American College of Neuropsychopharmacology

www.nature.com/npp

ARTICLE

Check for updates

Effects of psychedelic, DOI, on nucleus accumbens dopamine signaling to predictable rewards and cues in rats

David A. Martin[1]✉, Angel M. Delgado[1] and Donna J. Calu[1]

Psychedelics produce lasting therapeutic responses in neuropsychiatric diseases suggesting they may disrupt entrenched associations and catalyze learning. Here, we examine psychedelic 5-$HT_{2A/2C}$ agonist, DOI, effects on dopamine signaling in the nucleus accumbens (NAc) core, a region extensively linked to reward learning, motivation, and drug-seeking. We measure phasic dopamine transients following acute DOI administration in rats during well learned Pavlovian tasks in which sequential cues predict rewards. We find that DOI (0.0–1.2 mg/kg, i.p.) increases dopamine signals, photometrically measured using $GRAB_{DA}$ optical sensor, to rewards and proximal reward cues, but not to the distal cues that predict these events. We determine that the elevated dopamine produced by DOI to reward cues occurs independently of DOI-induced changes in reward value. The increased dopamine associated with predictable reward cues and rewards supports DOI-induced increases in prediction error signaling. These findings lay a foundation for developing psychedelic strategies aimed at engaging error-driven learning mechanisms to disrupt entrenched associations or produce new associations.

Neuropsychopharmacology (2024) 49:1925–1933; https://doi.org/10.1038/s41386-024-01912-4

INTRODUCTION

Psychedelic 5-HT_{2A} agonists including psilocybin, LSD, and 2,5-dimethoxy-4-iodoamphetamine (DOI) produce profound acute subjective effects. Accumulating evidence indicates psychedelics have significant clinical utility in the treatment of neuropsychiatric conditions including anxiety, depression, and addiction [1–5]. Acute psychedelic drug administration engenders positive alterations in mood and attitudes that persist for months [6, 7], suggesting that psychedelic effects on reward learning processes, which are disrupted in depression and addiction [8–12], may be important therapeutic mechanisms. Here we probe psychedelic effects on reward prediction error signaling, a fundamental element of associative learning.

Ventral tegmental dopaminergic neuron activity and phasic dopamine (DA) release in the nucleus accumbens core (NAc) are strongly implicated in reward prediction error signaling [13–16], learning [17], and drug-seeking [18–20]. DA is phasically released in NAc in response to unpredictable reward delivery, but as learning progresses reward-evoked DA release is suppressed and shifts to the earliest stimuli predictive of reward [13, 15]. While NAc DA signaling plays many roles (i.e. value encoding [21], motivation [22], and salience [23, 24]), its role in promoting conditioned behaviors [25, 26] and associative model-based learning [27], highlights its importance as a 'teaching' signal in the context of reward prediction error (RPE) based models of DA function [28, 29].

Psychedelics are theorized to affect RPE signaling [30, 31], and low-dose LSD impacts reward-evoked EEG activity related to increased RPE processing [32], and moderate LSD doses increase reinforcement learning rates, consistent with greater sensitivity to RPEs [33]. Psychedelics increase tonic striatal DA levels in humans [34] and in rodents [35]. However, to determine if psychedelics alter DA-mediated RPE signaling, it is critical to measure psychedelic impacts on fast, phasic DA transients.

To determine the effect of psychedelic 5-$HT_{2A/2C}$ agonist, DOI, on DA signaling during reward prediction we use fiber photometry to measure optical dopamine sensor, $GRAB_{DA}$, fluorescence in the NAc during Pavlovian tasks. Because psychedelics alter reward value [36, 37], parsing the influence of stimuli value versus stimuli predictability is important for interpreting effects on phasic DA. To achieve this, we utilize two reward types (water and food) in our studies to infer the influences of value and predictability on NAc DA signaling.

MATERIALS AND METHODS

For detailed methods, please see the Supplementary Material Online.

Experimental subjects

All subjects were wildtype Sprague-Dawley rats (Charles River). The water behavioral economics experiment used a cohort of 8 rats (n = 4 male; n = 4 female), and the food behavioral economics experiment used a separate cohort of 14 rats (n = 7 male; n = 7 female). For photometry experiments, two cohorts were run separately and their data were combined (combined n = 5 male, n = 3 female). Both cohorts were used in multiple experiments (timeline in supplementary material). All procedures were performed in accordance with the "Guide for the care and use of laboratory animals" (8th edition, 2011, US National Research Council) and were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee (IACUC).

[1]Department of Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA. ✉email: davidmartin@som.umaryland.edu

Received: 14 April 2024 Revised: 10 June 2024 Accepted: 24 June 2024
Published online: 6 July 2024

Surgical procedures

We infused 600 nL of AAV9-hsyn-DA2m(DA4.4) [38] ($GRAB_{DA}$) targeting the Nucleus Accumbens Core unilaterally at a 6-degree angle (coordinates from bregma: +1.8 mm AP), +2.15 mm ML, −6.6 mm DV. Histology was performed following experiments, and rats were excluded if viral expression and fiber optic placements were not in the NAc core.

Behavioral training and testing

Both food and water behavioral economics tasks used a within-session, escalating-price design wherein fixed ratios (FRs) were increased across the 30 min session comprised of 10 three-minute bins [37]. For the experiments using $GRAB_{DA}$ to measure dopamine, rats were trained and recorded during 14–18 Pavlovian training sessions with randomly intermixed 15 CS+ and 15 CS- trials with a 40–60 s inter-trial interval (ITI). The CS+ (tone/white noise, counterbalanced) sounded for 5 s, terminating simultaneously with audible syringe pump activation for 1.5 s to deliver water (95 µl). CS- trials used the opposite audio stimuli (tone or white noise), which signaled for 5 s with no reward delivery.

Following initial Pavlovian training sessions, the same rats were sequentially trained and tested under a variety of conditions using different cue types (auditory, levers), rewards (food, water), and timing to elucidate the effects of psychedelics on DA using diverse parameters, and selected results are presented here. The exact behavioral history of rats between the 2 cohorts was not identical. However, both photometry cohorts had similar amounts of DOI experience and similar training histories prior to collecting the data reported here (see supplementary material for timeline).

For Training and Test sessions with water reward and auditory cues (Fig. 3), the CS (distal cue) was 2 s, followed by a gap of 2 s, followed by activation of the audible syringe pump for 1.5 s (95 ul water per delivery). Sessions had 15 'Expected' trials in which the CS preceded the reward intermixed pseudorandomly with 12 'Unexpected' trials in which there was no CS. Test sessions with lever cues (Fig. 4) had 15 trials where a lever was extended for 3 s, followed by a 2 s gap, followed by another 3 s lever extension and another 2 s gap before proximal cue and reward delivery. In water experiments sequential levers were followed by syringe pump activation for 1.5 s (95 µl) and in food experiments (2) food pellets were delivered. Training and Test sessions were identical for all photometry experiments.

