNs in the NAc.

Besides DA projections from VTA, a growing body of literature indicated that glutamatergic and GABAergic signaling from the VTA to the NAc also play critical roles in reward learning and addictive behaviors (Van Bockstaele & Pickel, 1995; Wakabayashi et al., 2021). For example, GABAergic signals from VTA mainly regulate local DA neurons or targets on cholinergic interneurons in NAc by sending inhibitory signals, and are correlated with

aversive cue associative learning (Brown et al., 2012; Creed et al., 2014; Qi et al., 2016). However, it is important to note that the disruption of inhibitory GABAergic projections from the VTA to the NAc alone are insufficient to disrupt reward seeking behaviors (van Zessen et al., 2012; Brown et al., 2012). Despite this, the interaction between VTA GABA and DA neurons is crucial in coordinating the activity of aversion-related learning. (Brown et al., 2012; Creed et al., 2014). In addition, although glutamatergic neuronal activities only account for a small portion of VTA-NAc projection, it also has significant excitatory effects on NAc MSNs (Cai & Tong, 2022). It is believed that glutamatergic neurons from the VTA excites NAc local interneurons, which also plays a role in aversion. This is attributed to the GABAergic inhibition on NAc MSN by these local interneurons (Qi et al., 2016). Therefore, since we inhibited the entire VTA-NAc circuit, resulting not only in the inhibition of GABAergic projection to the NAc but also in the inhibition of DA and glutamatergic projection, this considerable inhibition could possibly withdraw aversive learning and make memory devaluation insufficient to disrupt cocaine seeking. Taken together, in the current study, we inhibited diverse neuronal subtypes within the VTA projecting to the NAc. This inhibition has the potential to induce both stimulatory and inhibitory effects on all neurons within the VTA-NAc circuit, and these neurons may contribute significantly to drug-seeking behaviors following mediated devaluation.

To fully understand the specific role of VTA-NAc circuit in memory devaluation approach, selectively targeting different neurons within the VTA-NAc circuit is necessary to study cue-induced drug seeking behaviors and innovative treatments (Want et al., 2018). Although we employed a dual viral infusion technique to successfully target the VTA-NAc circuit, a drawback of our current project lies in systematical targeting all neurons within the circuit, which induces a competition among neuronal activities and can be avoided by selectively

manipulating a specific neuronal subtype. The developments of special Cre recombinase expressed rat lines have allowed selective targeting of DA or GABA neurons with either DREADDs (Mahler et al., 2019) or optogenetic techniques (Witten et al., 2011). Future studies should take advantages of using TH::Cre transgenic rats for DA specific targeting or GAD::Cre transgenic rats for GABA targeting, combined with inhibitory Gi signaling, and explore the potentially distinct roles of these cell types in VTA- NAc mediated memory devaluation. (Witten et al., 2011).

The current study investigates the effect of memory devaluation on cue-induced and cocaine-primed reinstatement. However, it's necessary to consider the environmental context associated with cocaine, as it's also recognized as an important factor contributing to cocaine relapse and craving (Marchant et al., 2015). The current behavioral paradigm cannot entirely rule out the influence of context on lever responding during the first extinction session. Therefore, future study should look into access which particular features of this reward environment (e.g., cocaine itself, cocaine training context) undergo memory devaluation and following extinction testing. Additionally, another potential limitation in mediated devaluation could be attributed to biological factors, specifically sex. We only used male rats in this study, which means the result could not be generalized to females. Many studies have criticized the dominance of male-only subjects in the field of neuroscience (Beery & Zucker, 2011). Previous research has demonstrated that female rats not only self-administer drugs faster than males but also exhibit higher motivation compared to male rats (Becker & Koob, 2016). In addition, human females are more prone to drug addiction in comparison with males due to ovarian hormones (Anker & Carroll, 2011). Therefore, female rats will be a valuable model to study the sex difference in drug

addiction, and provide more insights into the memory devaluation approach for disrupting drug seeking behaviors in females.

Overall, this project provides initial findings to show a new approach to disrupt cocaine-seeking behaviors (i.e., mediated devaluation) in rats, which could benefit the field of neuroscience and addiction. Also, we indicated a new role for VTA-NAc circuitry that was necessary for disrupting cocaine seeking behaviors. Future studies examining the cell specificity (e.g., DA specific) and biological variables will be important to confirm the utility of this approach to treat cocaine addiction and addiction to other drugs.

