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Changes in dorsomedial striatum activity during expression of goal-directed vs. habit-like cue-induced cocaine seeking

Brooke N. Bender[a,b], **Sierra J. Stringfield**[a], **Mary M. Torregrossa**[a,b,*]

[a]Department of Psychiatry, University of Pittsburgh, 450 Technology Drive, Pittsburgh, PA 15219, United States

[b]Center for Neuroscience, University of Pittsburgh, 4200 Fifth Ave, Pittsburgh, PA 15213, United States

Abstract

A preclinical model of cue exposure therapy, cue extinction, reduces cue-induced cocaine seeking that is goal-directed but not habit-like. Goal-directed and habitual behaviors differentially rely on the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), but the effects of cue extinction on dorsal striatal responses to cue-induced drug seeking are unknown. We used fiber photometry in rats trained to self-administer cocaine paired with an audiovisual cue to examine how dorsal striatal intracellular calcium and extracellular dopamine activity differs between goal-directed and habit-like cue-induced cocaine seeking and how it is impacted by cue extinction. After minimal fixed-ratio training, rats showed enhanced DMS and DLS calcium responses to cue-reinforced compared to unreinforced lever presses. After rats were trained on goal-promoting fixed ratio schedules or habit-promoting second-order schedules of reinforcement, different patterns of dorsal striatal calcium and dopamine responses to cue-reinforced lever presses emerged. Rats trained on habit-promoting second-order schedules showed reduced DMS calcium responses and enhanced DLS dopamine responses to cue-reinforced lever presses. Cue extinction reduced calcium responses during subsequent drug seeking in the DMS, but not in the DLS. Therefore, cue extinction may reduce goal-directed behavior through its effects on the DMS, whereas habit-like behavior and the DLS are unaffected.

Keywords

Dorsal striatum; Cue extinction; Cocaine; Dopamine; habit; Goal-directed

*Corresponding author at: Department of Psychiatry, University of Pittsburgh, 450 Technology Drive, Pittsburgh, PA 15219, United States. torregrossam@upmc.edu (M.M. Torregrossa).

CRediT authorship contribution statement

Brooke N. Bender: Conceptualization, Methodology, Investigation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Sierra J. Stringfield:** Methodology, Visualization, Formal analysis, Writing – review & editing. **Mary M. Torregrossa:** Funding acquisition, Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.addicn.2024.100149.

1. Introduction

A major obstacle in the treatment of substance use disorders (SUDs) is maladaptive learning and memory, which can promote drug craving, use, and relapse [1–5]. Several types of associative learning contribute to persistent drug use, and drug exposure also enhances learning and the strength of these associative memories [6–12]. Response-outcome learning occurs when a desired outcome, such as a drug of abuse, becomes associated with a behavioral response, or action, that produces the drug effect [13–15]. Response-outcome associations can then promote goal-directed drug-seeking behavior [16]. As learning continues and an action repeatedly leads to the same outcome, stimulus-response associations begin to form where the environmental stimuli present during response-outcome learning (e.g., contexts or discrete cues) become sufficient to drive the behavioral response independent of the value of the outcome, which is defined as habitual behavior [13–15]. Therefore, over time these stimuli alone can promote putatively habitual drug-seeking behaviors [15,17]. Finally, in addition to action-related learning, Pavlovian associations also form between environmental stimuli and the effects of a drug, which can also promote motivated behavior [10,16,18]. The presentation of drug-associated stimuli, or cues, has been shown to enhance subjective levels of drug craving in individuals with SUDs, promote relapse, and activate implicated brain regions, including the nucleus accumbens, dorsal striatum, and regions of the cortex [1,4,5,19–24].

Therefore, the extinction of Pavlovian drug-cue associations has been proposed as a potential therapeutic target in the treatment of SUDs [25–27]. Cue exposure therapy, the repeated presentation of cues in the absence of the associated outcome, has been shown to be an effective behavioral treatment for others psychiatric disorders that involve mal-adaptive Pavlovian associations, such as phobias and post-traumatic stress disorder [28,29]. Additionally, cue extinction, a preclinical model of cue exposure therapy, reduces cue-induced cocaine seeking in rodent cocaine self-administration models [30–33]. However, the clinical application of cue exposure therapy to SUDs has yielded modest results [26,34]. There are likely several reasons for this difficulty in translation, including context dependency [35–37]. However, our lab has also shown that Pavlovian cue extinction reduces goal-directed cocaine seeking, but has no effect on habit-like cocaine seeking unless goal-directed control is restored [38]. Therefore, a lack of effect of cue extinction on habitual components of drug seeking may also be a contributing factor to this difficulty in translation.

Extensive literature has implicated distinct neural circuits in goal-directed and habitual behavior [18,39,40]. Dopaminergic inputs from the substantia nigra to the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) are important for the initiation of goal-directed and habitual behavior, respectively [41–44]. Additionally, other direct and indirect inputs to the dorsal striatum, including those from the cortex, thalamus, and amygdala, may be important for toggling between reliance on goal-directed and habitual behavior [45–50]. Dopamine release in the DMS and DLS during operant reward seeking can differ between regions depending on the operant task and extent of training [51–55]. Several studies using in vivo electrophysiology to compare DMS and DLS activity during operant reward-seeking behavior have also shown distinct patterns of neural activity in these regions and indicate these patterns change as habitual behavior develops [56–59]. However, the

specific contributions of dopaminergic and other inputs to the dorsal striatum's response to drug-associated cues and how they might be impacted by Pavlovian cue extinction remain unclear.

In the present study, we employed fixed-ratio (FR) and second-order (SO) schedules of reinforcement to facilitate either goal-directed (DMS dopamine-dependent) or habit-like (DLS dopamine-dependent) cocaine seeking, respectively, as previously described [38,42,50]. We utilized fiber photometry to examine dorsal striatal calcium and dopamine activity during drug seeking throughout the establishment of DLS-independent and -dependent cocaine self-administration and evaluated the effects of cue extinction on activity in these regions. We found distinct signatures of calcium and dopamine activity in the dorsal striatum in rats trained on FR or SO reinforcement schedules that promote goal-directed or habit-like cocaine seeking, respectively. Additionally, we showed that cue extinction impacted DMS, but not DLS, calcium and dopamine activity during subsequent drug seeking, which suggests that cue extinction does not impact the neural circuitry promoting DLS-dependent, habit-like behavior.