Test Sessions using DOI. For each of the behavioral conditions under study, rats were trained until their behavior and dopamine signals stabilized before being tested in a within-subject counterbalanced design in which each subject received DOI or saline on alternate test days, separated by a retraining session in which no injections were given. For behavioral economic tasks, DOI (0.8 mg/kg i.p) or vehicle (saline) were given 25 min prior to test sessions that were identical to training sessions. In test sessions measuring $GRAB_{DA}$ using audible cues and water reward (Fig. 3), 3 cumulative DOI doses were given as a series of 3 injections (400 µg/kg, i.p. for each injection), each before three closely spaced identical 15 min test sessions, with a gap of ~2 min between sessions to give injections. The 1st injection was given in homecage 15 min prior to 1st session. In test sessions using lever cues (Fig. 4), 2 cumulative DOI doses were given as a series of 2 injections (500 µg/kg, i.p. for each injection) immediately prior to each identical 15 min test session with ~2 min between sessions. We note that the peak effect of these cumulative DOI doses is likely not reached until later in the sessions due to lag between intraperitoneal injections and peak drug effects. Nevertheless, this design permits clear observation of dose-response relationships between cumulative DOI doses and DA signals and behavior. All injections were given i.p. at a volume corresponding to 1 ml/kg body weight. 2,5-Dimethoxy-4-iodoamphetamine (DOI, Cayman) was dissolved in sterile saline vehicle.

Data and statistical analyses

Photometry signal analysis. See Supplementary Materials for details. Briefly, raw data were analyzed in Matlab by z-scoring to a 5 s period prior to the distal cue. Significance across all bins in the trial was determined by calculating 95% confidence intervals (95% CIs) across trial lengths. To summarize data at important timepoints in trials across doses and reinforcers, we measured average $GRAB_{DA}$ signal peak heights (in terms of z-score) during the 1 s period following cues, and/or area under the curve (integrating z-score over time relative to baseline) for the 3 s following reward delivery (pump offset/ 1st pellet delivery). We analyzed this data using standard repeated measures, 2-Way ANOVAs using within subject factors across dose, reinforcer, and cue-type where appropriate. Sphericity was not assumed, and Geisser-Greenhouse corrections were employed when required. For the 0 mg/kg dose, data was averaged for each subject across control sessions. Post hoc comparisons between groups were corrected with Dunnett's multiple testing procedure using Prism software (GraphPad).

Behavioral analysis. The average probability of a leverpress or reward poke occurring during all timepoints across a trial was calculated for each rat. For within-subject comparisons of drug and control conditions, confidence intervals were calculated by subtracting the control from the drug probabilities for each subject. Periods where the 95% CI does not contain zero are labeled as significant on poking traces with no minimum period of significance. We summarized latency to enter reward well following cues across experiments and these data were analyzed with standard RM ANOVAs and multiple testing correction procedures for post-hoc tests (Dunnett's).

Behavioral economic analysis was performed by fitting the rewards earned at each price (consecutive bins with the same price were averaged) to the exponential behavioral economics equation [39] for each individual using the "fitnlm" function in matlab. We also analyzed the number of rewards earned at each price during drug and control conditions using 2-Way ANOVA. Post hoc comparisons between groups were corrected with Sidak's multiple testing procedure using Prism software (GraphPad).

Simple linear regression was performed between changes in proximal cue $GRAB_{DA}$ peak signals and changes in latency to enter the reward well during DOI experiments on an individual session basis. Changes in $GRAB_{DA}$ signal peak heights to proximal cues between drug and control sessions were normalized to the size of distal cue peaks during control sessions. For these analyses: $\Delta Latency = Latency_{DOI} - Latency_{CON}$; $\Delta DA = (DA_{DOI,Proximal} - DA_{CON,Proximal})/DA_{CON,Distal}$. All correlations were calculated in Prism.

RESULTS

DOI bidirectionally modulates water and food value

NAc DA release is influenced by both reward value and reward predictability [40]. We aimed to design an experiment to disambiguate motivational value and reward prediction in order to interpret psychedelic induced changes in DA signaling relating to these two factors. To do so, we first identified two reward types that show distinct motivational effects in the psychedelic state. We tested the effects of DOI on instrumental lever responding for either water or food reward in a behavioral economics task in which fixed ratio (FR) requirements increase across successive bins of the session. Fig. 1A, B show behavioral economic curves for food (Fig. 1A) and water (Fig. 1B) comparing saline and DOI (0.8 mg/kg, i.p.) conditions. We fit the exponential behavioral economic equation [39] to each subject's curves, revealing an interaction between Reward type and Treatment on consumption at low cost (Q_0 values: $F(1, 20) = 7.429$, $p = 0.013$, 2-Way ANOVA, Fig. 1C). Paired t-tests of Q_0 values indicate that DOI decreases Q_0 for food ($p = 0.041$) but increases Q_0 for water ($p = 0.0042$). These data show that motivation for food and water reward at low prices is bidirectionally modulated by administration of DOI. Consistent with opposite effects of DOI on Q_0 for food and water, at low work requirements (FR6), DOI (0.8 mg/kg, ip.) decreases food consumption, but increases water consumption (Fig. 1A, B); Food: $t(13) = 4.676$; $p = 0.0026$; Water: $(t(7) = 4.968$, $p = 0.0097$; Sidak's multiple comparison correction). At higher prices (FR40, FR63), DOI decreases water consumption ($t(7) > 5.487$, $p's < 0.006$, Sidak's, Fig. 1B). DOI also decreases food consumption significantly at FR10, FR16, and FR25 ($t(13) > 3.689$, $ps<0.0082$, Sidak's, Fig. 1A). Consistent with similar effects of DOI at high costs for both food and water, DOI increases economic demand elasticity (α), or the rate at which consumption decreases with increasing cost, for both water and food reward (main effect of Drug, $F(1, 20) = 16.37$, $p = 0.0006$, Fig. 1D). We found no interactions between Sex and Drug factors for Q_0 or α in food (Q_0: $F(1,12) = 1.293$, $p = 0.28$; α: $F(1,12) = 0.7184$, $p = 0.41$) or water (Q_0: $F(1,6) = 4.263$, $p = 0.085$; α: $F(1,6) = 0.887$, $p = 0.38$) experiments.

