Chemogenetic inhibition of ventral tegmental area dopamine neurons or their inputs to the nucleus accumbens disrupts cue-triggered reward seeking but not reward taking

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Abstract

Reward-paired cues acquire powerful incentive motivational properties that allow them to elicit reward-seeking behavior. This motivational influence is adaptive under normal conditions but can lead to compulsive behavior in addiction and related disorders. Here, we used pathway-specific chemogenetic inhibition of dopamine neurons to determine their role in mediating cue-triggered reward seeking vs. reward taking, which were parsed using a novel analytic approach based on microstructural analysis of the individual behaviors required for earning (lever pressing) and collecting rewards (food-cup approach), respectively. We found that inhibiting ventral tegmental area (VTA) dopamine neurons effectively abolished cue-motivated reward seeking, without impacting that cue's ability to increase reward-taking behavior, suggesting that this pathway supports the response-invigorating effects of reward-paired cues, but not their ability to signal reward availability. Inhibiting these dopamine neurons also spared rat's ability to select actions based on reward value, further defining the scope of this system's involvement in the regulation of reward-seeking behavior. We also found that locally inhibiting dopamine projections to nucleus accumbens (NAc), but not to medial prefrontal cortex (mPFC), disrupted cue-triggered reward seeking. Interestingly, response suppression induced by inhibiting NAc dopamine inputs was more marked in rats that tended to respond to reward-paired cues by engaging in exploratory reward seeking, without trying to collect reward, than in rats that engaged in complete bouts of seeking and taking, suggesting such responses vary in their dependence on mesolimbic dopamine. These findings provide important new insights into the behavioral and neural mechanisms underlying adaptive and compulsive forms of cue-motivated behavior.

The mesocorticolimbic dopamine system is strongly implicated in both adaptive and maladaptive forms of motivated behavior (for review, Berridge and Robinson, 2016; Farrell *et al*, 2018; Ostlund and Halbout, 2017; Salamone and Correa, 2012), though there is much left to learn about its specific contributions to such behavior. Dopamine appears to be most important when one must adapt to challenges while pursuing a reward (Beeler *et al*, 2014; Salamone *et al*, 2012), such as when reward-seeking behavior is first learned (Choi *et al*, 2005; Wassum *et al*, 2012) or when there is an abrupt increase in the effort needed to earn reward (Ostlund *et al*, 2012). Dopamine also mediates the tendency for reward-paired cues to motivate reward seeking (Dickinson *et al*, 2000; Wassum *et al*, 2011), suggesting that it helps reinvigorate such behavior when environmental cues signal that it may be adaptive to do so.

Although reward-paired cues can invigorate reward seeking through a nonspecific motivational, or arousal, process (Rescorla and Solomon, 1967), they can also influence action selection by engaging associative cognitive processes (Kruse *et al*, 1983; for review, see Corbit and Balleine, 2016). For instance, it has recently been proposed that reward-paired cues are able to temporarily inflate the perceived efficacy of reward-seeking behavior, which in turn increases the likelihood that such actions will be performed (Cartoni *et al*, 2013; see also Rescorla, 1994). This account is particularly useful for explaining why reward-paired cues seem to be so effective in reinvigorating reward-seeking actions that have been extinguished (for review, Cartoni *et al*, 2016; Holmes *et al*, 2010) or have a low probability of reinforcement (Cartoni *et al*, 2015).

To determine the specific involvement of dopamine in cue-motivated behavior, we developed a novel method to selectively assay how reward-paired cues influence animals' perception of the efficacy of their reward-seeking actions, based on the microstructural organization of reward-seeking behavior. In particular, when rats come to doubt their ability to earn food reward by pressing a lever, they perform that action in a more exploratory manner, without attempting to collect, or take, food (Joel and Avisar, 2001). We therefore propose that this *reward-taking behavior* (i.e., attempting to collect reward immediately after executing a reward-seeking action) reflects a rat's perception of the efficacy of their reward-seeking behavior. Given this, if reward-paired cues are able to inflate the perceived efficacy of reward-seeking behavior (e.g., lever pressing), then they should be effective in increasing the probability that individual reward-seeking actions are followed by reward-taking behavior (e.g., food-cup approach).

We used a Pavlovian-to-instrumental transfer (PIT) task (for review, Cartoni *et al*, 2016; Holmes *et al*, 2010) to assess the potentially dissociable influences of reward-paired cues on reward seeking and reward taking, and to disambiguate the involvement of dopamine in such processes. Previous studies using the PIT task have shown that cue-motivated reward seeking can be abolished by blocking dopamine receptors, either systemically (Dickinson *et al*, 2000; Ostlund and Maidment, 2011; Wassum *et al*, 2011) or locally in the nucleus accumbens (NAc) (Lex and Hauber, 2008), or by inactivating the ventral tegmental area (VTA) (Corbit *et al*, 2007; Murschall and Hauber, 2006). Importantly, if PIT depends on the ability of the reward-paired cue to inflate predictions about the efficacy of reward-seeking behavior, then it is

possible that dopamine is directly involved in this process, in which case disrupting dopamine signaling should block cue-motivated reward-seeking *and* reward-taking behaviors. However, if dopamine contributes to an incentive motivational process that directly results in invigoration of reward seeking behavior, than we would expect manipulations of dopamine signaling to spare the influence of reward-paired cues on reward-taking behavior.

The medial prefrontal cortex (mPFC) also receives substantial input from a subpopulation of VTA dopamine neurons (Lammel *et al*, 2008). Food-paired cues elicit dopamine release in the mPFC (Bassareo and Di Chiara, 1997; Feenstra *et al*, 1999), and mPFC neurons exhibit task-related activity during PIT testing (Homayoun and Moghaddam, 2009). Despite such findings, it remains unknown if mPFC dopamine signaling is also involved in the expression of PIT performance, let alone whether its involvement is specific to the influence of reward-paired cues on reward seeking versus reward taking.

In the current study, we introduce an analytic approach to parse the effects of reward-paired cues on reward-seeking versus reward-taking behavior during PIT, and investigate how chemogenetically inhibiting mesocorticolimbic dopamine circuits, (globally, or projections to NAc or mPFC), impacts these two distinct aspects of cue-motivated behavior. We also examined the influence of reward value on reward seeking and taking, and its dependence on mesocorticolimbic dopamine.

Materials and Methods

Animals: In total, 93 Long-Evans Tyrosine hydroxylase (Th):Cre+ rats (hemizygous Cre+) (Mahler *et al*, 2018; Mahler *et al*, 2014; Witten *et al*, 2011)

and wildtype (WT) littermates were used for this study. Subjects were at least 3 months of age at the start of the experiment and were single- or paired-housed in standard plexiglass cages on a 12h/12h light/dark cycle. Animals were maintained at ~85% of their free-feeding weight during behavioral procedures. All experimental procedures that involved rats were approved by the UC Irvine Institutional Animal Care and Use Committee and were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

Apparatus: Behavioral procedures took place in sound- and light-attenuated Med Associates chambers (St Albans, VT, USA). Each chamber was equipped with two retractable levers. Grain-based dustless precision pellets (45 mg, BioServ, Frenchtown, NJ, USA) were delivered into a food cup positioned in a recessed magazine between the levers. A photobeam detector was used to record food-cup approaches. Chambers were illuminated during all sessions.

Surgery: Th:Cre+ rats were anesthetized using isoflurane and placed in a stereotaxic frame for microinjections of a Cre-dependent (DIO) serotype 2 adeno-associated virus (AAV) vectors to induce dopamine neuron-specific expression of the inhibitory designer receptor exclusively activated by designer drug (DREADD) hM4Di fused to mCherry (AAV-hSyn-DIO-hM4Di-mCherry), or mCherry alone (AAV-hSyn-DIO- mCherry) (University of North Carolina Chapel Hill vector Core, Chapel Hill, NC, USA / Addgene, Cambridge, MA, USA; Experiment 2 was replicated with both sources). The AAV was injected bilaterally into the VTA (-5.5 mm AP, +/- 0.8 mm ML, -8.15 mm DV; 1uL / side). Experiment 3 rats were bilaterally implanted with guide

cannulae (22 gauge, Plastic One) 1 mm dorsal to NAc (+1.3 AP, +/-1.8 ML, -6.2 DV) or mPFC (+3.00 AP, +/-0.5 ML, -3.0 DV) for subsequent clozapine-n-oxide (CNO) microinjections. Animals were allowed at least 5 days of recovery before undergoing food restriction and behavioral training. Testing occurred at least 25 days after surgery to allow adequate time for viral expression of hM4Di throughout dopamine neurons, including in terminals within the NAc and mPFC.

Experiment 1: Microstructural analysis of reward seeking and taking under conditions of varying response-contingent feedback about reward delivery.

Instrumental learning. WT rats (n = 9) first underwent 2 d of magazine training (40 pellet deliveries in each 1-h daily session) before receiving 9 d of instrumental training. In each session, rats had continuous access to a single lever, which could be pressed to deliver food pellets into the food cup. The schedule of reinforcement for this was adjusted over days from continuous reinforcement (CRF) to increasing random intervals (RI), with one day each of CRF, RI-15s, RI-30s, and 6 days of RI-60s. Each session was terminated after 30 min or after 20 rewards deliveries.

