Chemogenetic activation of mesoaccumbal Gamma-Aminobutyric Acid projections selectively tunes responses to predictive cues when reward value is unexpectedly decreased

Ken T. Wakabayashi[1], Malte Feja[2]*, Martin P.K. Leigh[2], Ajay N. Baindur[2], Mauricio Suarez[2, 4] Paul J. Meyer[3], and Caroline E. Bass[2]*

*Authors contributed equally

[1]Department of Psychology, University of Nebraska-Lincoln, 1220 T. Street, Lincoln, NE 68588

[2] Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214, USA

[3] Department of Psychology, University at Buffalo, Buffalo, NY 14214, USA

[4]Clinical Research Institute on Addictions, University at Buffalo, State University of New York, Buffalo, NY 14203, USA

Abbr. Title: Mesoaccumbal GABA projection activation enhances reward learning

Text Info: (total 3923 without captions)

Abstract: 244
Introduction: 457
Methods/Data Analysis: 645
Results: 1543
Discussion: 1278
Number of figures: 7
Tables: 0

Corresponding author:

Caroline E. Bass
University at Buffalo, The State University of New York
955 Main St.
Buffalo, NY 14204
Email: cebass@buffalo.edu
Tel: 716-829-3790

Keywords (6): GABA, ventral tegmental area, chemogenetics, reinforcement, rat, prediction error

ABSTRACT

Background: Mesolimbic circuits regulate the attribution of motivational significance to incentive cues that predict reward, yet this network also plays a key role in adapting reward-seeking behavior when the contingencies linked to a cue unexpectedly change. Here we asked whether mesoaccumbal gamma-aminobutyric acid (GABA) projections enhance adaptive responding to incentive cues of unpredicted reward value, and whether these effects were distinct from global activation of all ventral tegmental area (VTA) GABA circuits.

Methods: We used a viral targeting system to selectively activate mesoaccumbal GABA projections during a novel cue-dependent operant Value Shifting (VS) task, in which the volume of a sucrose reward associated with a predictive cue is unexpectedly altered, from the beginning and throughout the session. We compared the results with global activation of VTA GABA neurons, which will activate local inhibitory circuits and long loop projections.

Results: We found that activation of mesoaccumbal GABA projections decreases responding to incentive cues associated with smaller than expected rewards. This tuning of behavioral responses was specific to cues associated with smaller than expected rewards, and did not impact responses to larger rewards, or measures more closely related to the reward itself. In marked contrast, activating all $VTA_{(GABA)}$ neurons resulted in a uniform decrease in responding to incentive cues irrespective of changes in the size of the reward.

Conclusions: Targeted activation of mesoaccumbal GABA neurons facilitate adaptation to advantageous reward-seeking behaviors. This suggests that these projections may play a very specific role in associative learning processes.

INTRODUCTION

Altering the motivational properties of a predictive cue when the value of a reward unexpectedly changes is a critical neurobehavioral component of survival. The mesolimbic system has been heavily implicated in incentive motivation processes, as well as in adapting behavior when reward contingencies unexpectedly change (1-6). While dopamine (DA) neurotransmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is fundamental to both these processes, γ-aminobutyric acid (GABA) neurons are also present in the VTA (7-9). Yet the role of these neurons in regulating reward-seeking behavior is not well understood. While the VTA contains primarily GABA interneurons, there is a subpopulation of $VTA_{(GABA)}$ neurons that project to the NAc ($VTA_{(GABA)}$→NAc, (10-12)). Approximately one third of the total VTA neurons that project to the NAc are GABAergic (13). Optogenetic stimulation of all $VTA_{(GABA)}$ neurons, including interneurons, decreases anticipatory licking in response to an odor predicting a reward during a classical conditioning task (14). Combined with electrophysiology data demonstrating that activation of $VTA_{(GABA)}$ neurons inhibits dopamine neuronal firing during reward expectancy, others have suggested that $VTA_{(GABA)}$ regulation of dopamine neurotransmission contributes to encoding reward prediction error during associative learning processes (14, 15). However, the contribution of $VTA_{(GABA)}$ projections to the NAc in mediating these processes was not evaluated in these studies, and its role remains largely unknown. One study demonstrated that activation of $VTA_{(GABA)}$ terminals in the NAc enhances discrimination between a cue predictive of an aversive stimulus (i.e. foot shock) from a non-paired cue via GABAergic inhibition of cholinergic interneurons (CINs) (16). Yet there is scant evidence $VTA_{(GABA)}$→NAc projections play any role in responding to reward predictive cues. Indeed, we and others have recently reported that directly activating $VTA_{(GABA)}$ terminals in the

NAc does not impact sucrose consumption, cue-induced anticipatory licking in mice, or operant responding to reward predictive cues in rats (17, 18).

The primary target of $VTA_{(GABA)}$→NAc projections are tonically active cholinergic interneurons (CINs) (16). CINs "pause" when learning new cue-reward associations (16) and are active when reward-seeking is disadvantageous (e.g. during satiety, (19, 20)). It is therefore possible that $VTA_{(GABA)}$→NAc projections may not have a strong influence on reward-seeking in animals with well-established reward associations, but are engaged when establishing or adapting to new associations and contingencies. Here we test the hypothesis that $VTA_{(GABA)}$→NAc neurotransmission specifically facilitates adapting advantageous behavioral responses when the reward value associated with predictive cues abruptly changes. To test this, we chemogenetically activated mesoaccumbal GABA projections selectively during a novel cue-dependent operant task in rats, in which the magnitude of a natural reward (i.e. sucrose) associated with a predictive cue is unexpectedly altered (Value Shifting, VS). We compared these effects to global activation of all $VTA_{(GABA)}$, which will activate GABA interneurons and projections throughout limbic and cortical brain regions.

METHODS AND MATERIALS (646)

Subjects

Long Evans rats (Envigo, Indianapolis, IN) weighing between 290-320 grams were individually housed in 12h/12h light/dark, with lights on at 3AM. Experiments were conducted at 9AM. Food and water were available *ad libitum*. All procedures complied with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Care and Use Committee at the University at Buffalo.

Adeno-associated virus (AAV)

We use a combinatorial viral method to target specific neuronal subtypes including dopamine (21-23) and GABA neurons (18). Details of the viral constructs, stereotaxic infusions and guide cannula implant surgeries, and CNO treatments are detailed in the Supplemental Materials.

Behavioral Apparatus

Operant chambers

Med-Associates operant chambers (Georgia, VT) in sound-attenuating cubicles were equipped with two illuminated nosepoke ports on either side of a liquid receptacle (i.e. reward cup) equipped with an infrared entry detector. A white houselight and tone generator speaker were located on the opposite wall. Each chamber was also equipped with a pump to deliver 10% sucrose solution to the receptacle. During incentive cue and value shifting sessions, a 10 ml syringe was used to deliver ~15 µl/s 10% sucrose solution, while a 30 ml syringe was used during free drinking sessions to deliver ~45 µl/s 10% sucrose solution. A dim white house light and speaker were located on the wall opposite the nosepokes and reward cup.