2. Materials and methods

2.1. Experimental design

Adult male and female rats expressing the fluorescent calcium indicator RCaMP1b and dopamine indicator dLight1.2 in the DMS and DLS were implanted with optic fibers in the DLS and DMS and jugular vein catheters. Rats were trained to self-administer cocaine paired with an audiovisual cue for 20 days, and were split into FR-trained and SO-trained groups and trained accordingly on different schedules of reinforcement to facilitate goal-directed (FR-trained) or habit-like (SO-trained) cocaine seeking behavior. Fiber photometry recordings under extinction conditions occurred during 15-min drug-seeking tests that occurred immediately before self-administration on days 9–20 on the rat's reinforcement schedule from the previous day. To determine how DLS and DMS calcium and dopamine responses are affected by lever presses that resulted in presentation of cocaine-associated cues, dorsal striatal responses were compared between active lever presses that resulted in cue presentation (cue-reinforced) and active lever presses that had no consequence (unreinforced). To examine how training schedule impacts dorsal striatal calcium and dopamine active, dorsal striatal responses were compared between FR-trained and SO-trained rats across early, middle, and late phases of training. Next, rats underwent fiber photometry recordings during cue extinction (120 20-s noncontingent cues) and a 1-h cue-induced drug-seeking test. To determine if cue extinction impacts dorsal striatal calcium or dopamine activity, dorsal striatal responses were compared between the cue-induced drug-seeking test that followed cue extinction and during the late phase of training.

2.2. Animals

Adult Sprague-Dawley rats (Envigo) were 8–9 weeks old upon arrival ($n = 26$; male $n = 14$; female $n = 12$). Animals were housed in auto-ventilated racks with automated watering in a temperature- and humidity- controlled room maintained on a 12-h light-dark cycle. Rats were given ≥4 days to acclimate to the facility before surgical procedures and were

pair-housed until catheter implantation. Rats had ad libitum access to food and water until 24 h before the start of training, when they were food restricted to maintain ~90 % of their free-feeding body weight. Behavioral experiments were run in the light cycle and began within ~3 h of the same time each day. Procedures were conducted in accordance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

2.3. Viral vectors

Viral vectors encoding the fluorescent dopamine indicator dLight1.2 (AAV5-hSyn-dLight1.2) (Addgene, titer ≥4×10^{12} vg/mL) and calcium indicator jRCaMP1b (AAV1.Syn.NES-jRCaMP1b.WPRE.SV40) (Addgene, titer ≥1×10^{13} vg/mL) were mixed in a 1:1 ratio and vortexed immediately prior to intracranial infusion surgeries.

2.4. Drugs

Cocaine hydrochloride (graciously provided by NIDA) was dissolved at 2 mg/ml in 0.9 % sterile saline (Thermo Fisher) and filter-sterilized.

2.5. Behavioral apparatus

Experiments were conducted in 4 standard operant conditioning chambers using MedPC software (Med Associates). Each animal underwent all training and testing in the same chamber. Each chamber was equipped with bar floors and a syringe pump connected to a swiveled leash. All chambers had 3 plexiglass walls and one wall containing two levers with cue lights above them, a head-entry magazine, a houselight, and a tone generator. Chambers were housed in a sound-attenuating box with a fan for background noise.

2.6. Surgery

2.6.1. Anesthesia—Rats were fully anesthetized with ketamine (100 mg/kg, Henry Schein) and xylazine (5 mg/kg, Butler Schein) intramuscularly, administered analgesic (5 mg/kg Rimadyl, Henry Schein) subcutaneously, and prepared for surgery as previously described [32,38].

2.6.2. Viral infusion—Viral infusion surgery took place at least 4 weeks prior to photometry recordings to allow for virus expression. Rats were placed in a stereotaxic frame and lidocaine (0.3 ml, Butler Schein) was injected subcutaneously above the skull as previously described [32]. A 26-gauge injection cannula connected to a Hamilton Syringe and pump was used to inject 1 μl of virus mixture at a rate of 0.05 μl/min unilaterally into the anterior DLS (in mm from bregma, anterior and posterior (AP): +0.8; medial and lateral (ML): ±2.8; dorsal and ventral (DV): −5.0) and, in the opposite hemisphere, the posterior DMS (AP: −0.2 mm; ML: ±2.2 mm; DV: −4.7 mm). The hemisphere receiving each injection was counter-balanced. We chose to target the anterior DLS and posterior DMS to remain consistent with previous experiments and because of inconsistencies in the literature regarding the role of the anterior DMS in goal-directed behavior [7,38,42,50,60].

2.6.3. Intravenous catheterization and optic fiber implantation—In a second surgery up to a week before rats began self-administration, rats were implanted with a

chronic indwelling intravenous catheter into the right jugular vein as previously described [32,38, 61]. Rats were then placed in a stereotaxic frame and lidocaine was injected subcutaneously above the skull. Fiber optic cannulae (Thorlabs, 2.5 mm ferrule, 400 μm core, 5 mm long) were lowered into the DLS (AP: +0.8 mm; ML: ±3.0 mm; DV: −4.5 mm) and the DMS (AP: −0.2 mm; ML: ±2.0 mm; DV: −4.0 mm) and were secured as previously described [32].

2.6.4. Post-operative care—Rats were administered analgesic and catheters were flushed daily to maintain patency as previously described [38].