Varying response-contingent feedback. Following training, rats were given a series of tests to assess the role of response-contingent feedback on reward-seeking and reward-taking behavior. Rats were given 3 tests (30-min each, pseudorandom order over days) during which lever pressing caused: 1) activation of the pellet dispenser to deliver a pellet into the food cup (RI-60s schedule), 2) activation of the pellet dispenser to deliver a pellet into an external cup not accessible to the rats, producing associated sound and

tactile cues but no reward (i.e., conditioned reinforcement; also RI-60s schedule), or 3) no dispenser activation (i.e., extinction; no pellets or cues).

Experiments 2 and 3: Role of mesocorticolimbic dopamine in cue-motivated reward seeking and taking:

Pavlovian conditioning. Th:Cre+ rats (n = 64) underwent 2 d of magazine training, as in Experiment 1, before they received 8 daily Pavlovian conditioning sessions, each of which consisted of a series of 6 presentations of a two-min audio cue (CS+; either 10Hz tone or white noise; 80dB), with trials separated by a variable 3-min interval (range 2-4-min). During each CS+ trial, pellets were delivered on a 30-sec random time schedule, resulting in an average of 4 pellets per trial. Rats were separately habituated to an unpaired auditory stimulus (CS-; see Supplemental Materials and Methods). Conditioning was assessed by comparing the rate of food-cup approaches during CS periods (between CS onset to first pellet delivery to avoid detection of unconditioned feeding) to the rate during pre-CS periods.

Instrumental training. Following Pavlovian conditioning, all rats were given 9 d of instrumental training with lever pressing ultimately reinforced on an RI-60s schedule, as in Experiment 1.

Pavlovian-to-instrumental transfer test. After a session of Pavlovian conditioning, rats were given a 30-min extinction session, during which lever presses were recorded but had no consequence (i.e., no pellets or cues). On the next day, rats were given a PIT test, during which the lever was continuously available but produced no rewards. Following 8 min of extinction, the CS+ and CS− were each presented 4 times (2 min per trial) in

pseudorandom order and separated by a 3-min fixed interval. Rats from Experiment 2 (effects of system-wide dopamine neurons inhibition) received CNO (5mg/kg, i.p.) or vehicle (5% DMSO in saline) injection 30 min prior the test. They were subjected to a second test following retraining (see supplemental Materials and Methods), during which they were given the alternative drug pretreatment. Because microinjection procedures produced additional variability in task performance, rats in Experiment 3 (effects of inhibiting dopamine release in NAc and mPFC) underwent a total of 4 tests. They received either CNO microinfusions (1mM, 0.5uL/side or 0.3uL/side, for NAc and mPFC respectively) or vehicle (DMSO 5% in aCSF) 5 min before the start of each test, and were given two rounds of testing each with CNO and vehicle (test order counterbalanced across other experimental conditions).

Experiment 4: *Role of mesocorticolimbic dopamine in goal-directed decision making*:

Instrumental Training. Th:Cre+ rats (n = 20) began with 2 d of magazine training, during which they received 20 grain-pellets and 20 liquid sucrose rewards (0.1 mL of 20% sucrose solution, w/v) on a random 30-s schedule. Rats were then given 10 d of instrumental training on two distinct action–outcome contingencies (e.g., left lever press → grain; right lever press→ sucrose) on a training schedule that gradually shifted on random ratio 20 (RR-20) schedule of reinforcement (see Supplemental Materials and Methods for details).

Devaluation Testing. To selectively devalue one of the food outcomes prior to testing, all rats were satiated on grain pellets or sucrose solution by

providing them with 90 min of unrestricted access to that food in the home cage. After 60 min of feeding, rats received CNO (5mg/kg, i.p.) or vehicle injections. After an additional 30 minutes of feeding, rats were placed in the chamber for a test in which they had continuous access to both levers. The test began with a 5-min extinction phase (no rewards or cues), which was immediately followed by a 15-min reinforced phase, during which each action was reinforced with its respective outcome (CRF for the first 5 rewards, then RR-20). Rats were given a total of 4 devaluation tests, 2 after CNO and 2 after vehicle, alternating the identity of the devalued outcome across the 2 tests in each drug condition (test order counterbalanced across training and drug conditions).

Drugs: CNO was obtained from NIMH (Experiments 2 and 4) or Sigma-Aldrich (St. Louis, MO, USA; Experiment 3), and dissolved in 5% DMSO in saline, or aCSF for microinjection.

Analysis: Lever presses were used to operationally define *reward-seeking behavior*. Because rats must approach the food cup in order to collect (take) any food that their reward seeking may have produced, we used this behavior to identify *reward taking*. We based our specific definition of reward taking on the natural microstructural pattern of food-cup approaches that surrounded individual lever presses, which was significantly elevated for the first 2.5 sec post-press period (Figure S1)(see also Marshall and Ostlund, *in press*). Given the inherent contingency between these measures of seeking and taking, we

focused our analysis on the probability that a reward-seeking action was followed by a bout of reward taking (i.e., at least one food cup approach within 2.5 sec). This measure (total reward-taking bouts / total reward-seeking actions) controls for variability across conditions in the opportunity that rats had to engage in reward taking. We operationally defined the subset of reward seeking actions that were not directly followed by reward taking as "exploratory" reward-seeking actions. Statistical analysis employed two-sided *t*-tests and general(ized) linear mixed-effects models (Pinheiro and Bates, 2000), the latter of which allows for simultaneous parameter estimation as a function of condition (fixed effects) and the individual rat (random effects). Using the Statistics and Machine Learning Toolbox in MATLAB, mixed-effects models provide *t*-values to reflect the statistical significance of the regression coefficient relative to the population mean (i.e., simple effects). These simple effects are indicative of main effects and interactions when a factor has at most two levels. For factors with at least 3 levels, *F*-tests were conducted to reveal the overall significance of the effect or interaction(s) involving this factor. A full description of the mixed-effects model analysis is available in Supplementary Materials and Methods.

Results

Microstructural characteristics of reward seeking and taking: effects of response-contingent feedback about reward delivery

We first characterized how the omission of response-contingent reward impacts the microstructural organization of instrumental reward-seeking and reward-taking actions, which is an important first step in understanding how

reward-paired cues influence such behavior. We analyzed the microstructural relationship between lever pressing (RI-60s schedule) and food-cup approach behavior to collect food pellets (see Methods and Figure 1A for illustration). As seen in Fig 1B, rats were more likely to approach the food cup within a few seconds of performing a lever-press response than they were at other times. Not surprisingly, rats were much more likely to approach the food cup when a lever press was reinforced than when it was not ($t(8) = 19.33$, $p < .001$), suggesting that they could detect the pellets being delivered based on sound and tactile cues produced by dispenser activation. Food-cup approaches also increased following nonreinforced presses (Fig 1B, Fig 1C), indicating that rats also made sporadic, uncued attempts to take rewards that were not actually delivered. To explore the factors controlling cued and uncued reward taking, rats were given a series of tests in which lever pressing produced either 1) pellet dispenser cues and pellet delivery, 2) pellet dispenser cues only (no pellet delivery), or 3) no pellet dispenser cues or pellet delivery (extinction). Reward-taking actions were defined as at least one food-cup approach response occurring within 2.5 sec of a lever press (see Fig S1 and Materials and Methods for further details). Given the inherent contingency between reward seeking and reward taking, we focused our analysis on the proportion of all reward-seeking actions (individual lever presses) that were followed by a reward-taking action (Fig 1D and 1E). We found that this measure of reward taking was highly sensitive to manipulations of reinforcement contingency. Reward taking was much more likely to occur after reinforced than nonreinforced trials, regardless of whether the pellet dispenser cues were presented alone or together with food delivery (Fig 1F;

*t*s(8) ≥ 13.74, *p*s < .001). When lever presses had no consequence at all (i.e., during the extinction test), rats rarely followed such actions with an attempt to collect food from the cup (Fig 1F). These data generally support the view developed above that animals adapt to reward omission by applying a more *exploratory* reward-seeking strategy that normally involves withholding any subsequent attempt to collect reward, as if they no longer perceived this reward-seeking action to be a reliable way to produce reward.

Inhibiting dopamine neurons during Pavlovian-to-instrumental transfer preferentially disrupts cue-motivated reward seeking, but not taking

We next conducted a PIT experiment to investigate the microstructural organization of cue-motivated reward seeking and taking, and determine how dopamine contributes to such effects. Rats with dopamine neuron-specific expression of hM4Di or mCherry (Fig 2) were trained on the PIT task (Fig 3A), which consisted a Pavlovian conditioning phase, in which two different auditory cues were paired (CS+) or unpaired (CS-) with food pellets, and a separate instrumental training phase, in which rats were trained to lever press for pellets as in Experiment 1. During PIT testing, we noncontingently presented the CS+ and CS- while rats freely engaged in nonreinforced reward seeking and taking.