Operant Training

Incentive cue (IC) task

Rats were trained on the Incentive Cue (IC) task (Figure IC), which has been modified from others and described elsewhere (18, 24-26), and in detail in the Supplementary Materials. In the initial IC training task, the rats respond during a distinct 8-second audiovisual cue to obtain a ~64 µl sucrose reward, and importantly this volume is kept constant throughout the 1-hr session (Figure 1D, left).

Value Shifting (VS) Task

Once rats reached criterion performance in the IC task, we probed rats with the Value Shifting (VS) task to assess the effect of altering the reward unexpectedly. In this task, diagrammed in Figure 1D (right panel), the IC predicting reward availability remained unchanged throughout all sessions. However, we divided the 1-hr session into three 20-min blocks where a successful response to the IC resulted in the delivery of one of three fixed volumes of sucrose (16 µl, 48 µl or 128 µl), which was achieved by changing the length of time the pump and associated CS+ was on (1s, 3s, 8s). There were ~35 ICs in each block, and every IC in that 20-min block received the same designated volume of sucrose per successful response. The order of the three 20-min blocks, and the corresponding reward sizes, were randomized by the computer during the 1 hr session. All possible order permutations were equally represented in the VS probe tests (Figure 1D (bottom panel). Rats were maintained on the standard IC task (~64 µl for 1-hr, Figure 1D, left) between VS task probes, with at least 2 days between probe sessions.

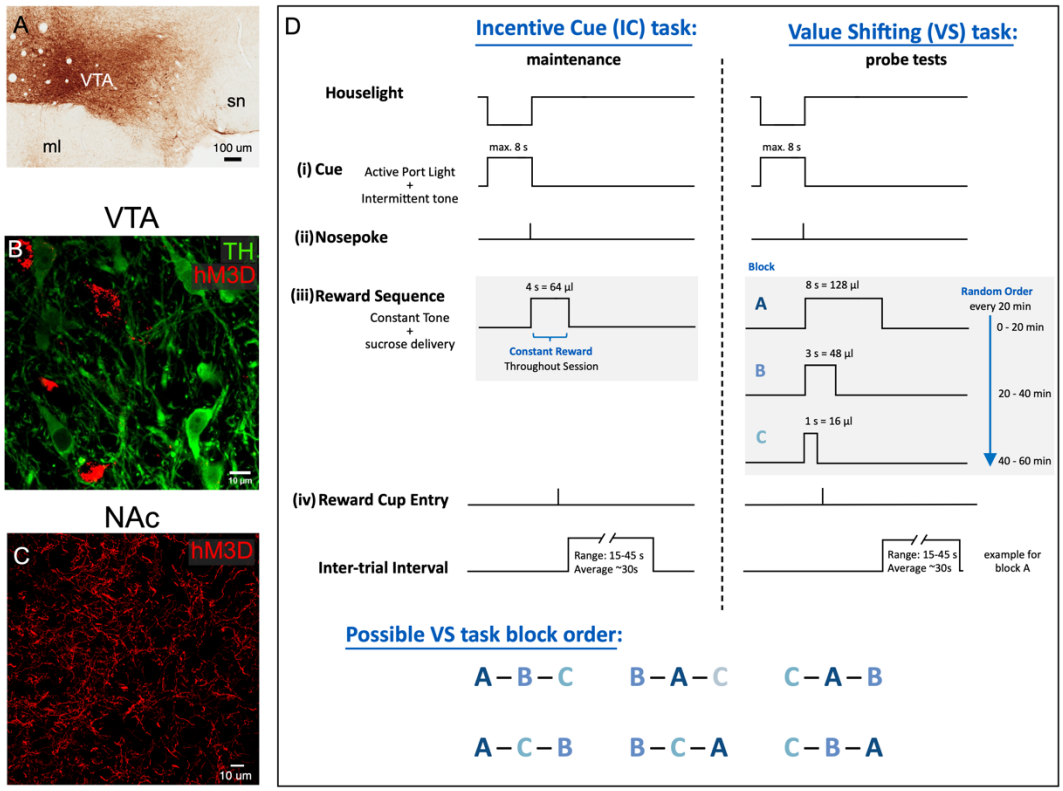

Figure 1 ***Viral targeting of activating DREADDs to VTA GABA neurons in the incentive cue and value shifting task.*** **(A)** Extent of virus expression within the VTA (scale bar 100 µm). Expression of hM3D mCherry DREADD expression in **(B)** VTA neuron bodies (TH in green, hM3D in red) and (**C**) NAc terminals. **(D)** Schematics depicting the events in the incentive cue task the rats were trained and maintained on (left panel), the VS probe test (right panel), and the potential order of reward blocks in any given probe session (bottom panel). ml, medial mammillary nucleus, SN, substantia nigra.

Free drinking and locomotor activity

Free drinking and locomotion tests are described in the Supplemental Materials.

Histological verification, immunohistochemistry, and visualization

Immunohistochemical procedures are detailed in the Supplemental Materials.

DATA ANALYSIS AND STATISTICAL ANALYSIS

For operant behavior, our primary metrics were the ***response ratio*** of responding to the IC predicting availability of sucrose (#nosepokes resulting in a reward/#IC presented), the ***latency to nosepoke*** in response to the IC ($Time_{nosepoke}$ - $Time_{IC}$), and the ***latency to enter the reward cup*** ($Time_{cup\ entry}$ – $Time_{nosepoke}$) during the VS probe trials. We adopted a sequential strategy of analysis for these experiments, which is described in detail in the Supplementary Materials. We also examined a number of secondary metrics to assess whether the treatment caused behavioral changes in unconstrained responses. These include ***accuracy*** (#total active nosepokes/#reward**),** ***active nosepokes per IC*** (#total active nosepokes/#IC), ***reward cup entries per reward*** (#total cup entries/#rewards earned), and ***total inactive nosepokes***. Details of statistical tests are found in the Supplemental Materials.

RESULTS

VIRAL TARGETING VTA$_{(GABA)}$ NEURONS

Our combinatorial viral system has been previously verified to drive transgene expression in VTA$_{(GABA)}$ neurons and to functionally inhibit dopamine neurons firing when chemogenetically activated (18). Here we demonstrate the spread of the hM3D-mCherry DREADD through the VTA (Figure 1A), hM3D-mCherry expression distinct from tyrosine hydroxylase (TH), a marker for dopamine neurons (Figure 1B), and terminal expression of hM3D in the NAc (Figure 1C).