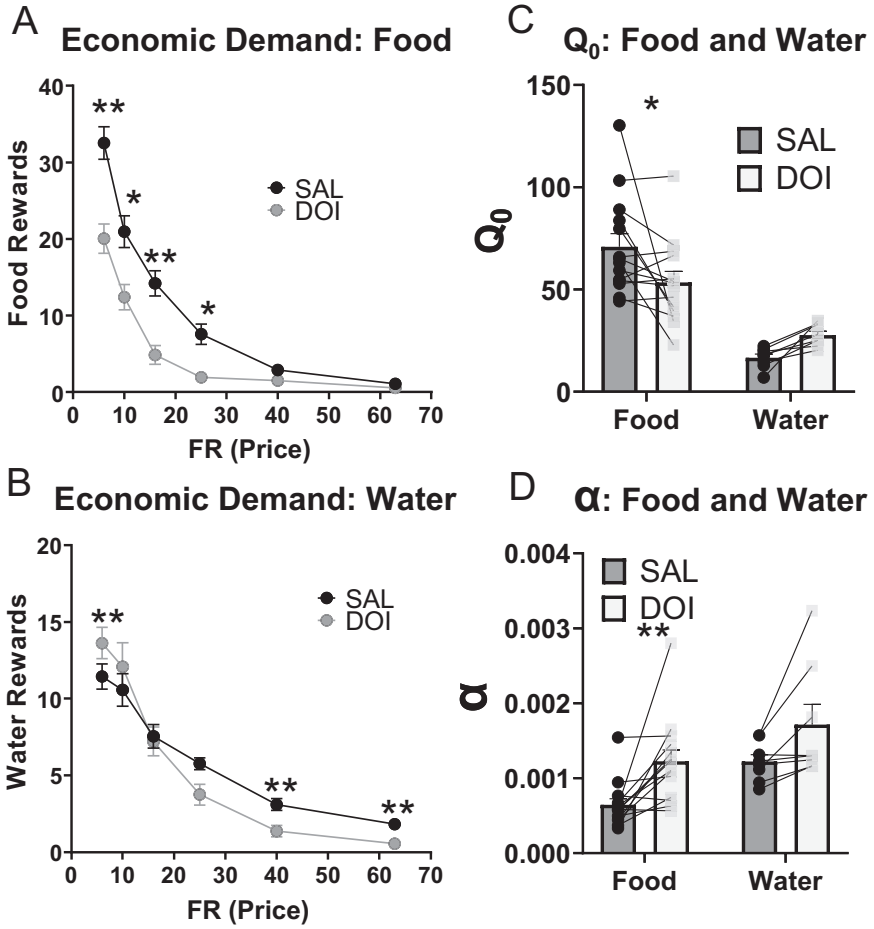

Fig. 1 DOI (0.8 mg/kg, i.p.) produces bidirectional effects on food ($N = 14$) and water ($N = 8$) consumption at low prices. A Food consumption. **B** Water consumption. **C** Fitted Q_0 values for food and water. Two-way AVOVA indicates an interaction between drug and reward type ($F(1,20) = 7.43$; $p = 0.013$). **D** Fitted alpha values for food and water. All Graphs: "*", "**" indicates $p < 0.05$, $p < 0.01$ at indicated post-hoc comparisons using two-way ANOVA and Sidak multiple testing correction.

In the following experiments, we examine the effects of DOI on NAc DA dynamics in Pavlovian conditioning tasks, in which rewards are earned with low effort - by merely approaching the reward when cues signal their availability. By identifying rewards for which motivation is bidirectionally altered in the psychedelic state, we are positioned to interpret NAc DA signal changes related to factors of predictability and value of rewards and reward cues in subsequent experiments.

Training shifts NAc dopamine release to distal cues

We infused optical dopamine sensors ($GRAB_{DA}$) and implanted an optical fiber targeting the nucleus accumbens core (NAc) in 8 rats (Fig. 2A, B). We water deprived and trained the rats on a Pavlovian task (Fig. 2C) in which water reward delivered by an audible syringe pump (proximal cue) was preceded by an audible CS+ (distal cue, tone or white noise) for 5 seconds. A CS- (tone or white noise) predicted no reward. During early training, photometrically recorded $GRAB_{DA}$ signals tended to peak following the proximal cue (syringe pump onset; Fig. 2C–E). As training progressed, peak $GRAB_{DA}$ signals migrated to the distal cue (CS+ onset; Fig. 2C–E). Analyzing $GRAB_{DA}$ peak height across training blocks reveals an interaction between Training block and Cue (proximal/distal) ($F(1.921, 13.45) = 5.698$, $p = 0.0169$, 2-Way ANOVA, Fig. 2D). The increase in NAc $GRAB_{DA}$ signaling to the distal cue across training was accompanied by and strongly correlated with increased nosepoking in the reward port during the period between CS+ and reward delivery (Fig. S1E, F). By the end of training, CS+ trials had higher peak $GRAB_{DA}$ signals for distal cues compared to CS- trials, and peak heights were higher for distal than proximal peaks (Fig. 2F). Migration of NAc dopamine signals to the most distal predictors of reward and reduction in reward and proximal cue dopamine signals is consistent with prior observations during Pavlovian learning [15].

DOI increases NAc DA signals to predictable, proximal water cues and rewards

After learning was established, we gave rats a low, medium, or high cumulative dose of psychedelic $5HT_{2A}$ agonist, DOI, or vehicle injections, prior to water reinforced sessions consisting of 'Expected' and 'Unexpected' trial types. For 'Expected' trials, the water pump onset was preceded by a CS+, whereas in 'Unexpected' trials no CS+ was present and the onset of the water pump was unpredictable. Dopamine traces and behavioral traces for each dose and trial type can be found in Supplementary Fig. S2, with the primary results summarized below and in Fig. 3.

During 'Expected' trials, we found DOI dose-dependently increased NAc $GRAB_{DA}$ signals to fully predictable proximal cues, without affecting $GRAB_{DA}$ signals to distal cues (Fig. 3A, B, Cue x Dose $F(2.26, 15.79) = 3.997$, $p = 0.035$; proximal post-hoc tests: 0.0 vs. 0.4 dose, $p = 0.0507$; 0.0 vs. 0.8 dose, $p = 0.0428$; 0.0 vs. 1.2 dose, $p = 0.0211$, Dunnett's multiple comparison test). We also observed small increases in $GRAB_{DA}$ peak heights to proximal cues on 'Unexpected' trials, when these cues were not predictable (Fig. 3C, D; 0 vs. 0.4 dose, $p = 0.034$; 0.0 vs. 0.8 dose, $p = 0.54$; 0.0 vs. 1.2 dose, $p = 0.033$, Dunnett's). For both trial types, we