BIBLIOGRAPHY

Anker, J. J., & Carroll, M. E. (2011). Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones. Current topics in behavioral neurosciences, 8, 73–96. https://doi.org/10.1007/7854_2010_93

Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S., & Roth, B. L. (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proceedings of the National Academy of Sciences of the United States of America, 104(12), 5163–5168. https://doi.org/10.1073/pnas.0700293104

Becker, J. B., & Koob, G. F. (2016). Sex Differences in Animal Models: Focus on Addiction. Pharmacological reviews, 68(2), 242–263. https://doi.org/10.1124/pr.115.011163

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. Neuroscience and biobehavioral reviews, 35(3), 565–572. https://doi.org/10.1016/j.neubiorev.2010.07.002

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?. Brain research. Brain research reviews, 28(3), 309–369. https://doi.org/10.1016/s0165-0173(98)00019-8

Buck, S. A., Torregrossa, M. M., Logan, R. W., & Freyberg, Z. (2021). Roles of dopamine and glutamate co-release in the nucleus accumbens in mediating the actions of drugs of abuse. The FEBS journal, 288(5), 1462–1474. https://doi.org/10.1111/febs.15496

Berridge, K. C., & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. The American psychologist, 71(8), 670–679. https://doi.org/10.1037/amp0000059

Brown, M. T., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Lüscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. Nature, 492(7429), 452–456. https://doi.org/10.1038/nature11657

Cai, J., & Tong, Q. (2022). Anatomy and Function of Ventral Tegmental Area Glutamate Neurons. Frontiers in neural circuits, 16, 867053. https://doi.org/10.3389/fncir.2022.867053

Cénat, J. M., Blais-Rochette, C., Morse, C., Vandette, M. P., Noorishad, P. G., Kogan, C., Ndengeyingoma, A., & Labelle, P. R. (2021). Prevalence and Risk Factors Associated With Attention-Deficit/Hyperactivity Disorder Among US Black Individuals: A

Systematic Review and Meta-analysis. JAMA psychiatry, 78(1), 21–28. https://doi.org/10.1001/jamapsychiatry.2020.2788

Creed, M. C., Ntamati, N. R., & Tan, K. R. (2014). VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems. Frontiers in behavioral neuroscience, 8, 8. https://doi.org/10.3389/fnbeh.2014.00008

Cregler, L. L. (1989). Adverse health consequences of cocaine abuse. Journal of the National Medical Association, 81(1), 27–38.

Cole, S. L., Robinson, M. J. F., & Berridge, K. C. (2018). Optogenetic self-stimulation in the nucleus accumbens: D1 reward versus D2 ambivalence. PloS one, 13(11), e0207694. https://doi.org/10.1371/journal.pone.0207694

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proceedings of the National Academy of Sciences of the United States of America, 85(14), 5274–5278. https://doi.org/10.1073/pnas.85.14.5274

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. Science (New York, N.Y.), 305(5686), 1014–1017. https://doi.org/10.1126/science.1099020

Gardner, M. P. H., Schoenbaum, G., & Gershman, S. J. (2018). Rethinking dopamine as generalized prediction error. Proceedings. Biological sciences, 285(1891), 20181645. https://doi.org/10.1098/rspb.2018.1645

Holtz, N. A., Anker, J. J., Regier, P. S., Claxton, A., & Carroll, M. E. (2013). Cocaine self-administration punished by i.v. histamine in rat models of high and low drug abuse vulnerability: effects of saccharin preference, impulsivity, and sex. Physiology & behavior, 122, 32–38. https://doi.org/10.1016/j.physbeh.2013.08.004

Holland, P. (1981). Acquisition of representation-mediated conditioned food aversions. Learning and Motivation, 12(1), 1-18. https://doi.org/10.1016/0023-9690(81)90022-9

Holland, P. (1998). Amount of training affects associatively-activated event representation. Neuropharmacology, 37(4-5), 461–469. https://doi.org/10.1016/s0028-3908(98)00038-0

Johansson, B. A., Berglund, M., & Lindgren, A. (2006). Efficacy of maintenance treatment with naltrexone for opioid dependence: a meta-analytical review. Addiction (Abingdon, England), 101(4), 491–503. https://doi.org/10.1111/j.1360-0443.2006.01369.x