ACTIVATION OF MESOACCUMBAL GABA PROJECTIONS DECREASES REWARD-SEEKING WHEN REWARD SIZE IS UNEXPECTEDLY DECREASED

To assess how selective activation of mesoaccumbal GABA projections regulates reward seeking behaviors during unpredicted changes in reward contingencies, we examined the response ratio during the VS probe task (Figure 2). When comparing the overall response ratio during each unexpected reward size across all treatments and groups, the response ratio was significantly decreased when the reward size decreased to 16 µl (Figure 2A). Three-way Mixed Effects analysis showed fixed effects of reward volume $F_{2,40}$=23.56, $p<0.0001$, and hM3D/tdTomato $F_{1,40}$=5.362 $p=0.0258$, and a hM3D/tdTomato x CNO/VEH treatment interaction $F_{1,40}$=9.470 $p=0.0038$. Importantly, the effects on response ratio performance during the 16 µl block was specific to rats expressing hM3D, and were not present with either vehicle or in DREADD-free control subjects. In comparing response ratio in the first and last 5 minutes of each reward bin, we found that a significant decrease only in the last 5 minutes of the 16 µl reward block compared to vehicle (Figure 2B). Three-way Mixed Effects analysis showed fixed

effects of reward volume $F_{2,24}$=7.016 p=0.004, CNO/VEH $F_{1,12}$=15.79 p=0.0018, and reward volume x within bin $F_{2,24}$=13.49 p=0.0001, CNO/VEH x within bin $F_{1,12}$=9.335 p=0.01, and reward volume x CNO/VEH x within bin $F_{2,24}$=6.081 p=0.0073 interactions. Statistical analyses showed that tdTomato controls were not sensitive to CNO between the first and last 5 minutes of any reward volume. However, they remained sensitive to unexpected changes in reward volume overall (Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,18}$=4.020 p=0.036). Moreover, tdTomato rats reduced their responding to the cue when comparing the beginning and end of the 16 µl reward block (Reward volume x within bin interaction $F_{2,18}$=5.222 p=0.0163).

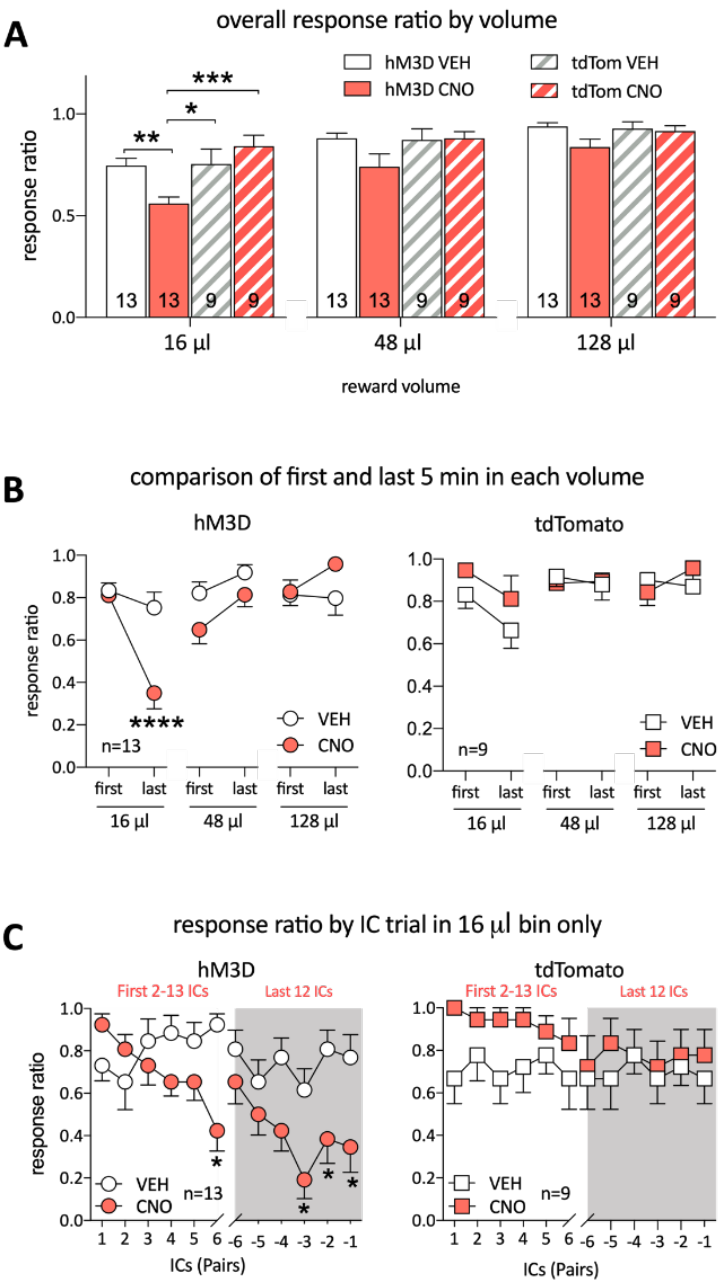

Figure 2 ***Activation of VTA$_{(GABA)}$ terminals in the NAc reduces responding to reward predictive cues reinforced by lower than expected rewards.*** All data are shown as mean±SEM. (**A**) The overall response ratio significantly decreased after intra-NAc CNO in rats expressing hM3D compared to vehicle, but not in rats expressing tdTomato, when the reward size was 16 µl. **(B)** Within the first and last 5 minutes of each reward size, intra-NAc injections of CNO only reduced IC performance in hM3D rats at the end of the 16 µl bin. **(C)** Responses to individual successive pairs of ICs during the 16 µl bin highlights an adaptive response only in CNO treated hM3D rats after experiencing a change in reward contingency. Asterisks represent significant differences between CNO and VEH determined by a Holms-Sidak post-hoc test (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001). The sample size for groups is found at the base of each column.

Dynamic changes in response ratio occurred on a cue by cue basis during the 16 µl reward block only after intra-accumbal CNO treatment in hM3D-expressing rats (Figure 2C, Two-way Mixed Effects analysis, fixed effects of cue pair $F_{11,132}$=4.743 p<0.0001, CNO/VEH $F_{1,12}$=14.88 p=0.0023, and a cue pair x CNO/VEH $F_{11,132}$=3.325 p=0.0006 interaction). This effect was not observed in tdTomato controls.

We next examined whether activation of $VTA_{(GABA)}$→NAc projections influences the vigor of reward-seeking in the VS probe task (Figure 3). Rats changed their latency to nosepoke to the IC relative to the reward size, and there was an overall effect of CNO treatment compared to vehicle. However, there were no overall effects on hM3D/tdTomato, and no significant interactions between reward size, hM3D/tdTomato, and CNO/VEH, (Figure 3A. Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,40}$=26.64, p<0.0001, and CNO/VEH $F_{1,20}$=5.347 p=0.0315). Within the first and last 5 minutes of each reward bin, we found that that nosepoke latency was significantly increased after CNO compared to vehicle only in the last 5 minutes of the 16 µl reward block (Figure 3B). Three-way Mixed Effects analysis demonstrated fixed effects of reward volume $F_{2,24}$=9.766 p=0.0008, CNO/VEH $F_{1,12}$=8.911 p=0.0114, and a reward volume x within bin $F_{2,24}$=8.093 p=0.0021 interaction). Although there was an overall difference in nosepoke latency depending on the reward size in the tdTomato controls (Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,16}$=6.973 p=0.0066) and the nosepoke latency at the beginning and end of each reward block differed according the reward size (reward volume x within bin interaction, $F_{2,16}$=4.670 p=0.0253), CNO treatment had no effect. We found no significant differences in the latency to enter the reward cup to consume reward after a successful nosepoke response to an IC, either as a function of reward size, virus type, or CNO/VEH (Figure 3C). Further, there were no differences in reward cup latencies

between the first and last 5 minutes of each reward bin across all of the variables tested (Figure 3D).