2.7. Behavioral procedures

2.7.1. Cocaine self-administration—Rats were trained to self-administer cocaine (1 mg/kg/infusion) in 1-h daily sessions for 20 days as previously described [38]. Briefly, cocaine infusions were paired with a 20-s audiovisual cue accompanied by a 20-s time-out when levers were retracted and the houselight was extinguished, and inactive lever presses were recorded but had no consequences. Rats were initially trained to self-administer cocaine on a fixed-ratio 1 (FR1) schedule for 7 days (acquisition) then on an FR3 schedule for 3 days (early training). Rats were then split into FR-trained (goal-directed) and SO-trained (habit-like) groups [38]. FR training typically promotes goal-directed behavior and produces cocaine seeking that is dependent on DMS dopamine in rats [15,42]. On fixed-ratio (FR) schedules of reinforcement, a fixed number of lever presses results in cocaine infusion and a 20-s timeout period, during which the audiovisual cue is presented (Fig. 1(A)). FR-trained rats were maintained on an FR3 schedule for 5 days (middle training) and then trained on an FR5 schedule for 5 days (late training). SO-trained rats were trained on second-order (SO) schedules of reinforcement, where a brief conditioned stimulus (secondary reinforcer) is presented upon the completion of one schedule, and the primary reinforcer is delivered under a secondary schedule of reinforcement [62,63]. For example, for an FR5 (FR2S) schedule, every second lever press (indicated by FR2S) results in a 1-s presentation of the audiovisual cue, and every fifth completion (indicated by FR5) of the FR2S schedule, after a total of 10 lever presses, results in cocaine infusion and timeout paired with the 20-s audiovisual cue (Fig. 1(B)). SO schedules promote drug seeking over longer periods without drug and increase the number of stimulus-response pairings, which results in putatively habitual DLS dopamine-dependent cocaine seeking in rats [10,13,38,42,50,64]. SO-trained rats were trained for 5 days on an FR5 (FR2S) schedule (middle training) followed by 5 days on an FR7(FR2S) schedule (late training). Levers were not retracted during 1-s cues during SO training.

2.7.2. Daily cue-induced drug-seeking tests—During fiber photometry recordings, rats underwent a 15-min drug-seeking test immediately before self-administration on days 9–20. To prevent cocaine from influencing extracellular dopamine, no cocaine was administered during these tests, but audiovisual cues and timeouts occurred on the same reinforcement schedule each rat self-administered under on the previous day. Standard self-administration sessions immediately followed each of these 15-min test sessions.

2.7.3. Pavlovian cue extinction—On the day immediately following the final day of self-administration, with levers retracted, rats were non-contingently exposed to 120 20-s audiovisual cues separated by 10 s during photometry recordings.

2.7.4. Cue-induced drug-seeking test—On the day following cue extinction, rats underwent a 1-h drug-seeking test during fiber photometry recordings. Cocaine was withheld and cues and timeouts occurred on the rat's previous reinforcement schedule. The cue-induced drug-seeking test was 1 h long because, in our hands, rats can take longer to resume cue-induced drug seeking after cue extinction, which was supported by our finding that multiple rats ($n = 4$) did not complete their reinforcement schedule within the first 15 min of this drug-seeking test.

2.8. Fiber photometry

2.8.1. Recordings—Photometry recordings were collected using a multi-wavelength photometry system (Plexon) and a branched low-autofluorescence fiber-optic patch cord (Doric: 2 branches, 400 μm core, 440 μm cladding, 0.37 NA). Laser output for each excitation wavelength (560 nm, 465 nm, and 410 nm) was set that laser intensity at the cable tip was 20–30 μW. Laser was passed through the patch cord for 30 min prior to daily photometry recordings to minimize autofluorescence of the cable during recordings. Recordings occurred using 3-phase cycling of 415, 465, and 560 nm LEDs. Fluorescence data were collected at 30 frames per second using Plexon software, and behavioral events were aligned to photometry fluorescence using TTL timestamp outputs from MedPC software. Rats were habituated to the optic cable setup in the operant chamber for at least 1 day prior to photometry recordings.

2.8.2. Processing and analysis—Data were processed and analyzed using custom MATLAB (Math-works, Natick, MA, USA) scripts, which are available at: https://github.com/brookebender/Code-for-DOI-10.1101–2023.07.24.550364.git. Fluorescent signals were forward and reverse low-pass filtered at 30 Hz. Traces were visualized and motion artifacts were removed. The isosbestic control trace was fitted to fluorescent signal traces at 465 nm (dLight) and 560 nm (RCaMP) using a least squares polynomial fit of degree 1. $\Delta F/F$ was calculated by subtracting the fitted isosbestic signal from the fluorescent signal and then dividing by the fitted isosbestic signal. Z-scores were calculated across the entire fluorescent signal trace by subtracting the mean $\Delta F/F$ value from each data point and dividing by the standard deviation. Traces around behavioral events (including 3 s before and 30 s after) were separated and averaged for each animal for each recording day, and SEM was calculated for the average trace.

The majority of the responses we observed occurred within 1 s after behavioral events. Therefore, for each training day, the traces for each event type were averaged, and the area under the curve (AUC) and peak z-score amplitude were calculated, with AUC defined as the area under the curve of the z-score during the 1-s period after each event and the peak z-score defined as the maximum z-score that occurred during the 1-s period after the event. The peaks and z-scores were then averaged across sessions in the same training phase for each animal. Therefore, each individual data point on bar graphs represents a single, unique

subject for each phase of training. Both calcium and dopamine responses were recorded in every rat. Although we recorded both calcium and dopamine from both the DMS and DLS in every rat, in some cases one region had to be excluded due to viral expression or fiber placement, which is why the number of individual data points may between DLS and DMS results. For results, “cue-reinforced active lever presses” refer to lever presses that resulted in cue presentation and timeout (20-s audiovisual cue, houselight off, and lever retraction). “Unreinforced active lever presses” refer to active lever presses that did not result in any cue presentation, and for which there were no active lever presses (and therefore no cue presentations) in the 3 s before or 5 s after.

2.9. Vaginal cytology

Estrous cycle phase was determined daily by cellular morphology as previously described [65,66].

2.10. Histology

Rats were anesthetized and perfused and brains were removed, sliced, mounted, and coverslipped as previously described [32]. Every fourth section containing the dorsal striatum was mounted and imaged at 10× magnification using an Olympus BX61VS epifluorescent slide-scanning microscope to verify fiber placement location and virus expression at the base of the fiber.