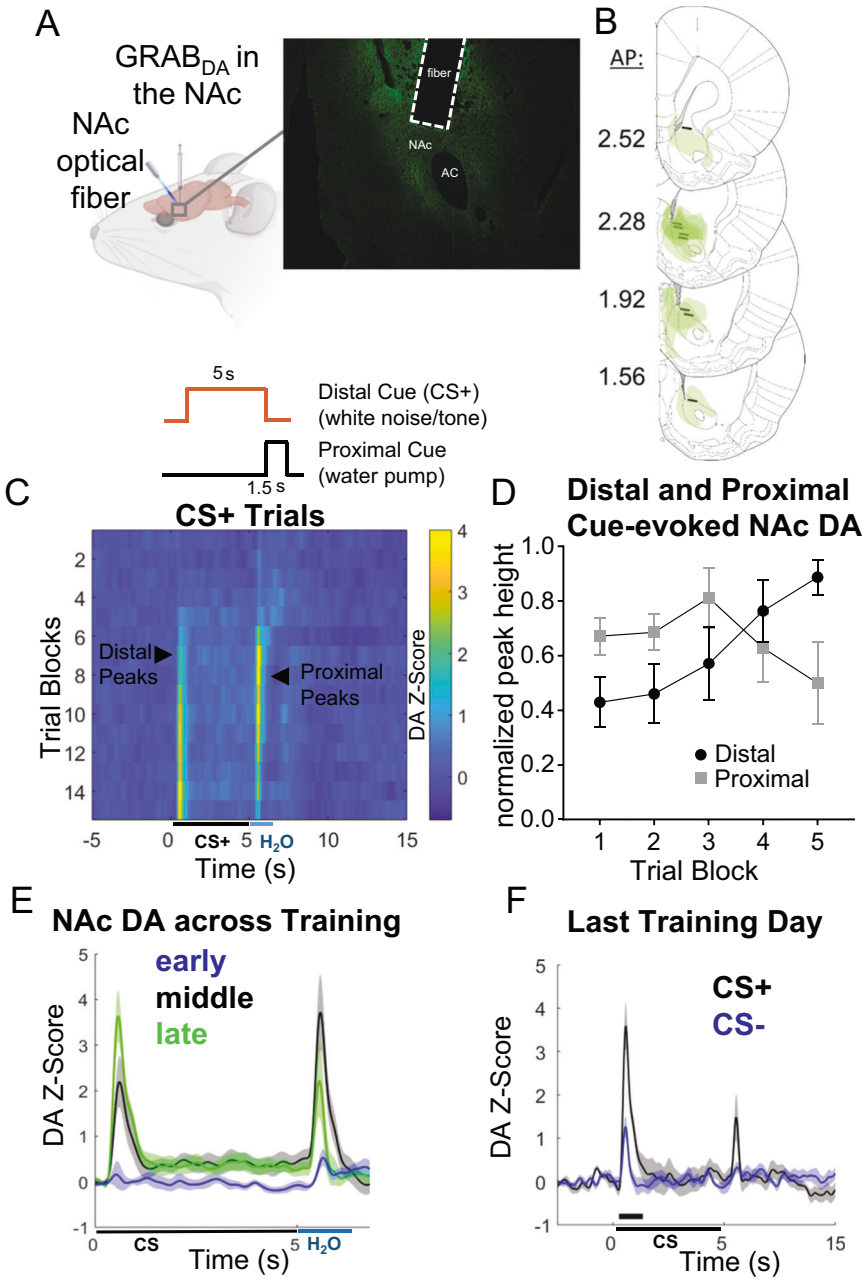

Fig. 2 Pavlovian training shifts dopamine signaling to distal cues. GRAB$_{DA}$ measurement of NAc dopamine during Pavlovian training: **A** surgical procedures (left), representative fiber placement (right). **B** Fiber tip placements (black bars) and GRAB$_{DA}$ expression (green shading). **C** Behavioral Paradigm for training (top), and heat plots of GRAB$_{DA}$ traces across training. CS+ trials are binned into 15 blocks. **D** Average distal and proximal GRAB$_{DA}$ peak heights, normalized to maximum peak height and binned into 5 blocks across training. **E** Average CS+ traces across all rats for the first, middle, and final third of training. **F** Average CS+ and CS− traces on the final session of training for all rats. Black bars indicate where CS+ DA signal is higher than CS− DA (95% confidence intervals do not overlap).

observed consistent, large increases in reward associated GRAB$_{DA}$ signals that were near maximal even at the lowest dose of DOI (Fig. 3E). Concurrent with DOI-induced GRAB$_{DA}$ signal changes, we observed dose-dependent increases in latency to enter the reward well following cues for both trial types (Fig. 3F; main effect of dose: $F(1.52, 10.64) = 6.669$, $p = 0.0177$). Post hoc testing showed that at the highest DOI dose, the latency to enter the well after the distal cue was significantly increased (0.0 vs. 1.2, $p = 0.019$, Dunnett's). Correspondingly, for both expected and unexpected trial types we observed reduced probability of reward well occupancy for a period following cue onset (Fig. 3G, H). Notably, there was no relationship between poke latency (relative to saline levels) and NAc GRAB$_{DA}$ proximal peak heights across individuals (Fig. 3I), because some rats with large increases in proximal DA peaks did not exhibit increased poke latencies. This suggests that the differences in response latencies in the psychedelic state are not driving changes in NAc DA signaling.

DOI similarly increases NAc DA signals to both food and water proximal cues

We sought to determine if DOI-induced increases in phasic DA are similar for water and food rewards that undergo opposite value shifts in the psychedelic state (see Fig. 1). We used sequential lever cues instead of auditory cues to determine the generality of DOI