Johnson, A. W. (2013). Eating beyond metabolic need: how environmental cues influence feeding behavior. Trends in neurosciences, 36(2), 101–109. https://doi.org/10.1016/j.tins.2013.01.002

de Jong, J. W., Roelofs, T. J., Mol, F. M., Hillen, A. E., Meijboom, K. E., Luijendijk, M. C., van der Eerden, H. A., Garner, K. M., Vanderschuren, L. J., & Adan, R. A. (2015). Reducing Ventral Tegmental Dopamine D2 Receptor Expression Selectively Boosts Incentive Motivation. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 40(9), 2085–2095. https://doi.org/10.1038/npp.2015.60

Kampman, K. M. (2019). The treatment of cocaine use disorder. Science advances, 5(10), eaax1532. https://doi.org/10.1126/sciadv.aax1532

Kravitz, A. V., Tye, L. D., & Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nature neuroscience, 15(6), 816–818. https://doi.org/10.1038/nn.3100

Lobo, M. K., Covington, H. E., 3rd, Chaudhury, D., Friedman, A. K., Sun, H., Damez-Werno, D., Dietz, D. M., Zaman, S., Koo, J. W., Kennedy, P. J., Mouzon, E., Mogri, M., Neve, R. L., Deisseroth, K., Han, M. H., & Nestler, E. J. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science (New York, N.Y.), 330(6002), 385–390. https://doi.org/10.1126/science.1188472

Marchant, N. J., Kaganovsky, K., Shaham, Y., & Bossert, J. M. (2015). Role of corticostriatal circuits in context-induced reinstatement of drug seeking. Brain research, 1628(Pt A), 219–232. https://doi.org/10.1016/j.brainres.2014.09.004

Mirenowicz, J., & Schultz, W. (1994). Importance of unpredictability for reward responses in primate dopamine neurons. Journal of neurophysiology, 72(2), 1024–1027. https://doi.org/10.1152/jn.1994.72.2.1024

Mahler, S. V., Brodnik, Z. D., Cox, B. M., Buchta, W. C., Bentzley, B. S., Quintanilla, J., Cope, Z. A., Lin, E. C., Riedy, M. D., Scofield, M. D., Messinger, J., Ruiz, C. M., Riegel, A. C., España, R. A., & Aston-Jones, G. (2019). Chemogenetic Manipulations of Ventral Tegmental Area Dopamine Neurons Reveal Multifaceted Roles in Cocaine Abuse. The Journal of neuroscience : the official journal of the Society for Neuroscience, 39(3), 503–518. https://doi.org/10.1523/JNEUROSCI.0537-18.2018

Matamales, M., Bertran-Gonzalez, J., Salomon, L., Degos, B., Deniau, J. M., Valjent, E., Hervé, D., & Girault, J. A. (2009). Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. PloS one, 4(3), e4770. https://doi.org/10.1371/journal.pone.0004770

Miech, R. A., Johnston, L. D., Patrick, M. E., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2023). Monitoring the Future National Survey Results on Drug Use, 1975–2022: Secondary School Students. Ann Arbor: Institute for Social Research, The University of Michigan.

Miles, F. J., Everitt, B. J., & Dickinson, A. (2003). Oral cocaine seeking by rats: Action or habit? Behavioral Neuroscience, 117(5), 927–938.

Mueller, L. E., Sharpe, M. J., Stalnaker, T. A., Wikenheiser, A. M., & Schoenbaum, G. (2021). Prior Cocaine Use Alters the Normal Evolution of Information Coding in Striatal Ensembles during Value-Guided Decision-Making. The Journal of neuroscience : the official journal of the Society for Neuroscience, 41(2), 342–353. https://doi.org/10.1523/JNEUROSCI.1755-20.2020

Panlilio, L. V., & Goldberg, S. R. (2007). Self-administration of drugs in animals and humans as a model and an investigative tool. Addiction (Abingdon, England), 102(12), 1863–1870. https://doi.org/10.1111/j.1360-0443.2007.02011.x

Pavlov, I. P. (1927). Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex. Oxford Univ. Press.