2.11. Exclusion criteria

Rats were excluded from all analysis due to death or illness after surgery ($n = 5$), loss of catheter patency ($n = 1$) (determined by a 0.1 ml intravenous infusion of 10 mg/ml sodium brevital, Covetrus), or bilateral histological misses ($n = 4$). For rats with a unilateral histological miss ($n = 6$), data for the region with the histological miss were excluded. One rat was excluded from the final cue-induced drug-seeking test due to loss of head cap. Rats were excluded from analysis during cue-induced drug-seeking tests if they failed to make enough lever presses to reach a long cue during all phases of training ($n = 2$, Figs. 3, 4, S2, and S3). Rats were excluded from comparing activity during the late training to activity during drug seeking after cue extinction if they failed to obtain a long cue or make an isolated unreinforced lever press during either test ($n = 3$, Fig. 5). We recognize that, due to many of these exclusions, some group sizes were small, which is one potential limitation to these studies.

2.12. Quantification and statistical analysis

Behavioral data were collected using MedPC software. All statistical analyses were performed using GraphPad Prism. For the 1-h cue-induced drug-seeking test after cue extinction, the ratio of responding was calculated by dividing the number of active lever presses during test by the number of active lever presses in the final self-administration session to normalize changes in responding across rats with different magnitudes of lever pressing behavior due to their training schedule.

For all statistical analyses, significance was set at $p < 0.05$. All data were determined to be normally distributed using the Shapiro-Wilk test, and Bartlett’s test was used to determine

that there were no significant differences in the estimated variance between groups. For analyses using repeated-measures ANOVAs, a Geisser-Greenhouse correction was used to account for potential lack of sphericity. Criteria for outlier data points was set at >2 standard deviations from the mean, and outlier points were excluded along with their paired data (Figs. 3(A), S1(E), S2(A), (D)).

Infusions were analyzed by two-way rmANOVA, using time and training schedule as factors. Lever presses during self-administration were analyzed by three-way rmANOVA with time, training schedule, and lever as factors. Ratio of active lever presses during the post-cue extinction cue-induced drug-seeking test was analyzed with an unpaired student's *t*-test. Calcium and dopamine peak z-score amplitude and AUCs during cue-induced drug-seeking tests were analyzed by two-way or three-way ANOVA with future training schedule, training schedule, sex, cue reinforcement, cue length, phase of training, or cue extinction as factors as indicated. Calcium and dopamine peak z-score amplitude and AUC during early training were analyzed to determine if there was an effect of estrous phase on signals by mixed-effects analysis with estrous phase and cue reinforcement as factors because one rat was never in estrus during those three days of testing. Calcium and dopamine peak z-score amplitude and AUCs during cue extinction were analyzed by one-way rmANOVA. For correlation analyses, Pearson's correlation coefficients were calculated with average active lever presses as the independent variable and peak z-score amplitude or AUC as the dependent variable. Calcium and dopamine peak z-score amplitude and AUCs after pre-learning stimuli exposure were analyzed by two-way ANOVA with stimulus type and stimulus presentation as factors. When a significant effect was detected by one-way ANOVA or an interaction was detected by two-way or three-way ANOVA analysis, significant effects were further analyzed by Tukey's or Sidak's post-hoc multiple comparisons analysis, respectively. Throughout our analyses, we report the peak z-score amplitude as our primary measure of calcium and dopamine responses. Results from analyses of AUC data were overall similar to results from the peak amplitude, and therefore AUC results are presented in the supplementary material for the sake of space and clarity. There were a few notable differences, and interested readers will find mention of these in the supplementary material.

3. Results

3.1. FR- and SO-trained rats do not differ in daily cocaine self-administration or cue-induced drug-seeking after cue extinction

The dopamine fluorescent sensor dLight and calcium sensor RCaMP were expressed contralaterally in the anterior DLS and posterior DMS in male and female rats ($n = 26$), and optic fibers and jugular vein catheters were implanted (Fig. 2(A)). After all experiments, virus expression at the base of the fiber (Fig. 2(B)) and fiber placement (Fig. 2(C)) were confirmed by fluorescent microscopy. A total of 10 rats were excluded due to death after surgery, loss of catheter patency, or bilateral fiber misplacement or virus expression. Remaining rats ($n = 16$; 9 males and 7 females) were trained to self-administer cocaine (1 mg/kg/inf) for 20 days before they underwent cue extinction and a subsequent cue-induced drug-seeking test (Fig. 2(D)). During daily self-administration, there was a main effect of training day on number of infusions ($F_{(4.776,66.86)} = 5.759$, $p = 0.0002$), but no main effect

of training schedule ($F_{(1,14)}$ = 0.002179, p = 0.9634) or interaction ($F_{(19,266)}$ = 1.486, p = 0.0899) (2-way rmANOVA) (Fig. 2(E)). For lever presses, there was a 3-way training day × schedule × lever interaction ($F_{(19,266)}$ = 9.801, p < 0.0001) (3-way rmANOVA) (Fig. 2(F)). These data indicate that both groups self-administered more cocaine infusions and made more active lever presses as training progressed, and that the increase in active lever presses was more pronounced in SO-trained rats, which is expected because these rats had to increase their lever presses to receive the same number of infusions. During the cue-induced drug-seeking test after cue extinction, there was no difference in the ratio of active lever presses during test to the final day of self-administration between FR-trained and SO-trained rats (p = 0.3229, η^2 = 0.08873) (unpaired t-test) (Fig. 2(G)).

In photometry recordings, which occurred daily on days 9–20 in 15-min sessions prior to self-administration, rats' responses resulted in cues based on the reinforcement schedule of the previous day, but cocaine infusions were withheld. For the number of schedule completions during photometry recordings each day, which would correspond with cocaine infusions if they were not withheld, there was no main effect of training schedule ($F_{(1,12)}$ = 1.912, p = 0.1919), but there was a main effect of testing day ($F_{(4.007,46.99)}$ = 5.393, p = 0.0012) and a testing day × training schedule interaction ($F_{(11,129)}$ = 1.979, p = 0.0355) (Mixed-effects analysis) (Fig. 2(H)). Post-hoc analyses (Sidak's multiple comparisons) did not detect any significant differences between testing days in either group, which suggest the number of schedule completions overall decreased throughout testing in SO-trained rats, likely because the number of lever presses required to complete the schedule increased when a new reinforcement schedule was introduced. However, for active lever presses, there was no main effect of testing day ($F_{(1.969,23.09)}$ = 2.314, p = 0.1220) and there was no testing day × training schedule interaction ($F_{(11,129)}$ = 1.836, p = 0.543), but there was a main effect of training schedule ($F_{(1,12)}$ = 6.527, p = 0.0252) (Mixed-effects analysis) (Fig. 2(I)). Post-hoc analyses (Sidak's multiple comparisons) did not detect any significant differences between groups on any individual training day. These results indicate that SO-trained rats made more active lever presses than FR-trained rats in tests, an effect that was more apparent when SO schedules were introduced, presumably because SO-trained rats increased their lever presses to complete the schedule. Therefore, rats did not reduce their responding across multiple testing days under extinction conditions, which suggests that there was no significant extinction learning occurring across phases of training.