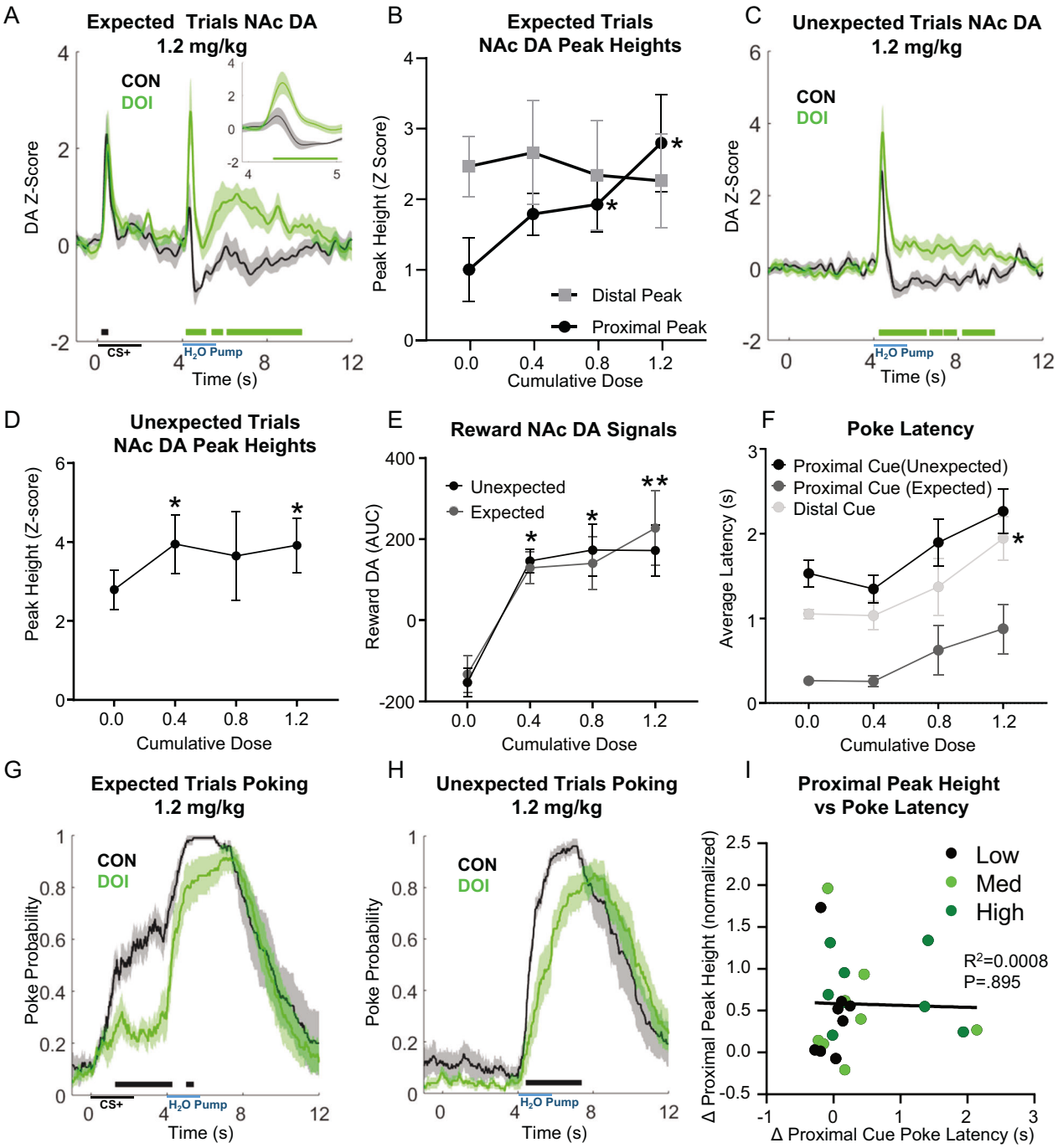

Fig. 3 DOI increases reward and proximal cue associated dopamine signaling during auditory-cued water delivery. DOI effects on NAc $GRAB_{DA}$ and behavior during auditory-cued water delivery: **A** average traces from expected trials (trials containing a distal cue) at 1.2 mg/kg DOI. Inset: Zoomed image of proximal cue epoch. **B** Average $GRAB_{DA}$ peak heights for proximal and distal cues during expected trials. **C** Average $GRAB_{DA}$ traces for unexpected trials (no distal cue) at 1.2 mg/kg DOI. **D** Unexpected trial $GRAB_{DA}$ peak heights. **E** Reward period (3 s following pump off) DA AUC across dose. **F** Latency to poke in reward well by cue and dose. **G** Average poke probabilities for expected trials at 1.2 mg/kg DOI. **H** Average poke probabilities for unexpected trials for 1.2 mg/kg DOI. **I** Linear correlation across individual sessions between change in poke latency (relative to control sessions) and change in $GRAB_{DA}$ proximal peak heights (relative to control sessions and normalized by control distal peak heights). All traces: Shading indicates SEM. Green and black bars above the *x*-axis indicate periods of significant differences between control and DOI traces. Line graphs: '*' and '**' indicate post hoc tests with $p < 0.05$, and $p < 0.01$, respectively (Dunnett's).

effects on NAc DA across cue modality and determine DOI effects on sign-tracking, a lever directed approach behavior that is associated with rigid associative learning [41, 42]. We presented sequential lever cues by inserting and retracting the lever twice prior to reward delivery to examine whether cue predictability or temporal proximity to reward influenced NAc DA signaling. With this design, only the first lever cue presentation is surprising, which allows us to test the effects of DOI on multiple predictable proximal cue presentations of different identities (lever and pump/food hopper). Dopamine traces and behavioral traces for each dose and reward type can be found in Supplementary Fig. S3, with the primary results summarized below and in Fig. 4.

As in the auditory-cued experiment, we observed a dose-dependent increase in proximal cue associated $GRAB_{DA}$ signals on DOI, and this effect was significant for both water and food reward, with no interaction between Dose and Reinforcer type (Fig. 4A–C; Main effect of Dose, $F(1.586,9.518) = 17.15$, $p = 0.001$, *water post-hoc tests*: 0.0 vs. 0.5 dose, $p = 0.003$; 0.0 vs. 1.0 dose, $p = 0.003$; *food post-hoc tests*: 0.0 vs. 0.5 dose, $p = 0.7227$, 0.0 vs. 1.0 dose, $p = 0.0369$, Dunnett's multiple comparison test). We also observed dose-dependent increases in reward-associated $GRAB_{DA}$ signals for both water and food reward that reached significance in post-hoc tests at the 1.0 mg/kg dose (Fig. 4D; main effect of Dose, $F(1.226,7.353) = 12.65$, $p = 0.0069$, *water* post hoc tests: 0.0 vs. 0.5 dose, $p = 0.067$; 0.0 vs. 1.0 dose, $p = 0.0039$; *food post-hoc tests*: 0.0 vs. 0.5 dose, $p = 0.1063$, 0.0 vs. 1.0 dose, $p = 0.0131$, Dunnett's multiple comparison test). For distal-cue associated $GRAB_{DA}$ signals, overall there was a dose-dependent reduction in distal cue height (Fig. 4E), with a significant interaction between Dose and Reinforcer type, (Fig. 4E, main effect of Dose, $F(1.141,6.844) = 6.458$, $p = 0.0367$, Dose X Reinforcer type interaction, $F(1.287,7.722) = 12.63$, $p = 0.006$), indicating a differential dose response on NAc DA distal cue signaling for the two reinforcer types. While post-hoc tests indicated that the highest dose tested (1.0 mg/kg) tended to reduce distal $GRAB_{DA}$ peaks for food (*Food Distal*: 0.0 vs 1.0 dose, $p = 0.0576$; *Water Distal*: 0.0 vs 1.0 dose, $p = 0.1795$, Dunnett's multiple testing correction), this effect did not reach significance. Sequential lever retractions and insertions following the first (distal) insertion also produced $GRAB_{DA}$ peaks, which were dose dependently increased in size in the water, but not food conditions by DOI (Fig. S4).

Behaviorally, DOI tended to nearly eliminate sign-tracking (Pavlovian lever pressing), affecting both reinforcers similarly, though this did not reach statistical significance (Fig. 4F, main effect of dose, $F(1.15,6.94) = 5.02$, $p = 0.0569$), consistent with its effects to reduce effortful behavior across reinforcers (Fig. 1) and previous reports that DOI reduces responding for conditioned reinforcers [43]. In contrast, DOI produced markedly differential effects on poking behavior depending on the reinforcer (Fig. 4G–J). Following proximal cues, DOI increased the latency to enter the reward well for food, but not water (Fig. 4I; Dose X Reinforcer type interaction, $F(1.287,7.722) = 12.63$, $p = 0.006$, *Food Proximal Latency:* 0.0 vs. 1.0 dose, $p = 0.003$; *Water Proximal Latency:* 0.0 vs. 1.0 dose, $p = 0.215$; Dunnett's multiple comparisons). Similarly, for distal cues, DOI increased the latency to enter the reward well for food, but not water (Fig. 4J; Dose X Reinforcer type interaction, $F(1.663,9.979) = 25.27$, $p = 0.0002$, *Food Distal Latency:* 0.0 vs. 1.0 dose, $p = 0.0017$; *Water Distal Latency:* 0.0 vs. 1.0 dose, $p = 0.3959$; Dunnett's multiple comparisons).

While differences in the effects of DOI on food and water poking latencies likely reflect differences in motivation for the respective rewards, response latencies do not account for the increases observed in proximal cue associated $GRAB_{DA}$ signals. Correlation analyses between individuals' poking latency differences between treatments and proximal $GRAB_{DA}$ signal differences between treatments reveal no significant relationships between these factors (Fig. 4K, L).

DISCUSSION

Here, we determined that the psychedelic drug, DOI, increases reward and proximal cue NAc DA signaling, despite those events being fully predictable. As learning progressed, DA responses were progressively inhibited to reward consumption and to fully predictable proximal reward cues in our study, replicating established results [13, 15]. Elevation of NAc DA signaling to predictable proximal cues during the psychedelic state resembles prediction error signals to these stimuli observed in earlier learning stages (e.g., Fig. 2E) and may reflect increased error signaling even to well established associations. We show that DOI bidirectionally affects the value of food and water rewards, while DOI increases DA to proximal reward cues associated with both rewards, suggesting that changes in reward value are unlikely to explain the observed increases in DA signaling in the psychedelic state.

Psychedelics produce a variety of behavioral disruptions that could affect NAc DA signaling. DOI produces hypolocomotion in rats [44], DOM and LSD increase pausing in operant responding for food [45], and DOI reduces motivation to work for rewards like food and opioids in behavioral economics tasks in rats [37], as does DOM in monkeys [46]. In the present study, we compare motivation for food and water in a behavioral economics task in the DOI-induced state, finding that while food is devalued, water increases in value. We also find that as price increases, work output decreases more quickly in the psychedelic state, irrespective of the value of the reward at low prices. These data suggest that as work demands increase, motor output may become more laborious in the psychedelic state. Consistently, in Pavlovian experiments, rats tend to be slower to approach the reward well in the DOI-induced state at higher doses. However, analysis of individual subjects demonstrated that many rats exhibited little or no changes in latency to approach the reward well with DOI treatment, yet exhibited large increases in DA associated with the predictable, proximal reward cue for food and water. Across individuals, there were no significant relationships between DOI-induced changes in approach latencies and NAc DA responses. However, we cannot rule out the possibility that behaviors we did not measure might correlate with the DOI-induced increases in NAc DA signaling observed here.