Pretreatment with the DREADD ligand CNO suppressed reward seeking specifically during CS+ periods in hM4Di but not mCherry rats (Fig 3B; significant Group * Drug * CS Period * CS Type interaction, $t(240) = -3.14$, $p = .002$; see Supplementary Information Table S1 for full generalized linear mixed-effects model output). Control rats in the mCherry group exhibited

elevated lever pressing during CS+ trials (relative to pre-CS response rates; CS Period * CS Type interaction, $p < .001$), and this effect was not altered by CNO (Drug * CS Period * CS Type interaction, $p = .780$). In contrast, CNO pretreatment disrupted expression of CS+ motivated reward seeking in the hM4Di group (Drug * CS Period * CS Type interaction, $p < .001$). Specifically, hM4Di rats showed a CS+ specific elevation in lever pressing when pretreated with vehicle (CS Period * CS Type interaction, $p < .001$) but not CNO (CS Period * CS Type interaction, $p = .684$). The increase in lever pressing during the CS+ (PIT score: CS+ - pre-CS) was significant for both vector groups when pretreated with vehicle (one-sample t-tests; *p*s < .001; Fig 3C), but was significantly suppressed by CNO in the hM4Di group ($t(17) = -3.83$, $p = .001$), and not in the mCherry group ($t(13) = -1.21$, $p = .249$; Fig 3C).

These findings indicate that inhibiting dopamine neurons disrupts the ability of a reward-paired cue to motivate reward-seeking behavior, so we next investigated the microstructural relationship between lever pressing and food cup-approach behavior to assess how the CS+ impacted the perceived efficacy of reward-seeking action and whether this influence was also sensitive to dopamine inhibition (Fig 3E and 3F). As in Experiment 1, we focused our analysis on the proportion of reward-seeking actions that were immediately followed by a food-cup approach to normalize for differences in reward-taking opportunities (Fig 3E; see Fig S2 and Table S2 for analysis of reward taking *frequency*). This measure revealed that the CS+ ($p < .001$) but not the CS- ($p = .501$) increased the probability that rats would attempt to take a reward after lever pressing. There was no evidence of an effect of dopamine

inhibition on this CS+ induced increase in reward taking (interactions involving Drug or Group, *p*s > .109; Table S3). Therefore, although noncontingent CS+ presentations caused rats to act as if they expected their reward-seeking behavior was once again effective in producing imminent reward delivery, this influence did not depend on VTA dopamine neuron activity.

Pathway-specific inhibition of dopamine projections to NAc, but not mPFC, disrupts cue-motivated reward seeking in Pavlovian instrumental transfer

As previously reported (Mahler *et al*, 2018), hM4Di expression in VTA dopamine neurons resulted in transport of DREADDs to axonal terminals in the NAc and mPFC (Fig 2). To investigate the roles of these two pathways on cue-motivated behavior, we conducted a PIT experiment in a separate set of rats with hM4Di or mCherry infection of VTA dopamine neurons, and guide cannulae aimed at the NAc or mPFC (Fig 4A; Fig S3). These rats underwent training and testing for PIT, as described above (Fig 4B), but were pretreated with intra-NAc or mPFC injections of CNO (1mM) or vehicle to achieve local inhibition of neurotransmitter release (Lichtenberg *et al*, 2017; Mahler *et al*, 2014; Stachniak *et al*, 2014), an approach previously shown to be effective in inhibiting these specific dopamine projections (Mahler *et al*, 2018). Figure 4C shows that, in hM4Di expressing rats, the CS+ specific increase in reward seeking was disrupted by CNO in a manner that depended on the microinjection site (Drug * CS Period * CS Type * Site interaction, $t(240) = -2.99$, $p = .003$; Table S4). After intracranial vehicle injections, rats showed a CS+ specific elevation in reward seeking (CS Period * CS Type interaction,

$p < .001$), which did not differ significantly across vehicle injection sites (CS Period * CS Type * Site interaction, $p = .151$). Unlike with systemic CNO, the CS+ remained effective in eliciting reward seeking after CNO microinjection into the mPFC (CS Type * CS Period interaction, $p < .001$) or NAc (CS Type * CS Period interaction, $p < .001$). However, this effect was significantly attenuated after CNO in NAc, relative to mPFC (CS Period * CS Type * Site interaction, $p = .012$). A more focused analysis (Fig 4D) of the net change (PIT score: CS+ - pre-CS+) in pressing on CS+ trials confirmed that both NAc and mPFC rats showed significant elevations when pretreated with vehicle (one-sample t-tests; *p*s ≤ .001), and that this effect was suppressed after intra-NAc ($t(6) = -2.49$, $p = .047$), but not intra-mPFC ($t(8) = 0.34$, $p = .746$) CNO. In contrast, CNO treatment had no effect in mCherry expressing rats (Fig S4).

As in the previous experiment, the CS+ was also effective in increasing the probability that rats would engage in reward taking after performing a reward-seeking action (Fig 4E). Specifically, we found that the CS+ ($p < .001$) increased the probability that a reward-seeking action was followed by an attempt to take reward, which was not affected by CNO at either site (*p*s > .187; Table S6; see Fig S5 and Table S5 for analysis of reward taking frequency). The CS- produced a decrease in the probability of reward taking given reward seeking compared to both pre-CS baseline ($p = .040$) and the CS+ ($p < .001$).

Given that inhibiting dopamine terminals in the NAc resulted in only a partial suppression of CS+ motivated reward seeking, but not its ability to promote reward taking, we hypothesized that variability in rats' sensitivity to

the suppressive effects of this treatment may be explained by individual differences in their tendency to respond to the CS+ by engaging in *exploratory* bouts of reward seeking (i.e., presses that were not followed by an immediate attempt to take reward). Consistent with this, we found that for NAc rats, CS+ elicited increases in exploratory reward seeking (CS+ - pre-CS+) during the vehicle test were negatively correlated with CNO-induced suppression of reward seeking (all presses; $r = -0.81$, $p = .027$; Fig 4F). No such relationship was seen between exploratory seeking on vehicle day and sensitivity to CNO in mPFC rats ($r = -0.19$, $p = .618$).

Inhibiting dopamine neurons spares use of outcome value when selecting between reward-seeking actions

We also investigated the role of the mesocorticolimbic dopamine system in an important aspect of uncued, goal-directed behavior – the ability to flexibly choose between actions based on the current value of the rewards they produce. Sensitivity to changes in the value of food rewards, induced by sensory-specific satiety, was assessed in a separate set of rats expressing mCherry or hM4Di in VTA dopamine neurons, following administration of CNO (5mg/kg) or vehicle (Fig 5A). We found that rats pressed significantly less for the devalued food than for the non-devalued food ($t(148) = -5.41$, $p < .001$; Fig 5B; Table S7). CNO treatment did not significantly affect sensitivity to devaluation in either hM4Di or mCherry rats (ps $\geq .146$), indicating that this aspect of goal-directed decision making is not disrupted by dopamine neuron inhibition. Inhibiting VTA dopamine neurons also failed to disrupt sensitivity to devaluation during reinforced testing (see Fig S6). Interestingly, devaluation

produced a modest but significant *increase* in the proportion of reward-seeking actions that were followed by reward taking, $p = .040$, suggesting that once rats initiated a reward-seeking action, they were at least as likely to attempt to take both a devalued reward as a nondevalued reward (i.e., when a reward is judged to be worth pursuing, it is also worth trying to collect). This measure of reward taking was not sensitive to dopamine inhibition (Fig 5C; *p*s ≥ .109; Table S9; see Fig S7 and Table S8 for analysis of reward taking frequency).

Discussion

Our findings support the view that reward-taking behavior (i.e., attempting to collect food immediately after lever pressing) scales with rats' perception of the efficacy of their reward-seeking behavior and that reward-paired cues influence this specific aspect of behavior, as if it had reinstated their expectation of response-contingent reward. Importantly, we found that inhibiting VTA dopamine neurons abolished cue-motivated reward *seeking* but not *taking*. Specifically, chemogenetic inhibition of dopamine neurons attenuated the ability of a food-paired CS+ to increase reward seeking, even though this cue continued to increase the likelihood that the few remaining presses were followed by an immediate attempt to take reward by approaching the food cup. A similar, albeit less pronounced, deficit in cue-motivated reward seeking was also observed when we inhibited dopamine terminals in the NAc, and, again, the ability of the CS+ to increase reward taking was spared. Inhibiting dopamine projections to the mPFC had no effect

on either of these behavioral effects of the CS+. Dopamine pathway inhibition also failed to alter the sensitivity of instrumental reward seeking to outcome devaluation, demonstrating the remarkable specificity of its role in cue-triggered incentive motivation.

The lack of effect of mPFC dopamine inhibition on cue-motivated reward seeking (or taking) reported here is notable given electrophysiological findings implicating this region in the expression of PIT (Homayoun *et al*, 2009), though it should be noted that lesions of the mPFC have typically found little evidence that it is necessary for this aspect of behavior (Cardinal *et al*, 2003; Corbit and Balleine, 2003b). Such findings suggest that the mPFC may be engaged during this task, but does not normally play a causal role in the expression of PIT.