3.2. After acquisition, dorsal striatal calcium responses are greater for cue-reinforced than unreinforced active lever presses

After 7 days of cocaine self-administration on an FR1 schedule, all rats self-administered on an FR3 schedule for 3 days. Following the first day of FR3 training, fiber photometry recordings took place in daily 15-min drug-seeking tests prior to self-administration. The first 3 days of recording to examine dorsal striatal responses during "early training" occurred after acquisition but before rats were split into FR- and SO-trained groups. Because we wanted to determine if there were any differences between rats later split into groups, during this early training phase we first compared dorsal striatal responses between rats that would later be separated into FR- and SO-trained groups. On the schedules of reinforcement used in these studies, some active lever presses resulted in cue presentation upon the completion

of the reinforcement schedule, but others that occurred before schedule completion had no consequences. Because we were particularly interested in the role of the drug-paired cue, we compared calcium and dopamine responses in the dorsal striatum between future training groups for cue-reinforced (those that completed the schedule and resulted in 20-s timeouts) versus unreinforced active lever presses (those that did not result in cue presentation and were isolated from other behavioral events) using 2-way ANOVAs. Therefore, any differences were due to differences in the response to cue presentation after lever press and were not a motion artifact of performing the lever press. Because we used both male and female rats, we first determined that there was no effect of sex or estrous phase on dorsal striatal responses to lever presses (Fig. S1). Therefore, males and females were combined for analyses throughout.

During early training prior to splitting animals into groups, there was a main effect of cue reinforcement ($F_{(1,8)} = 12.98$, $p = 0.0070$) on calcium peak z-score amplitude in the DLS during the 1 s after lever press, but no effect of future training schedule ($F_{(1,8)} = 4.263$, $p = 0.0728$) or interaction ($F_{(1,8)} = 2.214$, $p = 0.1751$) (Fig. 3(A)). Similarly, for calcium peak amplitude in the DMS, there was a main effect of cue reinforcement ($F_{(1,9)} = 9.035$, $p = 0.0148$), but no effect of future training schedule ($F_{(1,9)} = 0.006144$, $p = 0.9392$) or interaction ($F_{(1,9)} = 0.1558$, $p = 0.7022$) (Fig. 3(B)). For dopamine peak amplitude in the DLS, there were no main effects of cue reinforcement ($F_{(1,9)} = 0.06979$, $p = 0.7976$) or future training schedule ($F_{(1,9)} = 1.907$, $p = 0.2006$) or interaction ($F_{(1,9)} = 0.2988$, $p = 0.5979$) (Fig. 3(C)). Similarly, for dopamine peak amplitude in the DLS, there were no main effects of cue reinforcement ($F_{(1,9)} = 0.06176$, $p = 0.8093$), future training schedule ($F_{(1,9)} = 0.0007846$, $p = 0.9783$) or interaction ($F_{(1,9)} = 0.02546$, $p = 0.8767$) (Fig. 3(D)). Overall, these data suggest that after acquisition of cocaine self-administration, calcium activity in the DMS and DLS was greater in response to cue-reinforced lever presses compared to lever presses that did not result in cue presentation. Interestingly, cue reinforcement after lever press did not impact dopamine activity in either region during this early training phase. Throughout, we present peak amplitude data in the main figures, but we also calculated the area under the curve (AUC), which can be found in supplemental Figs. S1–S6. During early training, results from AUC data were similar to peak data (Fig. S2). After SO-training, SO-trained rats make more active lever presses during photometry recordings than FR-trained rats, so we were concerned that rats' rate of responding could impact calcium or dopamine responses to lever presses. Therefore, during the early phase of training prior to rats being separated into groups, we performed correlation analyses between the average number of active lever presses during early-phase drug seeking and the average calcium or dopamine peak amplitude or AUC for cue-reinforced lever presses (Fig. S2). We found no correlation between these measures, which suggests that different rates of lever pressing do not impact dorsal striatal responses to cues.

3.3. FR-trained rats, but not SO-trained rats, show greater DMS calcium activity after cue-reinforced compared to unreinforced lever presses

After 10 days of self-administration, rats were split into FR-trained and SO-trained groups and trained on different schedules of reinforcement accordingly. The next 10 days were split into 5 days of middle training and 5 days of late training. Comparing the middle

and late phase of training in addition to comparing FR- and SO-trained allowed us to determine if there were any consistent changes due to prolonged training in general, or if changes were unique to prolonged training on different schedules of reinforcement. During SO-schedule training, short 1-s cues are presented in addition to 20-s cues presented upon schedule completion, and for some measures SO-trained rats had different responses to short cues compared to long cues (Fig. S3(E)–(L)). Therefore, only long cues were compared between FR- and SO-trained rats. To determine if SO-trained rats had different dorsal striatal responses to lever presses than FR-trained rats, we compared cue-reinforced to un-reinforced lever presses between groups for each phase of training (middle or late) using 3-way ANOVAs. For DLS calcium peak amplitude, there was a main effect of cue reinforcement ($F_{(1,9)} = 31.21$, $p = 0.0003$), but no main effects of training schedule ($F_{(1,9)} = 4.259$, $p = 0.0691$) or phase of training ($F_{(1,9)} = 3.205$, $p = 0.1633$), and there were no cue reinforcement × training schedule ($F_{(1,9)} = 0.00748$, $p = 0.9318$), cue reinforcement × phase of training ($F_{(1,9)} = 0.6451$, $p = 0.4426$), phase of training × training schedule ($F_{(1,9)} = 3.954$, $p = 0.0780$), or 3-way interactions ($F_{(1,9)} = 0.6999$, $p = 0.4245$) (Fig. 4(A)). For DMS calcium peak amplitude, there was a main effect of cue reinforcement ($F_{(1,9)} = 9.569$, $p = 0.0129$) and a cue reinforcement × training schedule interaction ($F_{(1,9)} = 5.370$, $p = 0.0457$), but no main effect of training schedule ($F_{(1,9)} = 1.870$, $p = 0.2047$) or phase of training ($F_{(1,9)} = 1.130$, $p = 0.3155$), and no cue reinforcement × phase of training ($F_{(1,9)} = 0.003176$, $p = 0.9563$), phase of training × training schedule ($F_{(1,9)} = 4.718$, $p = 0.0579$), or 3-way interaction ($F_{(1,9)} = 0.01580$, $p = 0.9027$) (Fig. 4(B)). These data suggest that while both FR-trained and SO-trained rats had greater calcium responses in the DLS to cue-reinforced than unreinforced lever presses, this difference was only present in the DMS for FR-trained rats, but not in SO-trained rats. In other words, SO training led to a loss of cue-induced calcium-indicated activity selectively in the DMS.