NAc DA is canonically associated with RPE [47, 48], and its release decreases with the predictability of reward associated stimuli. One interpretation of the data is that the prediction (i.e., anticipation) of reward is disrupted by psychedelics. This result could be related to deficits in working memory in the psychedelic state [49, 50] or difficulty in estimating temporal intervals [50, 51], and this interpretation is also consistent with the view that psychedelics relax the strength of priors [30]. Another possibility is that reward prediction error signaling itself is enhanced - despite retained anticipation of the reward, per se. This interpretation is consistent with the observation that psychedelics can imbue ordinary stimuli with the sensation of novelty [52] (which DA is known to encode), as well as theories that posit enhancement of prediction error signaling as a core attribute of the psychedelic state [31].

NAc DA is associated with other functions besides RPE, such as the encoding of incentive salience [24], perceived salience [23], motivation [53], and costs [53–56]. An interpretation of the increased DA to proximal cues within these frameworks suggests that psychedelics may increase the salience and/or motivating aspects of proximal cues and rewards, while potentially decreasing the salience of the distal CS, evidenced by the tendency of DOI to reduce sign-tracking (associated with incentive salience) and distal cue DA - though we note DA was not consistently decreased to the distal CS for water (see Fig. 3 and 4) across experiments. A reduction in distal cue salience is consistent with pervious work showing that DOI decreased conditioned responding to water paired stimuli, without reducing responding for water [43]. Because stimuli salience is comprised of multiple factors, including predictability, novelty, intensity, and temporally discounted value - future experiments will be required to disambiguate between these non-mutually exclusive possibilities as factors influenced by psychedelics.

As mentioned, some theoretical accounts of psychedelic action posit disruptions in predictive coding to be fundamental mechanisms by which psychedelics produce many of their subjective effects [30, 31]. Two human studies show that surprising sensory stimuli produce altered EEG/MEG responses after psychedelics [57, 58], though two others showed null results

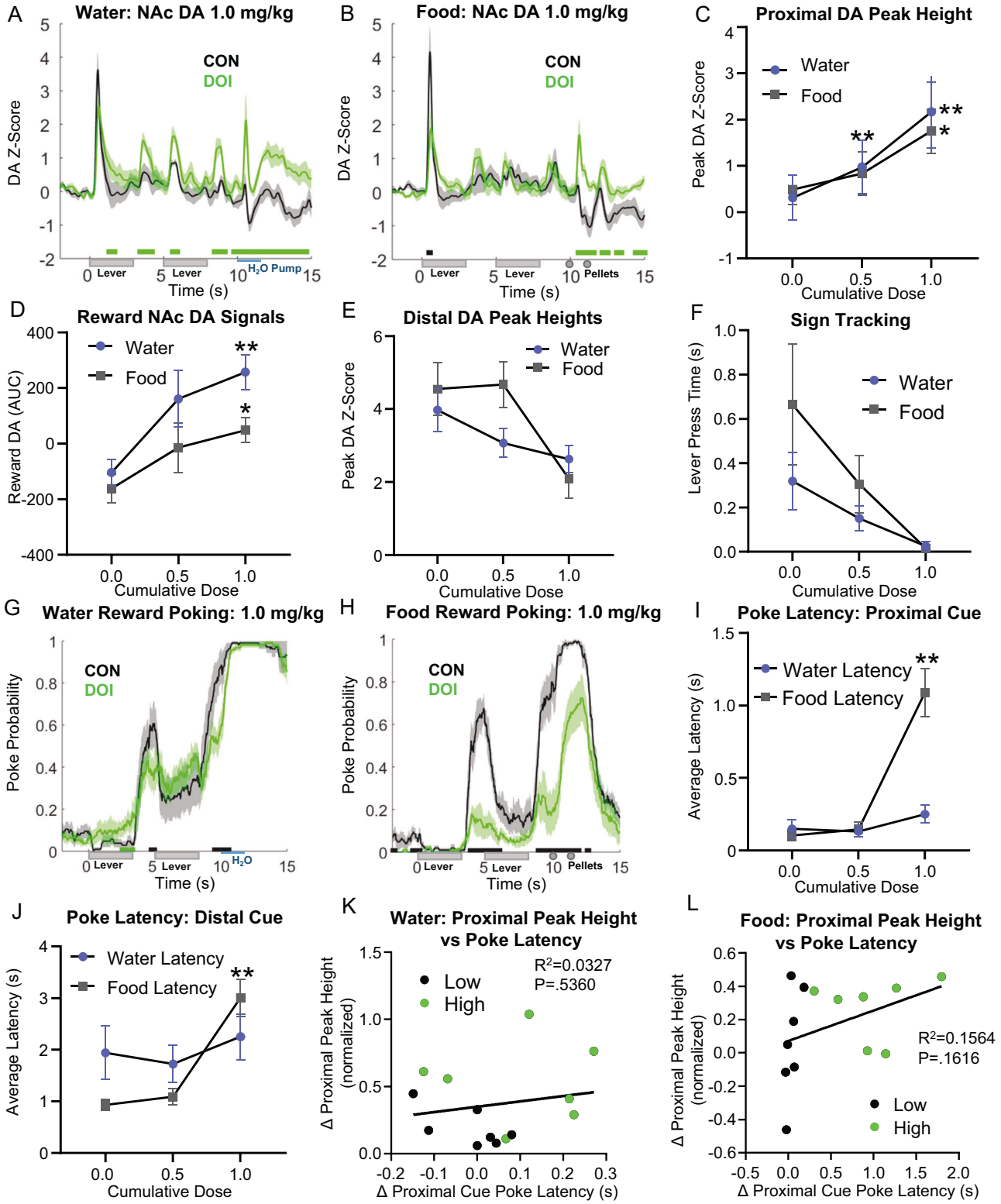

Fig. 4 DOI increases reward and proximal cue associated dopamine signaling during lever-cued water and food delivery. DOI effects on NAc $GRAB_{DA}$ and behavior during lever-cued water and food delivery: average $GRAB_{DA}$ traces during water (**A**) and food (**B**) conditions at 1.0 mg/kg. **C** Proximal cue average $GRAB_{DA}$ peak heights. **D** Average reward period AUC (3 s). **E** Distal cue average $GRAB_{DA}$ peak heights. **F** Average lever press time per trial. Average poking traces for water (**G**) and food (**H**) conditions at 1.0 mg/kg. Latency between proximal (**I**) and distal (**J**) cue and reward well poke. Linear correlation across individual DOI sessions between change in poke latency (relative to control sessions) and change in $GRAB_{DA}$ proximal peak heights (relative to control sessions and normalized to control distal peak heights) for water (**K**) and food (**L**) conditions. All traces: Shading indicates SEM. Green and black bars above the *x*-axis indicate periods of significance between traces. Line graphs: '*' and '**' indicate post hoc tests with $p < 0.05$, and $p < 0.01$, respectively (Dunnett's).