Our finding that inhibiting VTA dopamine neurons did not impact rats' sensitivity to outcome devaluation is consistent with previous findings demonstrating that this aspect of goal-directed decision making is dopamine-independent (Dickinson *et al*, 2000; Lex and Hauber, 2009; Lex and Hauber, 2010; Wassum *et al*, 2011), even though the NAc and mPFC make important contributions to this process (Bradfield and Balleine, 2017). Although our findings suggest that mesocorticolimbic dopamine signaling is not normally required for this aspect of cognitive control, they do not rule out its involvement when goal-directed decisions are more complex and/or require greater cognitive resources (Cools, 2015; Floresco, 2013; Westbrook and Braver, 2016).

Although PIT studies typically focus on the excitatory influence of reward-paired cues on reward-*seeking* behavior, previous studies (Corbit and

Balleine, 2003a; LeBlanc *et al*, 2012) using more complex action sequence tasks (e.g., press left lever → press right lever → food) have shown that such cues can also strongly invigorate an action that is needed to complete the instrumental contingency (i.e., the right lever press), which might therefore be considered a reward-*taking* behavior in that it is proximal to reward delivery. Here, we introduce an analytic approach allowing detection of cue-motivated changes in reward taking, distinct from reward seeking, using more conventional, well-characterized PIT testing procedures. This approach may help address a long-standing debate about the processes controlling food-cup approach behavior when animals are engaged in instrumental reward seeking. For instance, during PIT testing, it is generally assumed that food-cup approaches during the CS+ reflect a Pavlovian conditioned response (Holland and Gallagher, 2003; Holmes *et al*, 2010; Homayoun *et al*, 2009; Pecina *et al*, 2006), even though approaching the food cup is in fact required for collecting response-contingent food rewards and may therefore be instrumental in nature. The present PIT data show that food-cup approaches that occur during CS+ presentations are heterogeneous, with some occurring immediately after an instrumental lever press (attempted reward-taking actions) and others occurring at other times, most likely reflecting more conventional Pavlovian conditioned responses (see Marshall and Ostlund, *in press*). We found that these *instrumental* reward-taking food cup approaches were more likely to occur when a reward was expected, such as when response-contingent feedback signaled the delivery of reward, supporting the view that these responses relate to the perceived efficacy of the reward-seeking action.

We show that these distinct excitatory effects of the reward-paired cue on reward seeking and taking are neurochemically dissociable, such that inhibition of dopamine neurons, or their projections to NAc, disrupts the cue's influence on seeking, without impacting its influence on taking. This is consistent with findings that dopamine supports a general motivational process that facilitates the expression of preparatory, or exploratory, reward-seeking behavior, but is relatively unimportant for consummatory, or reward-taking, actions (Fibiger and Phillips, 1986; Ikemoto and Panksepp, 1996; Veeneman *et al*, 2012). For example, fast-scan cyclic voltammetry studies reveal that the mesolimbic dopamine system is strongly engaged during the initiation of reward seeking, but not during reward taking (Cacciapaglia *et al*, 2012; Collins *et al*, 2016; Klanker *et al*, 2015; Wassum *et al*, 2012). Likewise, our own prior studies have shown that phasic dopamine release in the NAc is correlated with the vigor of cue-motivated lever pressing (Ostlund *et al*, 2014; Wassum *et al*, 2013) and not food cup approaches (Aitken *et al*, 2016), and that individual transient dopamine release events are temporally correlated with the execution of individual lever-press responses (Ostlund *et al*, 2014). Altogether, such findings support the view that dopamine's role in regulating the pursuit of rewards varies as a function of reward proximity, contributing predominantly to the initiation of reward-seeking actions, rather than to their completion (at least under low effort conditions, as tested here).

The present results may also have implications for understanding the role of dopamine in pathologies of behavioral control such as obsessive-compulsive disorder (OCD). In the signal attenuation model of OCD (Joel *et al*, 2001; Ostlund *et al*, 2014), rats learn that they can no longer rely on

response-contingent cues to signal whether or not their reward-seeking behavior has been successful. When this happens, logical organization of their reward-seeking and reward-taking behavior disintegrates, and rats come to exhibit excessive reward seeking, typically without attempting to take reward. Importantly, blocking D1-dopamine receptors disrupts expression of these compulsive (incomplete) bouts of reward seeking, without affecting the production of complete bouts of seeking and taking, which continue to be infrequently performed on some test trials even though these rats no longer have reason to be confident in the efficacy of their reward-seeking behavior (Joel and Doljansky, 2003). Considered in this light, our findings suggest that mesolimbic dopamine circuits may mediate the tendency for reward-paired cues to promote this exploratory, or even compulsive, form of reward seeking, rather than more purposeful attempts to pursue reward. Interestingly, we found that the rats showing the greatest propensity to respond to the CS+ by exploratory seeking without taking were the same rats showing the greatest suppressive effect of NAc dopamine inhibition. Future research is warranted to further investigate this link between mesolimbic dopamine signaling and exploratory cue-motivated reward seeking, which may reflect a behavioral marker that is relevant to understanding compulsive disorders like OCD and addiction (Joel *et al*, 2008; Robinson *et al*, 2014).

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References

Aitken TJ, Greenfield VY, Wassum KM (2016). Nucleus accumbens core dopamine signaling tracks the need‐based motivational value of food‐paired cues. *Journal of neurochemistry* **136**(5): 1026-1036.

Bassareo V, Di Chiara G (1997). Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. *J Neurosci* **17**(2): 851-861.

Beeler J, Cools R, Luciana M, Ostlund S, Petzinger G (2014). A kinder, gentler dopamine… highlighting dopamine's role in behavioral flexibility. *Frontiers in Neuroscience* **8**(4).

Berridge KC, Robinson TE (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist* **71**(8): 670.

Bradfield L, Balleine B (2017). The Learning and Motivational Processes Controlling Goal-Directed Action and Their Neural Bases. *Decision Neuroscience*. Elsevier, pp 71-80.

Cacciapaglia F, Saddoris MP, Wightman RM, Carelli RM (2012). Differential dopamine release dynamics in the nucleus accumbens core and shell track distinct aspects of goal-directed behavior for sucrose. *Neuropharmacology* **62**(5-6): 2050-2056.

Cardinal RN, Parkinson JA, Marbini HD, Toner AJ, Bussey TJ, Robbins TW*, et al* (2003). Role of the anterior cingulate cortex in the control over behavior by Pavlovian conditioned stimuli in rats. *Behav Neurosci* **117**(3): 566-587.

Cartoni E, Balleine B, Baldassarre G (2016). Appetitive Pavlovian-instrumental Transfer: A review. *Neurosci Biobehav Rev* **71**: 829-848.

Cartoni E, Moretta T, Puglisi-Allegra S, Cabib S, Baldassarre G (2015). The relationship between specific Pavlovian instrumental transfer and instrumental reward probability. *Frontiers in psychology* **6**: 1697.

Cartoni E, Puglisi-Allegra S, Baldassarre G (2013). The three principles of action: a Pavlovian-instrumental transfer hypothesis. *Frontiers in Behavioral Neuroscience* **7**: 153.

Choi WY, Balsam PD, Horvitz JC (2005). Extended habit training reduces dopamine mediation of appetitive response expression. *J Neurosci* **25**(29): 6729-6733.

Collins AL, Greenfield VY, Bye JK, Linker KE, Wang AS, Wassum KM (2016). Dynamic mesolimbic dopamine signaling during action sequence learning and expectation violation. *Scientific reports* **6**.

Cools R (2015). The cost of dopamine for dynamic cognitive control. *Current Opinion in Behavioral Sciences* **4**: 152-159.

Corbit LH, Balleine BW (2003a). Instrumental and Pavlovian incentive processes have dissociable effects on components of a heterogeneous instrumental chain. *Journal of Experimental Psychology: Animal Behavior Processes* **29**(2): 99.

Corbit LH, Balleine BW (2003b). The role of prelimbic cortex in instrumental conditioning. *Behav Brain Res* **146**(1-2): 145-157.

Corbit LH, Balleine BW (2016). Learning and Motivational Processes Contributing to Pavlovian-Instrumental Transfer and Their Neural Bases: Dopamine and Beyond. *Curr Top Behav Neurosci* **27**: 259-289.

Corbit LH, Janak PH, Balleine BW (2007). General and outcome‐specific forms of Pavlovian‐instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. *European Journal of Neuroscience* **26**(11): 3141-3149.

Dickinson A, Smith J, Mirenowicz J (2000). Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behavioral neuroscience* **114**(3): 468.

Farrell MR, Schoch H, Mahler SV (2018). Modeling cocaine relapse in rodents: Behavioral considerations and circuit mechanisms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Feenstra MG, Teske G, Botterblom MH, De Bruin JP (1999). Dopamine and noradrenaline release in the prefrontal cortex of rats during classical aversive and appetitive conditioning to a contextual stimulus: interference by novelty effects. *Neurosci Lett* **272**(3): 179-182.