3.4. SO-trained rats, but not FR-trained rats, show greater DLS dopamine responses to cue-reinforced compared to unreinforced lever presses

For DLS dopamine peak amplitude during middle and late training, there was a main effect of cue reinforcement ($F_{(1,9)} = 11.42$, $p = 0.0081$) and a cue reinforcement × training schedule interaction ($F_{(1,9)} = 5.494$, $p = 0.0437$), but no main effect of training schedule ($F_{(1,9)} = 0.001535$, $p = 0.9696$) or phase of training ($F_{(1,9)} = 0.06291$, $p = 0.8076$), and no cue reinforcement × phase of training ($F_{(1,9)} = 0.3683$, $p = 0.5589$), phase of training × training schedule ($F_{(1,9)} = 0.8370$, $p = 0.3841$), or 3-way interaction ($F_{(1,9)} = 1.951$, $p = 0.1960$) (Fig. 4(C)). There were no main effects of cue reinforcement ($F_{(1,9)} = 0.2947$, $p = 0.1202$), training schedule ($F_{(1,9)} = 0.01592$, $p = 0.9024$), or phase of training ($F_{(1,9)} = 0.008296$, $p = 0.9294$) on DMS dopamine peak amplitude, and there were no cue reinforcement × training schedule ($F_{(1,9)} = 6271$, $p = 0.4488$), cue reinforcement × phase of training ($F_{(1,9)} = 0.1141$, $p = 0.7433$), phase of training × training schedule ($F_{(1,9)} = 0.2727$, $p = 0.6141$), or 3-way interactions ($F_{(1,9)} = 2.060$, $p = 0.1851$) (Fig. 4(D)). These data suggest that SO-trained rats, but not FR-trained rats, had increased DLS dopamine activity after cue-reinforced lever presses, but neither group had greater DMS dopamine in response to cue-reinforced compared to unreinforced lever presses. Data for AUC of calcium and dopamine responses during middle and late training are presented in the supplement (Fig.

S3(A)–(D)). Examples of traces for individual cue-reinforced and unreinforced lever presses on a single day of late-phase FR and SO training can be found in the supplement (Fig. S4).

3.5. Cue extinction reduces DMS calcium peak amplitude during drug seeking selectively in FR-trained rats

Given that we have previously found that SO-trained rats are resistant to the effects of cue extinction in modulating their cocaine-seeking behavior, and that this is reliant on activity in the DLS [38], we wanted to determine if cue extinction differentially affected dorsal striatal activity in a drug seeking test after cue extinction. Rats underwent a 1-h cue-induced drug-seeking test during which photometry recordings occurred. Dorsal striatal calcium and dopamine peak amplitudes after cue-reinforced or unreinforced lever presses during this post-cue extinction drug-seeking test (post-ext) were compared to the late phase of training (pre-ext) using 3-way ANOVAs. There was a main effect of cue reinforcement ($F_{(1,7)}$ = 8.302, p = 0.0236) on DLS calcium peak amplitude, but no main effects of training schedule ($F_{(1,7)}$ = 3.393, p = 0.2080) or cue extinction ($F_{(1,7)}$ = 0.4329, p = 0.5316) or cue reinforcement × training schedule ($F_{(1,7)}$ = 1.480, p = 0.2632), cue reinforcement × cue extinction ($F_{(1,7)}$ = 0.06984, p = 0.7992), training schedule × cue extinction ($F_{(1,7)}$ = 1.385, p = 0.2778), or 3-way interactions ($F_{(1,7)}$ = 0.3617, p = 0.5665) (Fig. 5(A)). These data suggest that cue extinction did not impact DLS calcium activity for either FR- or SO-trained rats. For DMS calcium peak amplitude, there was a main effect of cue reinforcement ($F_{(1,7)}$ = 11.42, p = 0.0118) and a main effect of cue extinction ($F_{(1,7)}$ = 6.514, p = 0.0380), as well as significant cue reinforcement × training schedule ($F_{(1,7)}$ = 6.362, p = 0.0397) and training schedule × cue extinction interactions ($F_{(1,7)}$ = 7.953, p = 0.0258) (Fig. 5(B)). There was no main effect of training schedule ($F_{(1,7)}$ = 2.980, p = 0.1279) on DMS dopamine peak amplitude, and there was no cue reinforcement × cue extinction ($F_{(1,7)}$ = 0.1234, p = 0.7358) or 3-way interaction ($F_{(1,7)}$ = 0.04091, p = 0.8455) (Fig. 5(B)). These results suggest that cue extinction resulted in a reduction in DMS calcium peak amplitude for FR-trained rats, while SO-trained rats were unaffected, which may be due to their already minimal DMS calcium response. Effects of cue extinction on calcium AUC can be found in the supplement (Fig. S5(A), (B)).