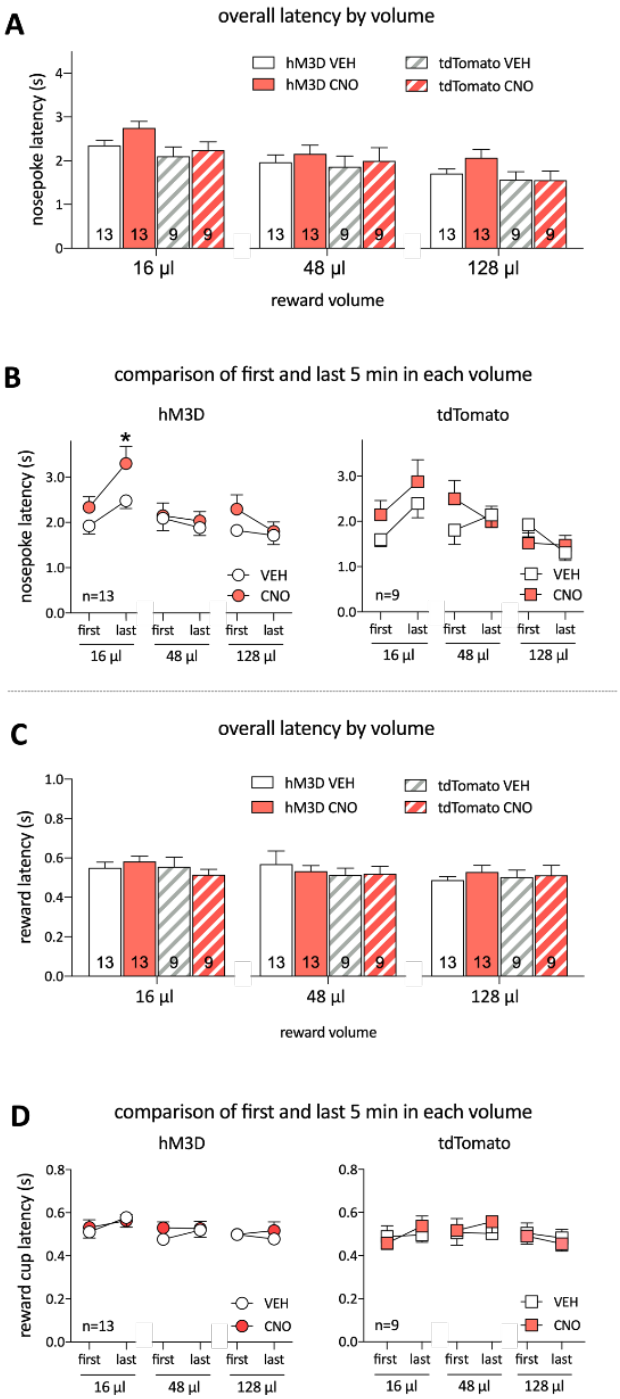

Figure 3 ***Activation of VTA$_{(GABA)}$ terminals in the NAc increases latency to respond to cues that predict lower than expected reward, but not latency to collect the reward.*** All data are shown as mean±SEM. (**A**) Overall

latency to nosepoke increased when the reward size was reduced to 16 µl, but there were no significant differences within each reward size. **(B)** Within the first and last 5 minutes of each reward size, intra-NAc injections of CNO only increased the latency to nosepoke to the IC in hM3D rats at the end of the 16 µl bin. **(C)** No effect of intra-NAc treatment was seen in latencies to enter the reward cup after responding to the IC. **(D)** No within-reward size effects on latency to enter the reward cup were seen after intra-NAc treatment. Asterisks represent significant differences between CNO and VEH determined by a Holms-Sidak post-hoc test (*p<0.05)

Finally, we examined how mesoaccumbal GABA activation during the VS probe task influenced unreinforced responses, specifically accuracy, total active nosepokes per IC, reward cup entries per reward, and responses in the inactive nosepoke (Figure 4). While three of the metrics of unconstrained behavior during the VS task showed a significant effect of reward size (Three-way Mixed Effects analysis, fixed effects of reward volume, Accuracy: $F_{2,40}$=4.663 p=0.0151, active nosepokes per IC $F_{2, 40}$=35.94, p< 0.0001, and reward cup entries per reward $F_{2, 40}$=10.39 p=0.0002), demonstrating that rats were adapting their responses as reward sizes changed, there were no effects of hM3D/tdTomato, or CNO/VEH in any group. There were no significant effects observed in inactive nosepoke responses.

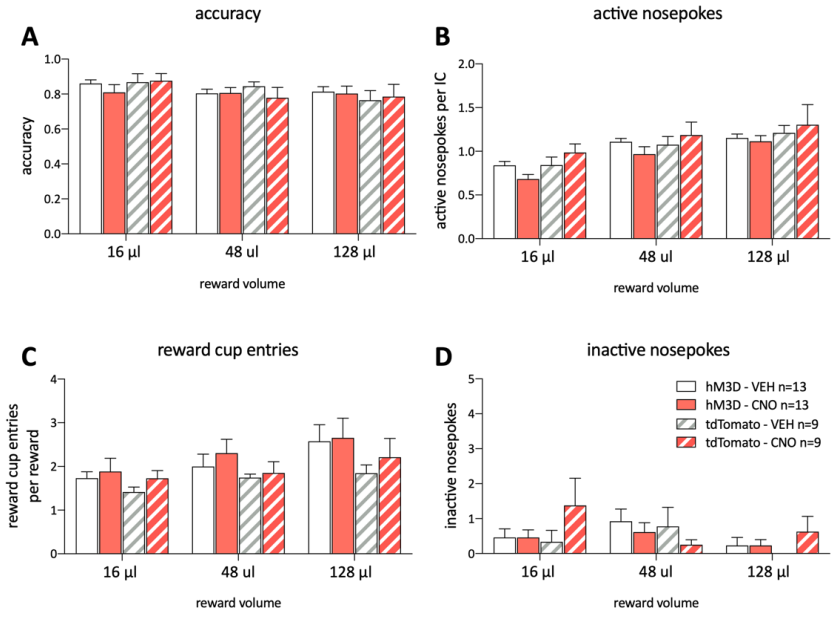

Figure 4 ***Activation of VTA$_{(GABA)}$ terminals in the NAc have no effect on the four possible response outcomes in the VS task.*** All data are shown as mean±SEM. There were no significant effects of intra-NAc treatment on accuracy (the number of active nosepokes per reward obtained) (**A**), active nosepokes per IC presented **(B)**,

reward consummatory behavior as measured by reward cup entries per reward earned **(C)**, or changes in discriminating between the active and inactive nosepoke **(D)**.