Fibiger H, Phillips A (1986). Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. *Comprehensive Physiology*.

Floresco SB (2013). Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Front Neurosci* **7**: 62.

Holland PC, Gallagher M (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *European Journal of Neuroscience* **17**(8): 1680-1694.

Holmes NM, Marchand AR, Coutureau E (2010). Pavlovian to instrumental transfer: A neurobehavioural perspective. *Neuroscience & Biobehavioral Reviews* **34**(8): 1277-1295.

Homayoun H, Moghaddam B (2009). Differential representation of Pavlovian–instrumental transfer by prefrontal cortex subregions and striatum. *European Journal of Neuroscience* **29**(7): 1461-1476.

Ikemoto S, Panksepp J (1996). Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. *Behavioral neuroscience* **110**(2): 331.

Joel D, Avisar A (2001). Excessive lever pressing following post-training signal attenuation in rats: a possible animal model of obsessive compulsive disorder? *Behavioural brain research* **123**(1): 77-87.

Joel D, Doljansky J (2003). Selective Alleviation of Compulsive Lever-Pressing in Rats by D 1, but not D 2, Blockade: Possible Implications for the Involvement of D 1 Receptors in Obsessive–Compulsive Disorder. *Neuropsychopharmacology* **28**(1): 77.

Joel D, Stein DJ, Schreiber R (2008). CHAPTER 6 - Animal Models of Obsessive–Compulsive Disorder: From Bench to Bedside via Endophenotypes and Biomarkers A2 - McArthur, Robert A. In: Borsini F (ed). *Animal and Translational Models for CNS Drug Discovery*. Academic Press: San Diego, pp 133-164.

Klanker M, Sandberg T, Joosten R, Willuhn I, Feenstra M, Denys D (2015). Phasic dopamine release induced by positive feedback predicts individual differences in reversal learning. *Neurobiology of Learning and Memory* **125**: 135-145.

Kruse JM, Overmier JB, Konz WA, Rokke E (1983). Pavlovian conditioned stimulus effects upon instrumental choice behavior are reinforcer specific. *Learning and motivation* **14**(2): 165-181.

Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J (2008). Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* **57**(5): 760-773.

LeBlanc KH, Ostlund SB, Maidment NT (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behavioral neuroscience* **126**(5): 681.

Lex A, Hauber W (2008). Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. *Learn Mem* **15**(7): 483-491.

Lex B, Hauber W (2009). The role of dopamine in the prelimbic cortex and the dorsomedial striatum in instrumental conditioning. *Cerebral cortex* **20**(4): 873-883.

Lex B, Hauber W (2010). The role of nucleus accumbens dopamine in outcome encoding in instrumental and Pavlovian conditioning. *Neurobiol Learn Mem* **93**(2): 283-290.

Lichtenberg NT, Pennington ZT, Holley SM, Greenfield VY, Cepeda C, Levine MS*, et al* (2017). Basolateral Amygdala to Orbitofrontal Cortex Projections Enable Cue-Triggered Reward Expectations. *The Journal of Neuroscience* **37**(35): 8374-8384.

Mahler SV, Brodnik ZD, Cox BM, Buchta WC, Bentzley BS, Cope ZA*, et al* (2018). Chemogenetic Manipulations of Ventral Tegmental Area Dopamine Neurons Reveal Multifaceted Roles in Cocaine Abuse. *bioRxiv*.

Mahler SV, Vazey EM, Beckley JT, Keistler CR, McGlinchey EM, Kaufling J*, et al* (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nat Neurosci* **17**(4): 577-585.

Marshall AT, Ostlund SB (in press). Repeated cocaine exposure dysregulates cognitive control over cue-evoked reward-seeking behavior during Pavlovian-to-instrumental transfer. *Learning & Memory*.

Murschall A, Hauber W (2006). Inactivation of the ventral tegmental area abolished the general excitatory influence of Pavlovian cues on instrumental performance. *Learning & Memory* **13**(2): 123-126.

Ostlund SB, Halbout B (2017). Chapter 29 - Mesolimbic Dopamine Signaling in Cocaine Addiction A2 - Preedy, Victor R. *The Neuroscience of Cocaine*. Academic Press: San Diego, pp 287-295.

Ostlund SB, Kosheleff AR, Maidment NT (2012). Relative Response Cost Determines the Sensitivity of Instrumental Reward Seeking to Dopamine Receptor Blockade. *Neuropsychopharmacology* **37**: 2653.

Ostlund SB, LeBlanc KH, Kosheleff AR, Wassum KM, Maidment NT (2014). Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology* **39**(10): 2441-2449.

Ostlund SB, Maidment NT (2011). Dopamine Receptor Blockade Attenuates the General Incentive Motivational Effects of Noncontingently Delivered Rewards and Reward-Paired Cues Without Affecting Their Ability to Bias Action Selection. *Neuropsychopharmacology* **37**: 508.

Pecina S, Schulkin J, Berridge KC (2006). Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress? *BMC biology* **4**: 8.

Pinheiro J, Bates D (2000). *Mixed-effects models in S and S-Plus* Springer: New York.

Rescorla RA (1994). Control of instrumental performance by Pavlovian and instrumental stimuli. *Journal of Experimental Psychology: Animal Behavior Processes* **20**(1): 44.

Rescorla RA, Solomon RL (1967). Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. *Psychological review* **74**(3): 151.

Robinson TE, Yager LM, Cogan ES, Saunders BT (2014). On the motivational properties of reward cues: individual differences. *Neuropharmacology* **76**: 450-459.

Salamone JD, Correa M (2012). The mysterious motivational functions of mesolimbic dopamine. *Neuron* **76**(3): 470-485.

Stachniak TJ, Ghosh A, Sternson SM (2014). Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus-->midbrain pathway for feeding behavior. *Neuron* **82**(4): 797-808.

Veeneman MMJ, van Ast M, Broekhoven MH, Limpens JHW, Vanderschuren LJMJ (2012). Seeking–taking chain schedules of cocaine and sucrose self-administration: effects of reward size, reward omission, and α-flupenthixol. *Psychopharmacology* **220**(4): 771-785.

Wassum KM, Ostlund SB, Balleine BW, Maidment NT (2011). Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. *Learning & memory* **18**(7): 475-483.

Wassum KM, Ostlund SB, Loewinger GC, Maidment NT (2013). Phasic mesolimbic dopamine release tracks reward seeking during expression of Pavlovian-to-instrumental transfer. *Biological psychiatry* **73**(8): 747-755.

Wassum KM, Ostlund SB, Maidment NT (2012). Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. *Biological psychiatry* **71**(10): 846-854.

Westbrook A, Braver TS (2016). Dopamine Does Double Duty in Motivating Cognitive Effort. *Neuron* **89**(4): 695-710.

Witten IB, Steinberg EE, Lee SY, Davidson TJ, Zalocusky KA, Brodsky M*, et al* (2011). Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron* **72**(5): 721-733.

Figure Legends

Figure 1. Microstructural organization of instrumental behavior. **A.** Hungry rats were trained to perform a self-paced task (RI-60s schedule of reinforcement) in which "reward-seeking" lever presses could be followed by immediate food-cup approaches to collect the food (i.e., "reward taking"). **B.** Probability of food-cup approaches as a function of time surrounding a reinforced or nonreinforced lever press. **C.** Representative organization of instrumental behaviors for an individual rat showing reward-seeking and -taking actions for each reinforced or nonreinforced press. Each line represents a reinforced trial during RI-60s training. **D, E.** Effects of the manipulation of reinforcement contingency on the organization of instrumental actions. Total reward-seeking (D) or -taking (E) actions at tests during which lever pressing was either reinforced under an RI-60s schedule of reinforcement with pellet delivery (Food), cues associated with food pellets delivery (Cue; i.e., activation of the pellet dispenser), or not reinforced (Extinction). **F.** The probability of reward taking given a reward-seeking action is highly dependent on the reinforcement contingency condition. Attempted reward taking was much more likely to occur after reinforced than nonreinforced trials, regardless of whether reinforcement was with Food, or Cue. Rats rarely followed lever press with an attempt to collect food from the cup in nonreinforced (including Extinction) conditions.

Figure 2. DREADD expression in Th:Cre+ rats. **A.** Th:Cre+ rats received bilateral injections of AAV-hSyn-DIO-hM4Di-mCherry or AAV-hSyn-DIO-mCherry in the VTA. **B.** Representative expression of the mCherry-tagged inhibitory DREADD hM4Di (red) in VTA Th positive neurons (green) of Th:Cre+ rats, as well as in neuronal terminals (**C.**) projecting to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). Scale bar is 500um.