3.6. Cue extinction reduces DMS dopamine peak amplitudes during drug seeking in both groups

For DLS dopamine peak amplitude, there was a main effect of cue reinforcement ($F_{(1,7)}$ = 6.724, p = 0.0358), but no effect of training schedule ($F_{(1,7)}$ = 0.1056, p = 0.7547) or cue extinction ($F_{(1,7)}$ = 0.2119, p = 0.6592), and there were no cue reinforcement × training schedule ($F_{(1,7)}$ = 0.02193, p = 0.8865), cue reinforcement × cue extinction ($F_{(1,7)}$ = 2.090, p = 0.1915), training schedule × cue extinction ($F_{(1,7)}$ = 0.02783, p = 0.8722), or 3-way interactions ($F_{(1,7)}$ = 2.151, p = 0.1859) (Fig. 5(C)). There was a main effect of cue extinction ($F_{(1,7)}$ = 8.889, p = 0.0205) on DMS dopamine peak amplitude, but there was no main effect of cue reinforcement ($F_{(1,7)}$ = 1.106, p = 0.3280) or training schedule ($F_{(1,7)}$ = 0.004638, p = 0.9476), and there were no cue reinforcement × training schedule ($F_{(1,7)}$ = 5.388, p = 0.0533), cue reinforcement × cue extinction ($F_{(1,7)}$ = 0.6134, p = 0.4592), training schedule × cue extinction ($F_{(1,7)}$ = 1.005, p = 0.3495), or 3-way interactions ($F_{(1,7)}$ = 0.07993, p = 0.7856) (Fig. 5(D)). These results suggest that cue extinction resulted in

an overall reduction in DMS dopamine peak amplitude after any lever press, regardless of cue reinforcement, in both groups, while DLS dopamine responses were not affected. This reduction in DMS dopamine signal is difficult to interpret, given that it occurred in both groups and for both cue-reinforced and unreinforced events, and there was not originally a significantly greater response to cue-reinforced presses. Effects of cue extinction on dopamine AUC can be found in the supplement (Fig. S5(C), (D)).

4. Discussion

In the present study, we show distinct patterns of calcium and dopamine activity during drug seeking in the dorsal striatum in rats trained on different schedules of reinforcement, where SO training results in an enhanced DLS dopamine response and a reduced DMS calcium response to cue-reinforced lever presses compared to FR training. Additionally, we show evidence that cue extinction impacts DMS, but not DLS, calcium and dopamine activity during later drug-seeking, which suggests that extinction of the Pavlovian cocaine-cue association impacts the DMS circuitry important for goal-directed drug seeking, but does not impact the DLS circuitry important for habitual drug seeking [42,50]. We have previously shown that cue extinction does not affect cue-induced drug seeking in rats trained on SO schedules of reinforcement to promote DLS dopamine-dependent behavior unless goal-directed behavior is restored [38]. In the present study, cue extinction's lack of effect on the DLS provides a biological explanation for why cue extinction does not affect DLS-dependent, habit-like drug seeking. These findings add to existing literature that indicates divergent roles of the DMS and DLS in goal-directed and habitual drug seeking and expand our understanding of how cocaine-cue associations facilitate dorsal striatal activity.

In these experiments, we chose to use fiber photometry, which allows for the in vivo comparison of bulk changes in fluorescent output of fluorescent indicators in the regions of interest. Fluorescent dopamine sensors provide the advantage of enhanced temporal resolution compared to other methods of monitoring dopamine release in vivo, including microdialysis and fast-scanning cyclic voltammetry (FSCV) [67–69]. Using fiber photometry also allowed us to simultaneously monitor dopamine release (via dLight) and intracellular calcium (via RCaMP) in the same rats [70]. Although in vivo electrophysiology has enhanced temporal sensitivity compared to fiber photometry, fiber photometry is more stable for long-term comparison across days, which was important for the present study [70]. Additionally, monitoring intracellular calcium may provide distinct information compared to in vivo electrophysiology. It was initially proposed that intracellular calcium activity reported by calcium sensors like GCaMP and RCaMP are proxy indicators of neural activity, or cell firing [71]. However, recent evidence suggests that at least in the dorsal striatum, where neurons have extensive dendritic arborization, changes in the fluorescent calcium signal reported by fiber photometry are more indicative of changes in non-somatic calcium and therefore do not reflect the same results as in vivo electrophysiology [72]. Importantly, this evidence suggests that the changes in calcium fluorescence we report here may be interpreted as the summation of excitatory and inhibitory input into dorsal striatal neurons [72]. Future experiments may use additional tools to elucidate the unique contributions of different populations of neurons in the dorsal striatum, given that direct and indirect pathway

medium spiny neurons play different roles in habitual behavior, and interneurons, though less densely populated, also contribute [47,73–77].

There are several limitations to these experiments that should be considered. In some cases, the number of subjects included in analyses was as few as 4 rats. Although signals were averaged across several trials for each rat, the low number of subjects may limit the detection of statistically significant results. Therefore, it is possible that some effects were masked by low power, and future experiments may follow up on these possibilities. We also did not directly compare DMS and DLS activity, and instead analyzed how activity in each region changed as a result of FR or SO training. Additionally, photometry recordings occurred under extinction conditions, primarily because we were concerned about cocaine's effects on dopamine impacting the dLight signal. Therefore, calcium and dopamine activity may differ under conditions when cocaine is available. Due to technical limitations, rats were not connected to optic cables during normal self-administration sessions, so it is possible they could learn over time that cocaine was unavailable when the cables were attached. Importantly, lever presses and schedule completions did not reduce across daily testing under extinction conditions, which indicates minimal extinction learning across testing sessions. However, it is possible that extinction learning within sessions could impact dorsal striatal calcium or dopamine activity. Therefore, it was important for us to compare between FR- and SO-trained groups that underwent the same amount of testing. Additionally, the dopamine signal we obtained was small, likely due to low expression of dLight and its relatively low affinity to dopamine. Although we did show enhanced DLS dopamine response to cue-reinforced compared to unreinforced lever presses in SO-trained rats, smaller differences in dopamine signals may be present, yet undetected, in the present study. We chose dLight as our dopamine sensor because of its relatively faster off kinetics, but future studies employing GRAB-DA, a dopamine sensor with higher affinity for dopamine, may gather additional insights into more subtle striatal dopamine dynamics [67,78]. We also expressed calcium and dopamine sensors throughout neurons in the DMS and DLS, but many different populations of neurons are present in the dorsal striatum. Evidence suggests that direct- and indirect-pathway medium spiny neurons and the more sparse local interneurons may have different roles in these goal-directed and habitual behavior [77,79–81]. Therefore, our results may occlude important differences between subsets of neurons, and future studies could determine how different neuronal sub-types as well as specific projections to the dorsal striatum may contribute to our findings.