[59, 60]. With respect to RPE specifically, one EEG study using low doses of LSD [32] and another human behavioral study of reinforcement learning [33] support the notion that psychedelics amplify RPE processing. The results reported here further suggest that RPE signaling is enhanced by DOI. We did not alter task contingencies during DOI sessions, so future experiments are necessary to understand how DOI-altered dopamine signaling may influence learning when contingencies change. Furthermore, the reported effects of DOI must be replicated with other psychedelic drugs to determine the generality of the effects we observe.

Currently, psychedelics are under intense clinical study for varied mental health conditions including depression and drug addiction, however, a lack of mechanistic clarity on how psychedelics work is a hindrance for maximizing benefits [61]. Many authors have emphasized the importance of preparation and context ('set and setting') in the therapeutic response [62], or that psychedelics may function as non-specific amplifiers of the placebo response, synergizing with placebo or expectancy effects [63, 64]. Others have emphasized neuroplastic actions of psychedelics on dendritic structure as likely therapeutic mechanisms [65]- though psychedelic-induced plasticity may be studied at several levels of inquiry- from synapses to circuit level plasticity mechanisms. As DA signaling is necessary for associative learning and behavioral conditioning, further work linking psychedelic effects on DA to various learning processes is uniquely positioned to identify psychedelic mechanisms for producing lasting behavioral changes.

DATA AVAILABILITY

All data made available upon request.

REFERENCES

1. Goodwin GM, Aaronson ST, Alvarez O, Arden PC, Baker A, Bennett JC, et al. Single-dose psilocybin for a treatment-resistant episode of major depression. N Engl J Med. 2022;387:1637–48.
2. Ross S, Bossis A, Guss J, Agin-Liebes G, Malone T, Cohen B, et al. Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. J Psychopharmacol. 2016;30:1165–80.
3. von Rotz R, Schindowski EM, Jungwirth J, Schuldt A, Rieser NM, Zahoranszky K, et al. Single-dose psilocybin-assisted therapy in major depressive disorder: A placebo-controlled, double-blind, randomised clinical trial. EClinicalMedicine. 2023;56:101809.
4. Savage C, McCabe OL. Residential psychedelic (LSD) therapy for the narcotic addict. A controlled study. Arch Gen Psychiatry. 1973;28:808–14.
5. Krebs TS, Johansen P-Ø. Lysergic acid diethylamide (LSD) for alcoholism: meta-analysis of randomized controlled trials. J Psychopharmacol. 2012;26:994–1002.
6. Griffiths RR, Johnson MW, Richards WA, Richards BD, McCann U, Jesse R. Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. Psychopharmacol (Berl). 2011;218:649–65.
7. Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, et al. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: a randomized double-blind trial. J Psychopharmacol. 2016;30:1181–97.
8. Gradin VB, Kumar P, Waiter G, Ahearn T, Stickle C, Milders M, et al. Expected value and prediction error abnormalities in depression and schizophrenia. Brain. 2011;134:1751–64.
9. Kumar P, Goer F, Murray L, Dillon DG, Beltzer ML, Cohen AL, et al. Impaired reward prediction error encoding and striatal-midbrain connectivity in depression. Neuropsychopharmacol. 2018;43:1581–8.
10. Rose EJ, Salmeron BJ, Ross TJ, Waltz J, Schweitzer JB, McClure SM, et al. Temporal difference error prediction signal dysregulation in cocaine dependence. Neuropsychopharmacol. 2014;39:1732–42.
11. Tanabe J, Reynolds J, Krmpotich T, Claus E, Thompson LL, Du YP, et al. Reduced neural tracking of prediction error in substance-dependent individuals. Am J Psychiatry. 2013;170:1356–63.
12. Queirazza F, Fouragnan E, Steele JD, Cavanagh J, Philiastides MG. Neural correlates of weighted reward prediction error during reinforcement learning classify response to cognitive behavioral therapy in depression. Sci Adv. 2019;5:eaav4962.
13. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. Sci (N. Y, NY). 1997;275:1593–9.
14. Bayer HM, Glimcher PW. Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron. 2005;47:129–41.
15. Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci. 2007;10:1020–8.
16. Cacciapaglia F, Saddoris MP, Wightman RM, Carelli RM. Differential dopamine release dynamics in the nucleus accumbens core and shell track distinct aspects of goal-directed behavior for sucrose. Neuropharmacology. 2012;62:2050–6.
17. Day JJ, Carelli RM. The nucleus accumbens and pavlovian reward learning. Neuroscientist. 2007;13:148–59.
18. Volkow ND, Wang G-J, Fowler JS, Tomasi D, Telang F. Addiction: beyond dopamine reward circuitry. Proc Natl Acad Sci. 2011;108:15037–42.
19. O'Neal TJ, Bernstein MX, MacDougall DJ, Ferguson SM. A conditioned place preference for heroin is signaled by increased dopamine and direct pathway activity and decreased indirect pathway activity in the nucleus accumbens. J Neurosci. 2022;42:2011–24.
20. Liu Y, McNally GP. Dopamine and relapse to drug seeking. J Neurochem. 2021. 23. https://doi.org/10.1111/jnc.15309.
21. Schelp SA, Pultorak KJ, Rakowski DR, Gomez DM, Krzystyniak G, Das R, et al. A transient dopamine signal encodes subjective value and causally influences demand in an economic context. Proc Natl Acad Sci. 2017;114:E11303–E11312.
22. Eshel N, Touponse GC, Wang AR, Osterman AK, Shank AN, Groome AM, et al. Striatal dopamine integrates cost, benefit, and motivation. Neuron. 2024;112:500–514.e5.
23. Kutlu MG, Zachry JE, Melugin PR, Cajigas SA, Chevee MF, Kelly SJ, et al. Dopamine release in the nucleus accumbens core signals perceived saliency. Curr Biol. 2021;31:4748–4761.e8.
24. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology. 2007;191:391–431.
25. Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, et al. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science. 2009;324:1080–4.
26. Nicola SM, Taha SA, Kim SW, Fields HL. Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. Neuroscience. 2005;135:1025–33.
27. Sharpe MJ, Chang CY, Liu MA, Batchelor HM, Mueller LE, Jones JL, et al. Dopamine transients are sufficient and necessary for acquisition of model-based associations. Nat Neurosci. 2017;20:735–42.
28. Hollerman JR, Schultz W. Dopamine neurons report an error in the temporal prediction of reward during learning. Nat Neurosci. 1998;1:304–9.
29. Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH. A causal link between prediction errors, dopamine neurons and learning. Nat Neurosci. 2013;16:966–73.
30. Carhart-Harris RL, Friston KJ. REBUS and the anarchic brain: toward a unified model of the brain action of psychedelics. Pharm Rev. 2019;71:316–44.
31. De Filippo R, Schmitz D. Synthetic surprise as the foundation of the psychedelic experience. Neurosci Biobehav Rev. 2024;157:105538.
32. Glazer J, Murray CH, Nusslock R, Lee R, de Wit H. Low doses of lysergic acid diethylamide (LSD) increase reward-related brain activity. Neuropsychopharmacology. 2023;48:418–26.
33. Kanen JW, Luo Q, Kandroodi MR, Cardinal RN, Robbins TW, Nutt DJ, et al. Effect of lysergic acid diethylamide (LSD) on reinforcement learning in humans. Psychological Med. 2023;53:6434–45.
34. Vollenweider FX, Vontobel P, Hell D, Leenders KL. 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man-a PET study with [11C]raclopride. Neuropsychopharmacology. 1999;20:424–33.
35. Sakashita Y, Abe K, Katagiri N, Kambe T, Saitoh T, Utsunomiya I, et al. Effect of psilocin on extracellular dopamine and serotonin levels in the mesoaccumbens and mesocortical pathway in awake rats. Biol Pharm Bull. 2015;38:134–8.
36. Aulakh CS, Hill JL, Yoney HT, Murphy DL. Evidence for involvement of 5-HT1C and 5-HT2 receptors in the food intake suppressant effects of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Psychopharmacol (Berl). 1992;109:444–8.
37. Martin DA, Gyawali U, Calu DJ. Effects of 5-HT2A receptor stimulation on economic demand for fentanyl after intermittent and continuous access self-administration in male rats. Addiction Biol. 2021;26:e12926.
38. Sun F, Zhou J, Dai B, Qian T, Zeng J, Li X, et al. Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. Nat Methods. 2020;17:1156–66.
39. Koffarnus MN, Franck CT, Stein JS, Bickel WK. A modified exponential behavioral economic demand model to better describe consumption data. Exp Clin Psychopharmacol. 2015;23:504–12.