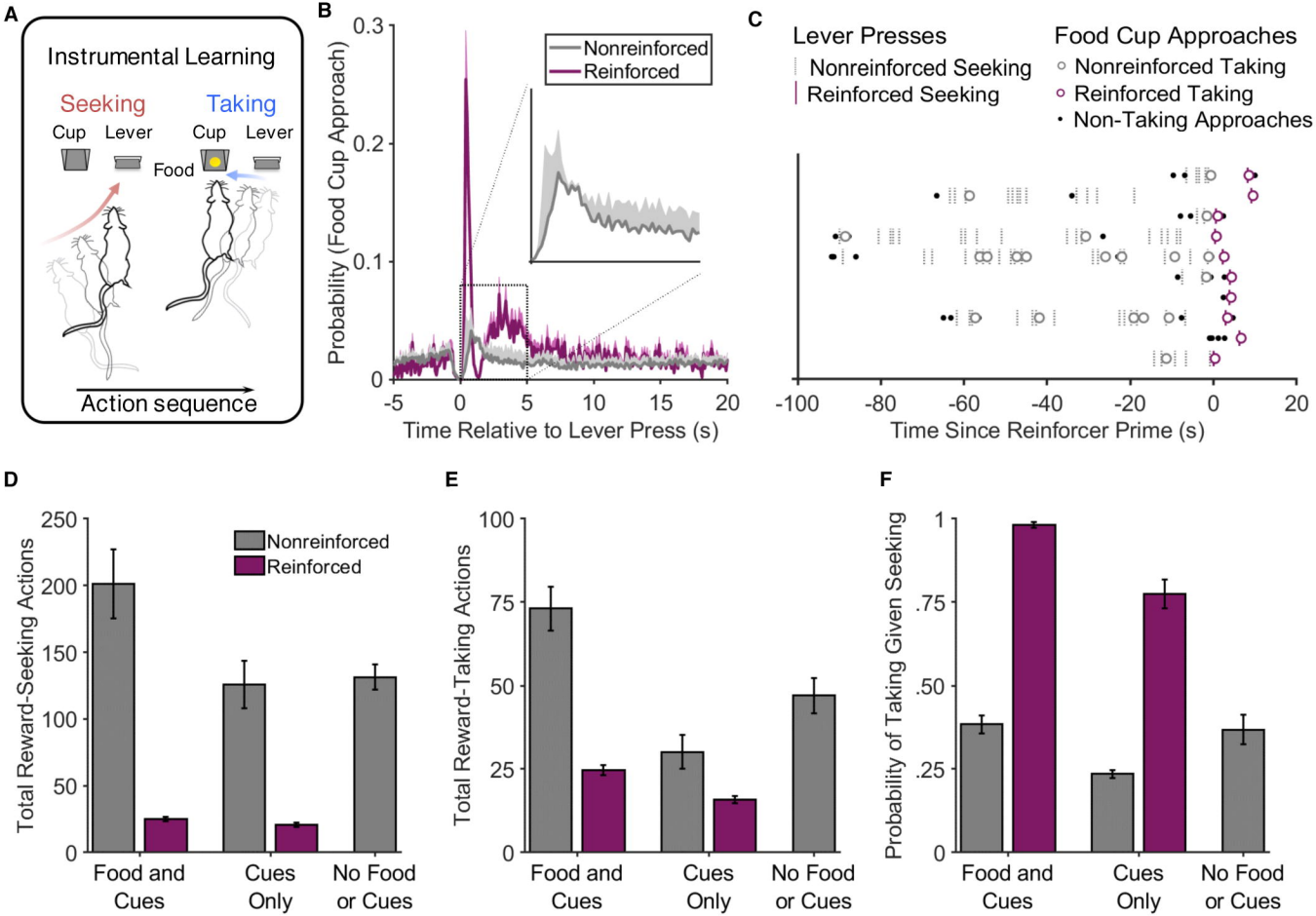
Figure 3. Chemogenetic inhibition of dopamine neurons in Pavlovian to instrumental transfer (PIT). **A.** Experimental design: Following viral vectors injections and recovery, rats received Pavlovian training, during which they learned to associate an auditory cue (CS+) with food pellet delivery. During instrumental learning rats learned to lever press according to the same task used in Experiment 1. Lever pressing was extinguished (Ext) before rats were submitted to a Pavlovian to Instrumental Transfer test, during which CS+ (or a control CS-) were randomly presented. We studied the effect of CNO (0 or 5mg/kg within-subject design) pre-treatment on reward-seeking and reward-taking at test. **B.** Chemogenetic inhibition of VTA dopamine neurons disrupt cue-motivated reward seeking. Reward-seeking actions in rats expressing the inhibitory DREADD hM4Di or mCherry following vehicle (left) or CNO (5mg/kg, right) treatment prior to test. Grey bars represent instrumental actions during the pre-CS period, red bars represent reward-seeking actions during CSs presentation. **C.** PIT expression is specifically impaired in hM4Di expressing Th:Cre+ rats. Left panel, analysis of PIT scores after vehicle treatment (Reward seeking during CS minus reward seeking during Pre-CS) show a significant elevation of reward-seeking behaviors during CS+ period for both groups; right panel, analysis of the CNO suppression score (PIT score following vehicle treatment minus PIT score following CNO treatment)

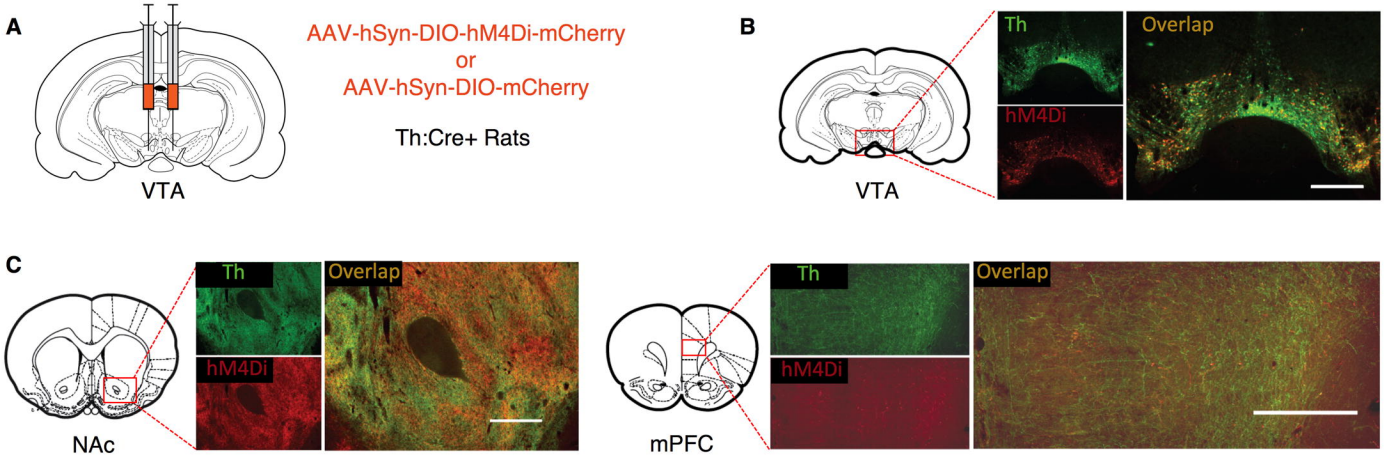
show a significant effect of CNO only in hM4Di expressing rats. *p<0.05 vs 0. **D.** Proportions of reward-seeking actions that were immediately followed by an attempt to take the reward during different PIT periods do not differ as a function of treatment or group, but were increased during CS+ as opposed to CS- or pre-CS periods. **E.** Representative organization of instrumental behaviors in reward-taking and -seeking actions in PIT. Data show responses for two individual Th:Cre+ rats expressing mCherry and receiving vehicle treatment during pre-CS and CS presentation periods at test.