GLOBAL ACTIVATION OF VTA$_{(GABA)}$ NEURONS DECREASED REWARD-SEEKING REGARDLESS OF CHANGE IN REWARD SIZES

In contrast to targeted activation of the mesoaccumbal GABA projections, global activation of VTA$_{(GABA)}$ neurons using systemic CNO, including local interneurons and projections to limbic and cortical regions, uniformly reduced responding to reward predictive incentive cues during the VS task (Figure 5). The response ratio was significantly decreased across all reward sizes after systemic CNO treatment (Figure5A. Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,40}$=16.40, p<0.0001, and hM3D/tdTomato $F_{1,40}$=11.14 p=0.0018, CNO/VEH $F_{1,20}$=28.47, p<0.0001, and a hM3D/tdTomato x CNO/VEH interaction $F_{1,40}$=18.36 p=0.0001). The effects of systemic CNO was limited to rats expressing hM3D targeted to VTA$_{(GABA)}$ neurons. Global activation decreased the response ratio within both the first and last 5 minutes across most reward bins compared to vehicle treatment (Figure 5B. Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,24}$=6.148 p=0.007, within bin $F_{1,12}$=88.41, p<0.0001, and a reward volume x within bin $F_{2,24}$=5.826 p=0.0087 interaction). Within the tdTomato controls we again observed a modest proportional change in response ratio with reward volume (Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,16}$=7.827 p=0.0043), but also observed a reward volume x treatment x within bin interaction ($F_{2,16}$=6.173 p=0.0103). However, no clear trend was apparent and comparing individual responses between CNO and vehicle revealed no significant differences.

When conducting a cue by cue analysis of the response ratio performance in the 16 µl reward block, we found that global VTA$_{(GABA)}$ activation produced a uniform reduction in response ratio from the beginning of the block (Figure 5C, Two-way Mixed Effects analysis,

fixed effects of cue pair $F_{11,132}$=3.115 p=0.0009 and VEH/CNO $F_{1,12}$=43.11 p<0.0001). In tdTomato controls, there was no effect of CNO treatment, although there was an overall modest decrease in responding on a cue by cue basis in the 16 µl reward block (Two-way Mixed Effects analysis, fixed effects of cue pair $F_{11,88}$=1.932 p=0.0457).

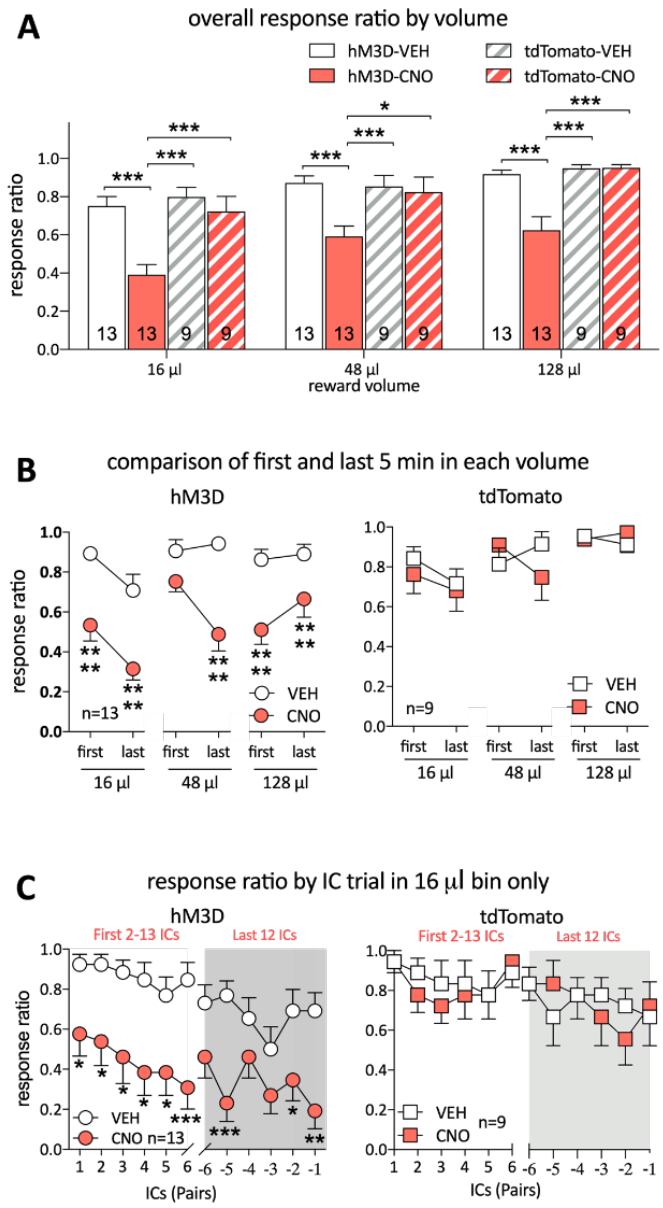

Figure 5 ***Global activation of VTA$_{(GABA)}$ neurons reduces responding to reward predictive cues regardless of reward size.*** All data are shown as mean±SEM. (**A**) Overall response ratio significantly decreased after systemic CNO in rats expressing hM3D compared to vehicle and tdTomato rats across all reward sizes. **(B)**

Within the first and last 5 minutes of each reward size, systemic CNO treatment reduced IC performance in hM3D rats at both the beginning and end of the 16 µl and 128 µl reward bin. While CNO also reduced response ratios at the beginning and end of the 48 µl bin, it was only significant at the end. No differences between treatments were seen in tdTomato controls. **(C)** Responses to individual successive pairs of ICs during the 16 µl bin highlights a uniform decrease in responding to cues in CNO treated hM3D rats. Asterisks represent significant differences determined by a Holms-Sidak post-hoc test (*p<0.05, **p<0.01, ***p<0.005 ****p<0.001).

In examining the effects of global $VTA_{(GABA)}$ activation on other VS probe task metrics, we observed an overall increase in nosepoke latencies in all reward blocks in hM3D rats treated with systemic CNO (Figure 6A. Three-way Mixed Effects analysis, fixed effects of reward volume $F_{2,40}$=26.78, p<0.0001, hM3D/tdTomato $F_{1,40}$=8.026 p=0.0072, CNO/VEH $F_{1,20}$=6.994 p=0.0155, and a virus x treatment interaction $F_{1,20}$=7.884 p=0.0077). The nosepoke latency was increased in hM3D, CNO treated rats compared to vehicle, between the first and last 5 minutes of each reward block (Figure 6B fixed effects of reward volume $F_{2,24}$=9.226 p=0.001, CNO/VEH $F_{1,12}$=11.65 p=0.0051), although post-hoc tests revealed that this effect was not consistently statistically significant across all reward sizes. No effects were seen in tdTomato controls. Likewise, after global $VTA_{(GABA)}$ activation, rats showed an increase in latency to enter the reward cup after a successful response regardless of reward size (Figure 6C. Fixed effects of hM3D/tdTomato $F_{1,40}$=6.808 p=0.0127, CNO/VEH $F_{1,20}$=13.39 p=0.0016, and a hM3D/tdTomato x CNO/VEH interaction $F_{1,40}$=21.89 p<0.0001). Examining changes in the reward cup latency between the first and last 5 minutes of each reward bin showed that the latency increased regardless of whether it was at the beginning or end of the reward bin, and occurred during all reward sizes compared to vehicle (Figure 6D. Fixed effects of treatment $F_{1,12}$=31.30 p=0.0001).