Pierce, K. L., Premont, R. T., & Lefkowitz, R. J. (2002). Seven-transmembrane receptors. Nature reviews. Molecular cell biology, 3(9), 639–650. https://doi.org/10.1038/nrm908

Qi, J., Zhang, S., Wang, H. L., Barker, D. J., Miranda-Barrientos, J., & Morales, M. (2016). VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. Nature neuroscience, 19(5), 725–733. https://doi.org/10.1038/nn.4281

Ranaldi, R., & Wise, R. A. (2001). Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. The Journal of neuroscience : the official journal of the Society for Neuroscience, 21(15), 5841–5846. https://doi.org/10.1523/JNEUROSCI.21-15-05841.2001

Reppucci, C. J., & Petrovich, G. D. (2012). Learned food-cue stimulates persistent feeding in sated rats. Appetite, 59(2), 437–447. https://doi.org/10.1016/j.appet.2012.06.007

Robinson, A. M., Lacy, R. T., Strickland, J. C., Magee, C. P., & Smith, M. A. (2016). The effects of social contact on cocaine intake under extended-access conditions in male rats. Experimental and clinical psychopharmacology, 24(4), 285–296. https://doi.org/10.1037/pha0000078

Saddoris, M. P., Sugam, J. A., Stuber, G. D., Witten, I. B., Deisseroth, K., & Carelli, R. M. (2015). Mesolimbic dopamine dynamically tracks, and is causally linked to, discrete

aspects of value-based decision making. Biological psychiatry, 77(10), 903–911. https://doi.org/10.1016/j.biopsych.2014.10.024

Self, D. W., Barnhart, W. J., Lehman, D. A., & Nestler, E. J. (1996). Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science (New York, N.Y.), 271(5255), 1586–1589. https://doi.org/10.1126/science.271.5255.1586

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. Science (New York, N.Y.), 275(5306), 1593–1599. https://doi.org/10.1126/science.275.5306.1593

Substance Abuse and Mental Health Services Administration (SAMHSA). (2022). Key Substance Use and Mental Health Indicators in the United States: Results from the 2021 National Survey on Drug Use and Health. https://www.samhsa.gov/data/sites/default/files/reports/rpt39443/2021NSDUHFFRRev010323.pdf

Surdyka, M. M., & Figiel, M. (2021). Retrograde capabilities of adeno-associated virus vectors in the central nervous system. Biotechnologia, 102(4), 473–478. https://doi.org/10.5114/bta.2021.111111

Vanderschuren, L. J., & Everitt, B. J. (2004). Drug seeking becomes compulsive after prolonged cocaine self-administration. Science (New York, N.Y.), 305(5686), 1017–1019. https://doi.org/10.1126/science.1098975

Van Bockstaele, E. J., & Pickel, V. M. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain research, 682(1-2), 215–221. https://doi.org/10.1016/0006-8993(95)00334-m

van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. Neuron, 73(6), 1184–1194. https://doi.org/10.1016/j.neuron.2012.02.016

Wakabayashi, K. T., Feja, M., Leigh, M. P. K., Baindur, A. N., Suarez, M., Meyer, P. J., & Bass, C. E. (2021). Chemogenetic Activation of Mesoaccumbal Gamma-Aminobutyric Acid Projections Selectively Tunes Responses to Predictive Cues When Reward Value Is Abruptly Decreased. Biological psychiatry, 89(4), 366–375. https://doi.org/10.1016/j.biopsych.2020.08.017

Wang, T. R., Moosa, S., Dallapiazza, R. F., Elias, W. J., & Lynch, W. J. (2018). Deep brain stimulation for the treatment of drug addiction. Neurosurgical focus, 45(2), E11. https://doi.org/10.3171/2018.5.FOCUS18163

Witten, I. B., Steinberg, E. E., Lee, S. Y., Davidson, T. J., Zalocusky, K. A., Brodsky, M., Yizhar, O., Cho, S. L., Gong, S., Ramakrishnan, C., Stuber, G. D., Tye, K. M., Janak, P. H., & Deisseroth, K. (2011). Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron, 72(5), 721–733. https://doi.org/10.1016/j.neuron.2011.10.028

Yamaguchi, T., Sheen, W., & Morales, M. (2007). Glutamatergic neurons are present in the rat ventral tegmental area. The European journal of neuroscience, 25(1), 106–118. https://doi.org/10.1111/j.1460-9568.2006.05263.x

Zapata, A., Minney, V. L., & Shippenberg, T. S. (2010). Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. The Journal of neuroscience : the official journal of the Society for Neuroscience, 30(46), 15457–15463. https://doi.org/10.1523/JNEUROSCI.4072-10.2010