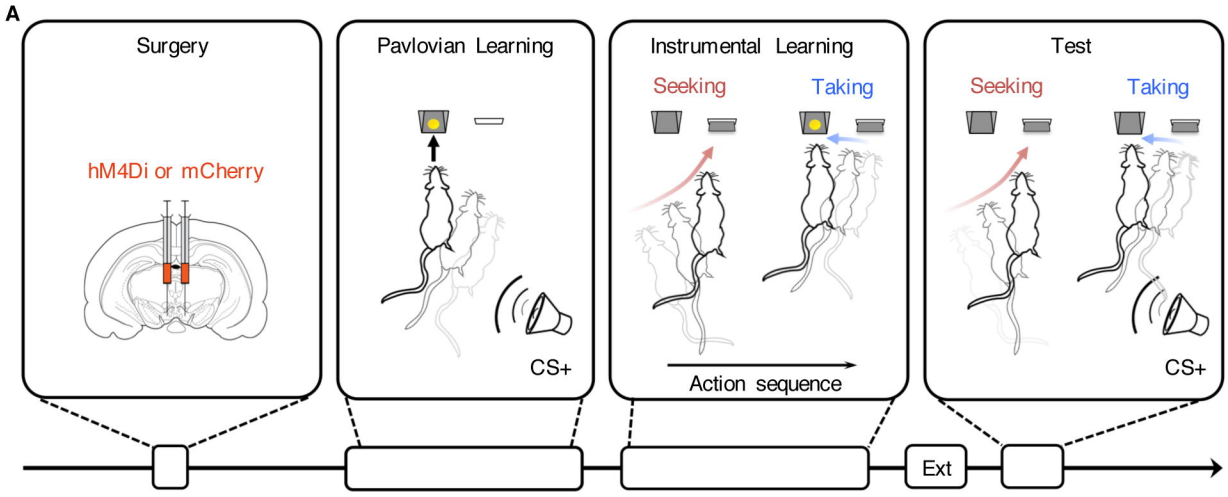
Figure 4. Pathway specific chemogenetic inhibition of dopamine in PIT. A. Th:Cre+ rats initially received VTA AAV-hSyn-DIO-hM4Di-mCherry injections and were implanted with guide cannulas aimed at the nucleus accumbens (NAc) or medial prefrontal cortex (mPFC) for microinjection of CNO (1mM) or vehicle (veh) to inhibit dopamine release at terminals at test. B. Following surgery, rats underwent PIT training and testing as described above (Pavlovian: Pavlovian Learning; Instr: Instrumental Learning; Ext: Extinction). We analyzed the microstructural organization of behavior (reward-seeking and reward-taking actions) at test. C. Pathway specific inhibition of dopamine release in the nucleus accumbens (NAc) but not the medial prefrontal cortex (mPFC) disrupts reward seeking in PIT. Reward-seeking actions during PIT in rats expressing the inhibitory DREADD hM4Di and receiving CNO or vehicle microinfusions in either the NAc or mPFC. Grey bars represent instrumental actions during the pre-CS period, red bars represent reward-seeking during CS presentation. D. PIT expression is specifically impaired following NAc CNO treatment. Left panel, analysis of PIT scores followingy vehicle treatment (Reward seeking during CS minus reward seeking during Pre-CS) show a significant elevation of reward-seeking behaviors during CS+ period for both groups; right panel, analysis of the CNO suppression score (PIT score following vehicle treatment minus PIT score following CNO treatment) show a significant effect of CNO only when injected in the NAc. *p<0.05 vs 0. E. Proportions of reward-seeking actions that were immediately followed by an attempt to take the reward during different PIT periods do not differ as a function of treatment or group, but were increased during CS+ as opposed to CS- or pre-CS periods. F. Scatter plot showing the relationship of individual differences in the effect of CS+ on exploratory reward-seeking actions in the vehicle condition (Δ Exploratory Seeking; i.e., presses non followed by an attempt to collect reward) and the suppressive effect of CNO on reward seeking. Data points represent individual rats receiving intra-mPFC (left panel) or –NAc (right panel) CNO microinjections.

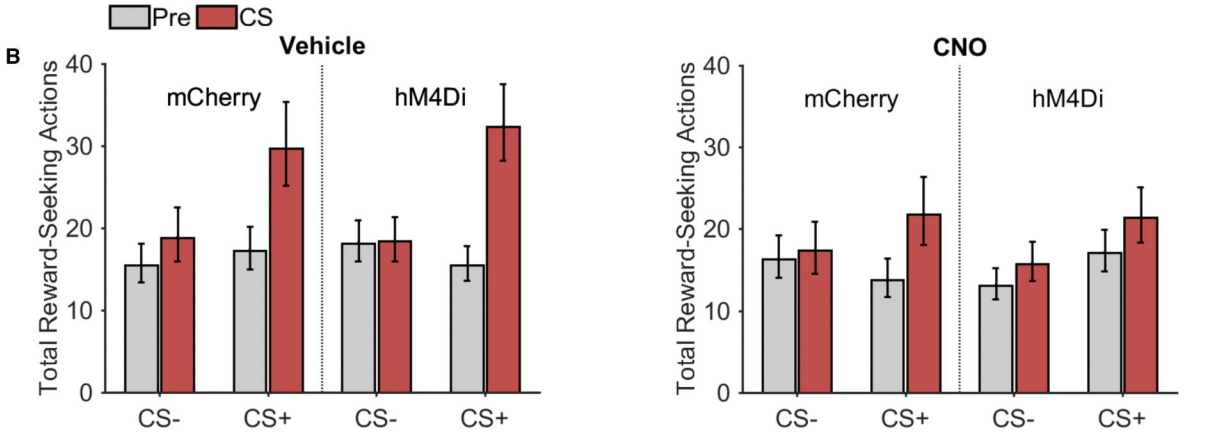
Figure 5. Chemogenetic inhibition of dopamine neurons in outcome specific devaluation test. **A.** Th:Cre+ rats received VTA injections of AAV-hSyn-DIO-hM4Di-mCherry or AAV-hSyn-DIO-mCherry. Following recovery, rats were trained on two distinct lever press actions for two different rewards (Instrumental Learning). Rats then underwent specific outcome devaluation testing following treatment with CNO (5mg/kg) or vehicle. **B.** Chemogenetic VTA dopamine inhibition does not affect outcome specific devaluation. Total reward seeking actions at test on the valued (red bars) and devalued (grey) levers in hM4di or mCherry expressing Th:Cre+ rats, following CNO (5 mg/kg)

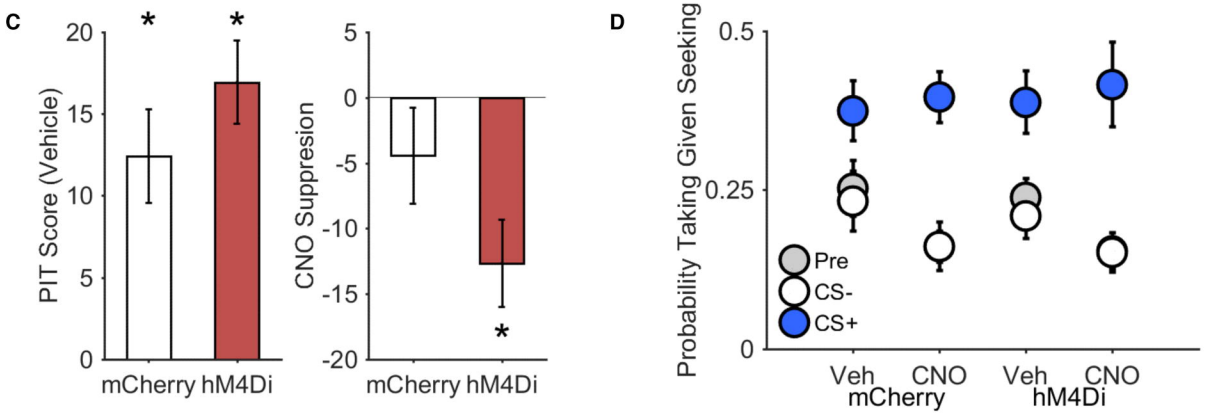
or vehicle treatments. **C.** Probability of engaging in a taking action given a seeking action for the different treatments and virus expression conditions for the valued and devalued levers.

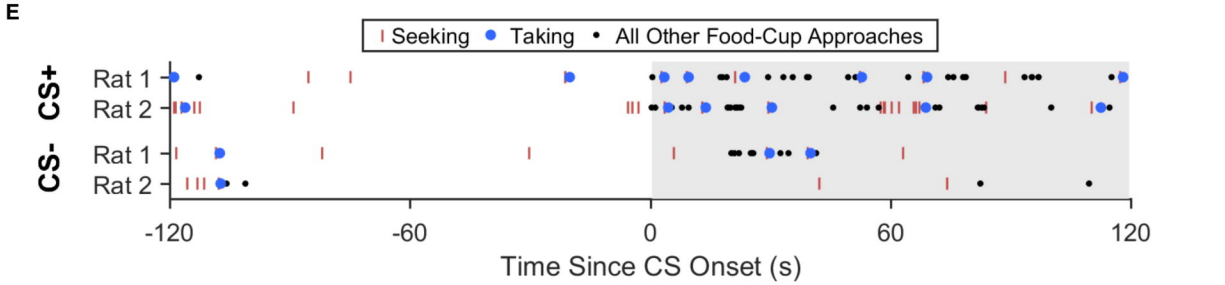

A
Instrumental Learning
Seeking
Taking
Cup
Lever
Cup
Lever
Food
Action sequence
B
Nonreinforced
Reinforced
Probability (Food Cup Approach)
0.3
0.2
0.1
0
-5
0
5
10
15
20
Time Relative to Lever Press (s)
C
Lever Presses
Nonreinforced Seeking
Reinforced Seeking
Food Cup Approaches
Nonreinforced Taking
Reinforced Taking
Non-Taking Approaches
-100
-80
-60
-40
-20
0
20
Time Since Reinforcer Prime (s)
D
Total Reward-Seeking Actions
250
200
150
100
50
0
Nonreinforced
Reinforced
Food and Cues
Cues Only
No Food or Cues
E
Total Reward-Taking Actions
100
75
50
25
0
Food and Cues
Cues Only
No Food or Cues
F
Probability of Taking Given Seeking
1
.75
.50
.25
0
Food and Cues
Cues Only
No Food or Cues