40. Saddoris MP, Sugam JA, Stuber GD, Witten IB, Deisseroth K, Carelli RM. Mesolimbic dopamine dynamically tracks, and is causally linked to, discrete aspects of value-based decision making. Biol Psychiatry. 2015;77:903–11.
41. Nasser HM, Chen Y-W, Fiscella K, Calu DJ. Individual variability in behavioral flexibility predicts sign-tracking tendency. Front Behav Neurosci. 2015;9:289.
42. Keefer SE Bacharach SZ, Kochli, DE, Chabot, JM, Calu, DJ. Effects of limited and extended Pavlovian training on devaluation sensitivity of sign-and goal-tracking rats. Front Behav Neurosci. 2020. 2020. https://doi.org/10.3389/fnbeh.2020.00003.
43. Fletcher PJ, Zeeb FD, Browne CJ, Higgins GA, Soko AD. Effects of 5-HT1A, 5-HT2A and 5-HT2C receptor agonists and antagonists on responding for a conditioned reinforcer and its enhancement by methylphenidate. Psychopharmacology. 2017;234:889–902.
44. Wing LL, Tapson GS, Geyer MA. 5HT-2 mediation of acute behavioral effects of hallucinogens in rats. Psychopharmacology. 1990;100:417–25.
45. Mokler DJ, Rech RH. Behavioral effects of intracerebroventricular administration of LSD, DOM, mescaline or lisuride. Pharmacol Biochem Behav. 1984;21:281–7.
46. Maguire DR, Li J-X, Koek W, France CP. Effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and quipazine on heroin self-administration in rhesus monkeys. Psychopharmacology. 2013;225:173–85.
47. Diederen KMJ, Fletcher PC. Dopamine, prediction error and beyond. Neuroscientist. 2021;27:30–46.
48. Keiflin R, Janak PH. Dopamine prediction errors in reward learning and addiction: from theory to neural circuitry. Neuron. 2015;88:247–63.
49. Barrett FS, Carbonaro TM, Hurwitz E, Johnson MW, Griffiths RR. Double-blind comparison of the two hallucinogens psilocybin and dextromethorphan: effects on cognition. Psychopharmacology. 2018;235:2915–27.
50. Wittmann M, Carter O, Hasler F, Cahn BR, Grimberg U, Spring P, et al. Effects of psilocybin on time perception and temporal control of behaviour in humans. J Psychopharmacol. 2007;21:50–64.
51. Asgari K, Body S, Bak VK, Zhang Z-Q, Rickard JF, Glennon JC, et al. Effects of 5-HT2A receptor stimulation on the discrimination of durations by rats. Behav Pharm. 2006;17:51–59.
52. Nichols DE. Psychedelics. Pharm Rev. 2016;68:264–355.
53. Salamone JD, Correa M. The neurobiology of activational aspects of motivation: exertion of effort, effort-based decision making, and the role of dopamine. Annu Rev Psychol. 2024;75:1–32.
54. Bakhurin K, Hughes RN, Jiang Q, Hossain M, Gutkin B, Fallon IP, et al. Force tuning explains changes in phasic dopamine signaling during stimulus-reward learning. 2023:2023.04.23.537994.
55. Day JJ, Jones JL, Wightman RM, Carelli RM. Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. Biol Psychiatry. 2010;68:306–9.
56. Roesch MR, Calu DJ, Schoenbaum G. Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. Nat Neurosci. 2007;10:1615–24.
57. Duerler P, Brem S, Fraga-González G, Neef T, Allen M, Zeidman P, et al. Psilocybin induces aberrant prediction error processing of tactile mismatch responses—a simultaneous EEG–FMRI study. Cereb Cortex. 2021;32:186–96.
58. Timmermann C, Spriggs MJ, Kaelen M, Leech R, Nutt DJ, Moran RJ, et al. LSD modulates effective connectivity and neural adaptation mechanisms in an auditory oddball paradigm. Neuropharmacology. 2018;142:251–62.
59. Umbricht D, Vollenweider FX, Schmid L, Grübel C, Skrabo A, Huber T, et al. Effects of the 5-HT2A agonist psilocybin on mismatch negativity generation and AX-continuous performance task: implications for the neuropharmacology of cognitive deficits in Schizophrenia. Neuropsychopharmacol. 2003;28:170–81.
60. Schmidt A, Bachmann R, Kometer M, Csomor PA, Stephan KE, Seifritz E, et al. Mismatch negativity encoding of prediction errors predicts S-ketamine-induced cognitive impairments. Neuropsychopharmacol. 2012;37:865–75.
61. van Elk M, Fried EI. History repeating: guidelines to address common problems in psychedelic science. Ther Adv Psychopharmacol. 2023;13:20451253231198466.
62. Thal SB, Bright SJ, Sharbanee JM, Wenge T, Skeffington PM. Current perspective on the therapeutic preset for substance-assisted psychotherapy. Front Psychol. 2021;12:617224.
63. Dupuis D, Veissière S. Culture, context, and ethics in the therapeutic use of hallucinogens: psychedelics as active super-placebos? Transcult Psychiatry. 2022;59:571–8.
64. Szigeti B, Heifets B. Expectancy effects in psychedelic trials. Biol Psychiatry Cogn Neurosci Neuroimaging. 2024:S2451-9022 00055-7.
65. Olson DE. Biochemical mechanisms underlying psychedelic-induced neuroplasticity. Biochemistry. 2022;61:127–36.

ACKNOWLEDGEMENTS

We thank Drs.Yulong Li and Jessie Feng for providing the $GRAB_{DA}$ construct used in these studies.

AUTHOR CONTRIBUTIONS

DAM conceived of experiments, collected and analyzed data, and wrote the manuscript; AD collected and analyzed data; and DJC conceived of experiments and critically revised the manuscript.

FUNDING

This work was supported by NIDA RO1DA043533 to DJC, and the Department of Neurobiology, University of Maryland, Baltimore.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-024-01912-4.

Correspondence and requests for materials should be addressed to David A. Martin.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.