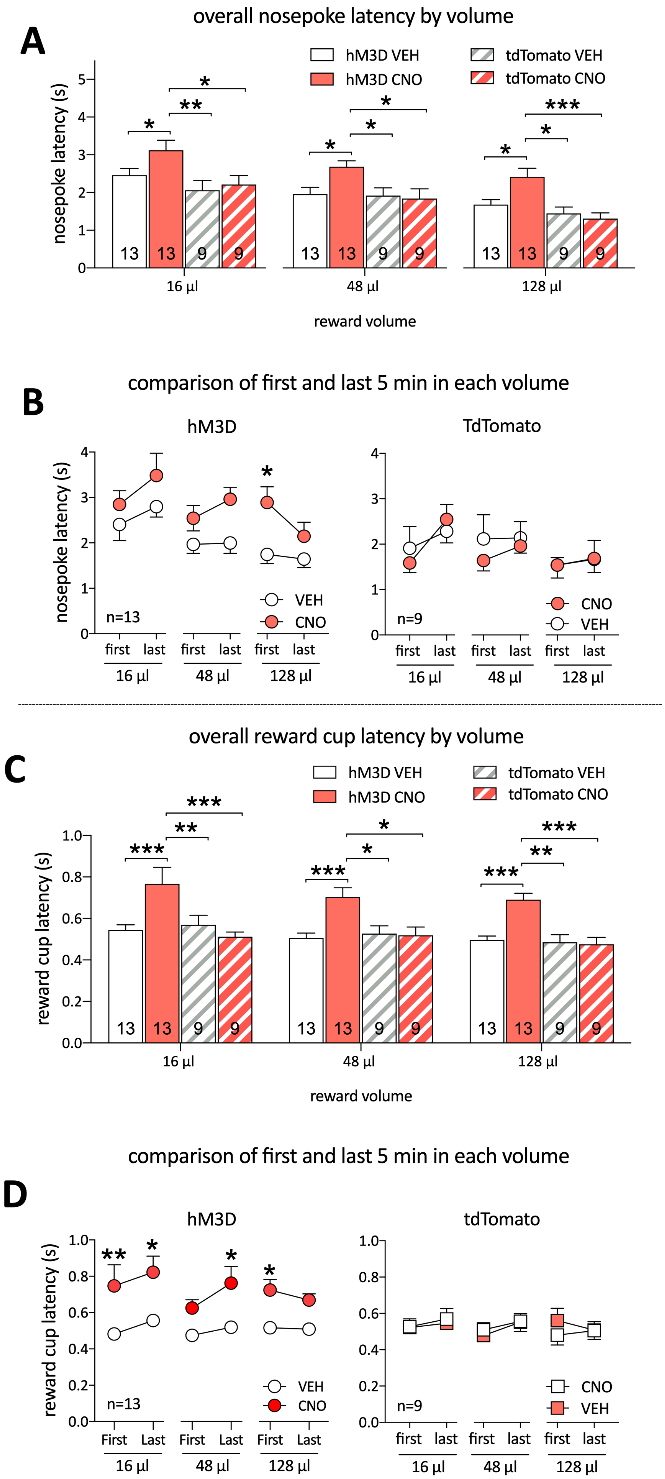

Figure 6 ***Global activation of VTA$_{(GABA)}$ neurons increases the latencies to respond to cues and collect the reward irrespective of reward size.*** All data are shown as mean±SEM. (**A**) Overall latency to nosepoke increased with all reward sizes after systemic CNO pretreatment compared to controls. **(B)** Within the first and last 5 minutes of each reward size, VTA$_{(GABA)}$ activation increased nosepoke latencies overall in both the first and last 5 minutes of each reward bin, although most of this could be attributed to the first 5 min during

the 128 µl reward block. No changes were seen in tdTomato controls. **(C)** Latencies to enter the reward cup significantly increased in hM3D rats given systemic CNO across all reward sizes compared to controls **(D)**. Latencies to enter the reward cup were significantly greater in the first 5 min of the 16 and 128 µl reward blocks, and the last 5 minutes of the 48 µl bin. No within-reward block effects on latency to reward were seen in tdTomato controls. Asterisks represent significant differences determined by a Holms-Sidak post-hoc test (*$p<0.05$, **$p<0.01$, ***$p<0.005$).

Finally, we examined how global $VTA_{(GABA)}$ activation mesoaccumbal during the VS probe task influenced accuracy, total active nosepokes per IC, reward cup entries per reward, and responses in the inactive nosepoke. Similar to $VTA_{(GABA)}$→NAc projection activation, rats were adapted their responses as reward sizes changed, except for responses in the inactive nosepoke (Figure 7. Three-way Mixed Effects analysis, fixed effects of reward volume, Accuracy: $F_{2,40}=3.594$ $p=0.0367$. Active nosepokes per IC $F_{2, 40}=12.48$ $p< 0.0001$, and Reward cup entries per reward $F_{2, 40}=4.049$ $p=0.025$). However, in contrast to targeted $VTA_{(GABA)}$→NAc projections activation, global $VTA_{(GABA)}$ activation resulted in a decrease in active nosepokes per IC after treatment across in all reward blocks (Fixed effects of hM3D/tdTomato $F_{1,40}=8.002$ $p=0.0073$, CNO/VEH $F_{1,20}=27.61$, $p<0.0001$, and a significant hM3D/tdTomato x CNO/VEH interaction, $F_{1,40}$ $p=12.26$ $p=0.0012$). No significant effects were observed in inactive nosepoke responses.

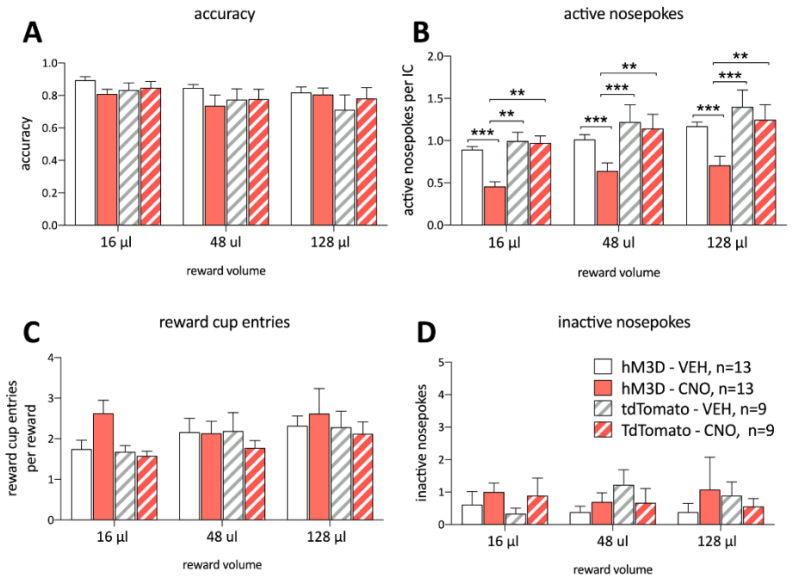

Figure 7 ***Systemic activation of VTA$_{(GABA)}$ neurons selectively impacts measures of responding to the IC in the VS task.*** All data are shown as mean±SEM. There were no significant effects of CNO treatment on accuracy (the number of active nosepokes per reward) (**A**), while there was a decrease in active nosepoke per IC as a result of treatment across all reward sizes compared to controls **(B)**. There were no effects of VTA-GABA activation on reward cup entries per reward earned **(C)**, or changes in the number of inactive nosepokes **(D)**. Asterisks represent significant differences determined by a Holms-Sidak post-hoc test (**p<0.01, ***p<0.005).