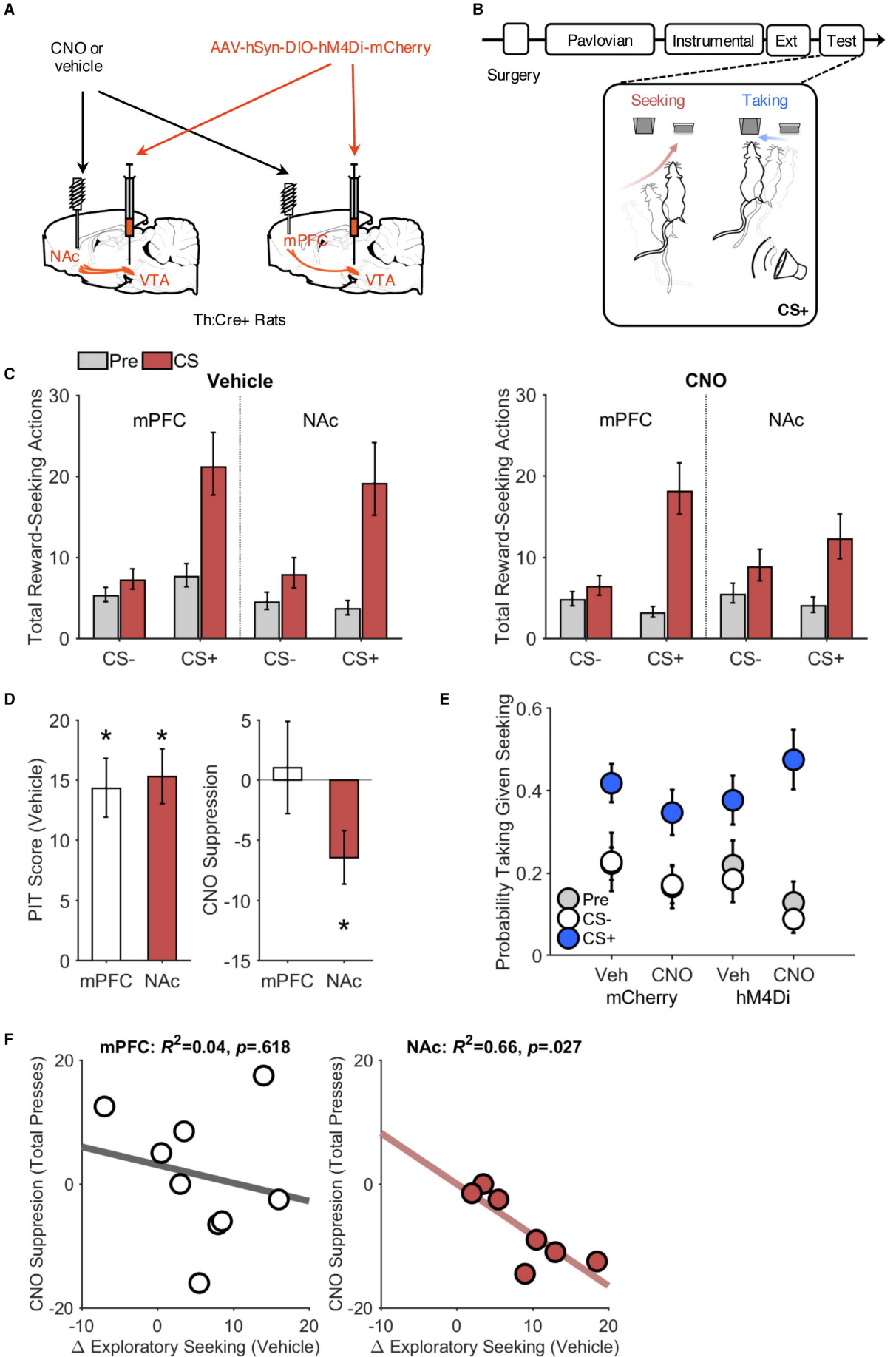

A
AAV-hSyn-DIO-hM4Di-mCherry
or
AAV-hSyn-DIO-mCherry
Th:Cre+ Rats
VTA
B
VTA
Th
hM4Di
Overlap
C
NAc
Th
hM4Di
Overlap
mPFC
Th
hM4Di
Overlap

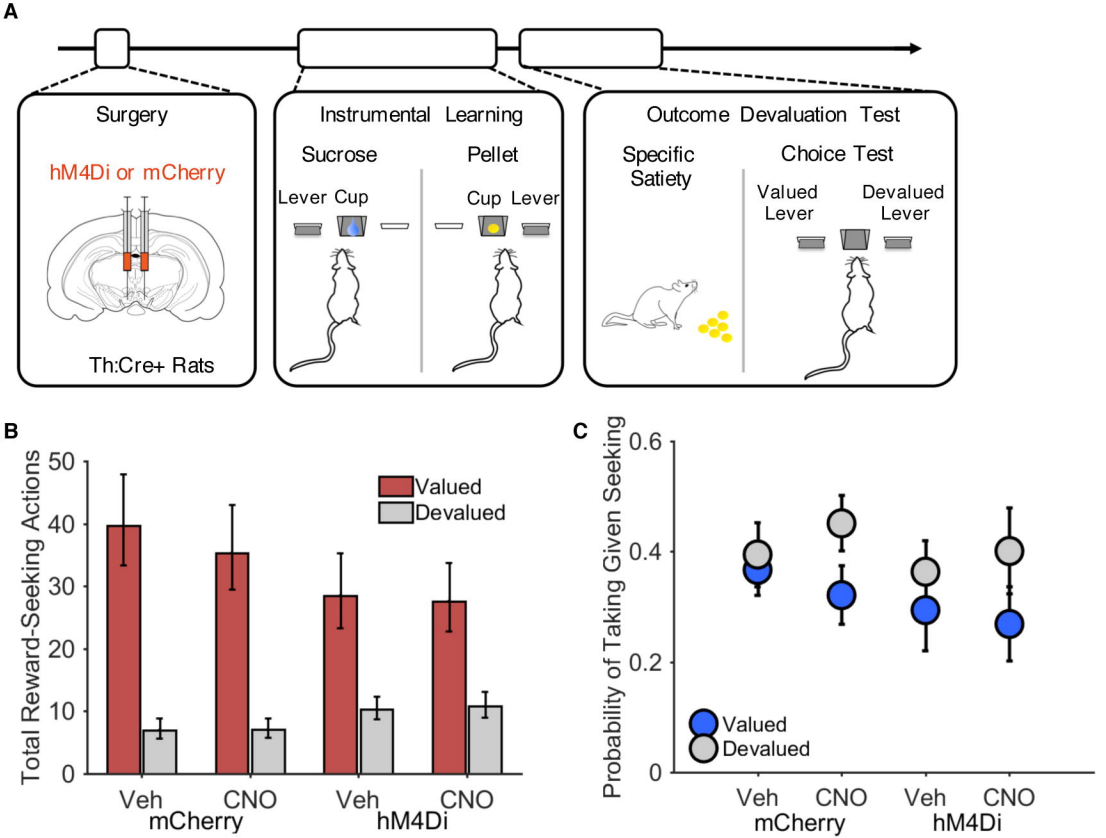

A
Surgery
hM4Di or mCherry
Pavlovian Learning
CS+
Instrumental Learning
Seeking
Taking
Action sequence
Test
Seeking
Taking
CS+
Ext


Pre
CS
B
Vehicle
mCherry
hM4Di
Total Reward-Seeking Actions
CS-
CS+
CS-
CS+
CNO
mCherry
hM4Di
Total Reward-Seeking Actions
CS-
CS+
CS-
CS+


C
PIT Score (Vehicle)
mCherry
hM4Di
CNO Suppresion
mCherry
hM4Di
D
Probability Taking Given Seeking
Pre
CS-
CS+
Veh
CNO
Veh
CNO
mCherry
hM4Di


E
Seeking
Taking
All Other Food-Cup Approaches
CS+
Rat 1
Rat 2
CS-
Rat 1
Rat 2
-120
-60
0
60
120
Time Since CS Onset (s)


A
CNO or vehicle
AAV-hSyn-DIO-hM4Di-mCherry
NAc
VTA
mPFC
VTA
Th:Cre+ Rats
B
Pavlovian
Instrumental
Ext
Test
Surgery
Seeking
Taking
CS+
C
Pre
CS
Vehicle
CNO
mPFC
NAc
Total Reward-Seeking Actions
CS-
CS+
D
PIT Score (Vehicle)
CNO Suppression
mPFC
NAc
E
Probability Taking Given Seeking
Pre
CS-
CS+
Veh
CNO
mCherry
hM4Di
F
mPFC: R^2=0.04, p=.618
NAc: R^2=0.66, p=.027
CNO Suppression (Total Presses)
Δ Exploratory Seeking (Vehicle)


A
Surgery
hM4Di or mCherry
Th:Cre+ Rats
Instrumental Learning
Sucrose
Pellet
Lever Cup
Cup Lever
Outcome Devaluation Test
Specific Satiety
Choice Test
Valued Lever
Devalued Lever
B
Total Reward-Seeking Actions
50
40
30
20
10
0
Valued
Devalued
Veh
CNO
Veh
CNO
mCherry
hM4Di
C
Probability of Taking Given Seeking
0.6
0.4
0.2
0
Valued
Devalued
Veh
CNO
Veh
CNO
mCherry
hM4Di