MESOACCUMBAL GABA ACTIVATION DOES NOT ALTER SUCROSE PALATABILITY OR LOCOMOTOR ACTIVITY.

In a series of control experiments, we determined that mesoaccumbal VTA$_{(GABA)}$ activation does not change the primary reinforcing effects of sucrose or baseline locomotor activity (see Supplemental Results). Further, we assessed the effect of potential conversion of CNO to clozapine (CZP) by administering an equivalent intra-NAc dose of CZP in DREADD-free controls (Supplemental Figure S1). CZP alone did not alter any of the metrics in the VS task.

DISCUSSION

This study was undertaken to test the hypothesis that VTA$_{(GABA)}$ projections to the NAc specifically facilitate adapting behavioral responses in the face of changing reward

contingencies. We achieved this by selectively activating mesoaccumbal GABA terminals using DREADDs, while unexpectedly changing the volume of a sucrose reward associated with a reward-predictive cue. We found three main novel findings, first, that activation of mesoaccumbal GABA projections selectively tunes behavioral responses to predictive cues when the reward is smaller than expected. Secondly, this decrease in responding induced by $VTA_{(GABA)}$→NAc projection activation occurred with experience, and became more pronounced over the 20-min bin. Third, the tuning was specific to responding to the incentive cue associated with the reward, and did not impact reward consumption. In contrast, global activation of all $VTA_{(GABA)}$ neurons, which affects both GABA projections to different regions and GABA interneurons, attenuated responding irrespective of experience with the new reward contingency, and attenuated reward consumption.

Reward seeking is a multifaceted process, and engages multiple reward-related systems and neural circuits (10, 27-29). Several different reward processes have been ascribed to mesolimbic circuitry, including evaluating the outcome of a goal-directed action against an established, learned value (14, 15). For example, negative reward prediction is a type of behavioral learning that occurs when an earned reward is worse than predicted (30), resulting in a subsequent decrease in seeking that reward. In our study, rats were well trained to expect a fixed volume of reward (64 µl), but during Value Shifting probe tests the sucrose reward was unpredictably decreased from that obtained during training sessions, or in relation to larger rewards obtained in earlier reward blocks (48 or 128 µl) within the session. Under these conditions, mesoaccumbal $VTA_{(GABA)}$ activation selectively decreased the response ratio and increased the nosepoke latency to the cue suddenly predicting the much smaller volume of sucrose reward (16 µl). A central component of a learned process is that the changed reward

outcome must be experienced before an alteration in reward-seeking behavior can occur. Indeed, in the Value Shifting probe rats initially responded to the cue predicting the smallest reward at levels identical to controls. Yet as they gained experience with the new reward contingency upon subsequent cue presentations, activation of $VTA_{(GABA)}$→NAc projections facilitated a decrease in responding to the new, less than expected reward. In addition, the latency to respond to the cue predicting the smallest reward increased at the end of the bin, compared to saline vehicle controls. Together, this suggests that activation of $VTA_{(GABA)}$→NAc projections selectively facilitated a progressive decrease in the choice to respond to the cue over time, along with a decrease in the vigor of the response (31). These effects were limited to behaviors specific to the cue itself, as there were no changes in the latency to enter the reward cup to obtain a reward, or other task measures (e.g. accuracy, reward cup entries, inactive nosepokes). Interestingly, a comparable increase in either choice or vigor of response was not seen when there was an unpredicted larger than expected reward. It is possible that $VTA_{(GABA)}$→NAc projections specifically contribute to forming new stimulus-outcome associations only when rewards are less than expected. However, we cannot preclude the possibility the rats reached a performance ceiling during this instrumental task with sucrose reinforcement, thereby masking increases in responding with larger than expected reward values.

To our knowledge, this is the first demonstration that $VTA_{(GABA)}$→NAc projections can alter cue processing for rewards. We and others have generally shown that selectively activating these projections does not alter cue-induced reward seeking (17, 18). However, one study demonstrated that this pathway enhances discrimination of cues predicting an aversive stimulus (16) . In that study, mice generalized freezing to two distinct tones, but optogenetic activation of $VTA_{(GABA)}$ terminals in the NAc during conditioning resulted in the mice freezing less to the

non-predictive tone, while freezing was maintained for the tone predicting the foot shock (16). In this way, the non-predictive tone was marked as less motivationally relevant. The authors determined this effect occurred through selective inhibition or "pausing" of local CINs in the NAc. Future studies examining whether our results also occur through accumbal CINs will be useful in determining if this is a common pathway for facilitating learning new cue-reinforcement contingencies regardless of valence.

These results substantially expand our earlier work, in which we observed no change in cue-induced responding after activating $VTA_{(GABA)}$ projections to the NAc in the standard IC task, where reward size was kept constant (18). The Value Shifting task has different reward contingencies and is likely engaging reward-seeking processes that are distinct from those in the standard IC task. The Value Shifting challenge differs notably from the IC task in that the reward sizes are randomly assigned to the same predictive cue within the session. To prevent rats from becoming over-trained on this Value Shifting task and to preserve the novelty of the changed reward contingencies, these sessions were conducted sparingly and always after several days of IC task sessions. A significant aspect of our data is that activation of $VTA_{(GABA)} \rightarrow$NAc projections altered responding in the Value Shifting challenge even though the rats were very well-trained, and continued to run in the IC task on intervening days. Thus, our subjects have a strong pre-established cue-reward expectancy established by prior training in the IC task. In contrast, after global activation of $VTA_{(GABA)}$ neurons, we are able to show a decrease in responding, increase in nosepoke and reward latencies, and an increase in the active nosepokes per IC during the Value Shifting challenge, irrespective of the reward size. This supports our earlier findings that global $VTA_{(GABA)}$ activation preferentially inhibits the incentive salience of the reward-predictive cues (18) possibly through $VTA_{(GABA)}$ interneuron inhibition of dopamine

neurons, activity of $VTA_{(GABA)}$ projections to other brain regions, or a combination of both effects. During the Value Shifting probe, changes in performance after global $VTA_{(GABA)}$ activation are present at the beginning of each reward bin. This is a defining characteristic of incentive motivational processes, in that changes in reward-seeking behavior occur before experience with the reward itself (2).

Similar to our previous work, we found that the behavioral effects we report here after targeted $VTA_{(GABA)}$→NAc activation do not a result from a change in the generalized palatability of sucrose, as there was no effect on sucrose consumption under free-drinking conditions. Similarly, there was no effect of $VTA_{(GABA)}$→NAc activation on generalized locomotion, suggesting that the decreases in responding to cue associated with a lower than expected reward did not result from a suppression of locomotor activity. Indeed, our data demonstrate that when the rats do respond in the other reward bins, they do so with similar speed, and are thus capable of emitting faster responses (Figure 3). In addition, since it has recently been shown that high doses of CNO are converted to CZP in vivo (32), all of our behavioral metrics for both intra-NAc CNO and systemic administration of CNO included comparisons with DREADD-free controls. We also determined that systemic CZP in DREADD-free rats did not alter the behavioral metrics of the VS probe, demonstrating that there is no confound from the potential metabolism of CNO to CZP in this study.

In summary, our data demonstrate that targeted activation of $VTA_{(GABA)}$ projections to the NAc preferentially regulates associative learning related to new stimulus-outcomes, possibly underlying negative reward prediction processes. This specific behavioral consequence in a robust operant model is distinct from global $VTA_{(GABA)}$ activation, which appears to preferentially regulate the attribution of incentive salience to reward predictive cues, regardless

of the value of the subsequent reward. Further studies will be needed to determine the precise local circuitry within the NAc that contribute to these reward-seeking behaviors.

Acknowledgements

This research was supported by the State University of New York BRAIN Network of Excellence Post-doctoral Fellow program and T32 AA007583 (K.T.W) as well as The Whitehall Foundation (C.E.B), DA043190 (C.E.B) and AA024112 (P.J.M). An earlier version of this manuscript has been posted on the bioRxiv preprint server (33).

Financial Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

References

1. Berridge KC, Robinson TE (1998): What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*. 28:309-369.
2. Berridge KC (2012): From prediction error to incentive salience: mesolimbic computation of reward motivation. *Eur J Neurosci*. 35:1124-1143.
3. Berridge KC (2007): The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)*. 191:391-431.
4. Schultz W, Dayan P, Montague PR (1997): A neural substrate of prediction and reward. *Science*. 275:1593-1599.
5. Schultz W, Dickinson A (2000): Neuronal coding of prediction errors. *Annu Rev Neurosci*. 23:473-500.
6. Keiflin R, Janak PH (2015): Dopamine Prediction Errors in Reward Learning and Addiction: From Theory to Neural Circuitry. *Neuron*. 88:247-263.
7. Margolis EB, Toy B, Himmels P, Morales M, Fields HL (2012): Identification of rat ventral tegmental area GABAergic neurons. *PLoS One*. 7:e42365.
8. Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA (2008): Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*. 152:1024-1031.
9. Saddoris MP, Cacciapaglia F, Wightman RM, Carelli RM (2015): Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. *J Neurosci*. 35:11572-11582.
10. Fields HL, Hjelmstad GO, Margolis EB, Nicola SM (2007): Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu Rev Neurosci*. 30:289-316.
11. Taylor SR, Badurek S, Dileone RJ, Nashmi R, Minichiello L, Picciotto MR (2014): GABAergic and glutamatergic efferents of the mouse ventral tegmental area. *J Comp Neurol*. 522:3308-3334.
12. Creed MC, Ntamati NR, Tan KR (2014): VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems. *Front Behav Neurosci*. 8:8.
13. Van Bockstaele EJ, Pickel VM (1995): GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res*. 682:215-221.
14. Eshel N, Bukwich M, Rao V, Hemmelder V, Tian J, Uchida N (2015): Arithmetic and local circuitry underlying dopamine prediction errors. *Nature*. 525:243-246.
15. Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N (2012): Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature*. 482:85-88.
16. Brown MT, Tan KR, O'Connor EC, Nikonenko I, Muller D, Luscher C (2012): Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature*. 492:452-456.
17. van Zessen R, Phillips JL, Budygin EA, Stuber GD (2012): Activation of VTA GABA neurons disrupts reward consumption. *Neuron*. 73:1184-1194.
18. Wakabayashi KT, Feja M, Baindur AN, Bruno MJ, Bhimani RV, Park J, et al. (2019): Chemogenetic activation of ventral tegmental area GABA neurons, but not mesoaccumbal GABA terminals, disrupts responding to reward-predictive cues. *Neuropsychopharmacology*. 44:372-380.
19. Mark GP, Rada P, Pothos E, Hoebel BG (1992): Effects of feeding and drinking on acetylcholine release in the nucleus accumbens, striatum, and hippocampus of freely behaving rats. *J Neurochem*. 58:2269-2274.

20. Avena NM, Rada P, Moise N, Hoebel BG (2006): Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience*. 139:813-820.
21. Gompf HS, Budygin EA, Fuller PM, Bass CE (2015): Targeted genetic manipulations of neuronal subtypes using promoter-specific combinatorial AAVs in wild-type animals. *Front Behav Neurosci*. 9:152.
22. Budygin EA, Bass CE, Grinevich VP, Deal AL, Bonin KD, Weiner JL (2020): Opposite Consequences of Tonic and Phasic Increases in Accumbal Dopamine on Alcohol-Seeking Behavior. *iScience*. 23:100877.
23. Mikhailova MA, Bass CE, Grinevich VP, Chappell AM, Deal AL, Bonin KD, et al. (2016): Optogenetically-induced tonic dopamine release from VTA-nucleus accumbens projections inhibits reward consummatory behaviors. *Neuroscience*. 333:54-64.
24. Wakabayashi KT, Fields HL, Nicola SM (2004): Dissociation of the role of nucleus accumbens dopamine in responding to reward-predictive cues and waiting for reward. *Behav Brain Res*. 154:19-30.
25. Ambroggi F, Ghazizadeh A, Nicola SM, Fields HL (2011): Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. *J Neurosci*. 31:6820-6830.
26. Yun IA, Wakabayashi KT, Fields HL, Nicola SM (2004): The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J Neurosci*. 24:2923-2933.
27. Kelley AE, Berridge KC (2002): The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci*. 22:3306-3311.
28. Meyer PJ, King CP, Ferrario CR (2016): Motivational Processes Underlying Substance Abuse Disorder. *Curr Top Behav Neurosci*. 27:473-506.
29. Belin D, Jonkman S, Dickinson A, Robbins TW, Everitt BJ (2009): Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction. *Behav Brain Res*. 199:89-102.
30. Schultz W (2016): Dopamine reward prediction error coding. *Dialogues Clin Neurosci*. 18:23-32.
31. Niv Y, Daw ND, Joel D, Dayan P (2007): Tonic dopamine: opportunity costs and the control of response vigor. *Psychopharmacology (Berl)*. 191:507-520.
32. Gomez JL, Bonaventura J, Lesniak W, Mathews WB, Sysa-Shah P, Rodriguez LA, et al. (2017): Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*. 357:503-507.
33. Wakabayashi KT, Feja M, Leigh MPK, Baindur AN, Suarez M, Meyer PJ, et al. (2020): Chemogenetic activation of mesoaccumbal Gamma-Aminobutyric Acid projections selectively tunes responses to predictive cues when reward value is unexpectedly decreased. *bioRxiv*.2020.2003.2004.977371.