We initially recorded dorsal striatal dopamine and calcium activity during cue-induced drug seeking after acquisition. Notably, we did not show any significant differences in dopamine or calcium peak amplitudes between rats that would later be separated into FR- and SO-trained groups, which suggests that later differences in calcium and dopamine responses were the result of effects of training on different schedules of reinforcement and were not due to baseline differences in sensor expression. Additionally, our comparison between cue-reinforced and unreinforced active lever presses controls for motion artifacts that could be produced by the lever press action and isolates the contribution of cocaine-paired cue presentation on dorsal striatal activity [82].

After just one week of cocaine self-administration, there was an enhanced calcium response to cue-reinforced lever presses in both the DMS and DLS. Because these recordings took place after limited self-administration training at a timepoint when behavior would presumably be dependent on the DMS [39,42], the DLS calcium response to reinforced lever presses was somewhat surprising. Importantly, the response in both regions were much greater when lever presses were accompanied by cue presentation than when lever presses had no consequence, which rules out the possibility that the response is a result of the animal's movement. There is some uncertainty about when the stimulus-response associations that later guide habitual behavior are learned. Typically, habitual behavior can be differentiated from goal-directed behavior through its lack of sensitivity to outcome devaluation and outcome contingency degradation [15]. There is evidence that even after minimal operant training, DMS inhibition, DMS lesions, or exposure to stimulants can render behavior insensitive to outcome devaluation or contingency degradation [7,8,39,60,83]. Insensitivity to these paradigms could suggest reliance on habitual behavior, but in some cases it could also be attributed to impaired execution of goal-directed behavior [84]. Our results showing increased calcium activity after cue-reinforced lever presses in the DLS at this early timepoint, when behavior is presumably DMS-dependent and goal-directed, support the theory that stimulus-response learning occurs during early training, even though the behavioral response may still be goal-directed. Unfortunately, because of technical challenges involved in devaluing or degrading contingency for intravenous cocaine delivery, interpretations of our findings are limited by pharmacologically defined habitual behavior that is impaired by DLS dopamine inhibition [13–15, 85].

Interestingly, we did not show increased DMS dopamine release in response to cue-reinforced lever presses compared to unreinforced lever presses in either group at any phase of training. Because previous experiments have shown that after similar training, DMS dopamine antagonism reduces drug-seeking behavior, this finding was particularly surprising in the early training phase, as well as during the middle and late phase in FR-trained rats [42]. The lack of effect is not likely due to limitations of the dopamine sensor dLight, as we did detect a DMS dopamine response to novel stimuli prior to training, in accordance with a previous study showing that midbrain dopamine neurons, including those that project to the dorsal striatum, respond to novel stimuli [86]. It is well-established that dopamine release in the nucleus accumbens core occurs upon the presentation of a reward-predictive or reward-associated cue [51,87]. However, the impact of reward-associated cues on DMS dopamine release is less clear. Because the DMS is particularly important for goal-directed drug seeking, which is promoted by the association between the lever press behavior and reward, DMS dopamine release may not be directly tied to cue presentation, but to reward delivery. Indeed, in mice that were trained to self-administer sucrose, calcium activity in dopamine neuron terminals in the DMS did show increased responses to nosepokes that were reinforced by sucrose delivery [88]. Therefore, the data in the present study may not show a DMS dopamine response because we recorded DMS dopamine under extinction conditions, when lever presses were reinforced by the cue alone and not cocaine, due to technical limitations and to prevent cocaine exposure from impacting DMS dopamine. However, in rats trained to self-administer sucrose after a discriminative stimulus was presented, presentation of the discriminative stimulus resulted

in increased DMS, but not DLS, dopamine release as measured by FSCV [55]. In this case, the stimulus does not signal reward delivery, but the imminent availability of a lever that, when pressed, results in sucrose delivery [55]. Therefore, future experiments should investigate the conditions for which DMS dopamine release occurs for reward-predictive or reward-associated cues. Interestingly, results from the present study did suggest DMS dopamine overall during drug seeking was reduced after cue extinction, even though this reduction was not specific to cue-reinforced lever presses.

On the other hand, our results indicate that SO, but not FR training, does enhance DLS dopamine release after cue-reinforced lever presses. These results agree with those of another study using in vivo microdialysis to measure dopamine release in the DLS in rats trained to self-administer cocaine on second-order schedules of reinforcement, which also showed enhanced DLS dopamine release during cue-induced drug seeking [52]. Our findings complement these by providing enhanced temporal resolution and showing that this increased DLS dopamine release is specific to cue-reinforced lever presses. In another study, rats were trained to self-administer alcohol or sucrose on a variable-interval schedule, which usually promotes habitual behavior, and striatal dopamine release was measured with FSCV during operant responding for reward-associated cues [15,51]. They observed enhanced dopamine release after cue-reinforced compared to unreinforced lever presses in the DLS, but not in the DMS [51]. Our results extend these findings by indicating that SO-training to self-administer cocaine facilitates a similar pattern of dorsal striatal dopamine release, which suggests this enhanced DLS dopamine response to cue-reinforced lever presses is consistent across multiple reinforcers (cocaine, ethanol, and sucrose) and is also generalized between multiple schedules of reinforcement known to facilitate habit-like behavior. Although FR-trained rats in the present study did not develop an enhanced DLS dopamine response to cue-reinforced lever presses, one previous study did report enhanced dopamine release in the DLS, measured by FSCV, after just 2–3 weeks of similar cocaine self-administration, but rats also received cocaine along with the cocaine-associated cue when they made an active nose poke [54]. Therefore, reward delivery may also be required to promote this DLS dopamine response in FR-trained rats, and future experiments should determine what experimental parameters are required to facilitate a DLS dopamine response to cue-reinforced drug seeking.

With regard to calcium responses, as in early training, both FR- and SO-trained rats showed increased DLS calcium responses to cue-reinforced compared to unreinforced lever presses throughout middle and late training. Where FR- and SO-trained rats did differ was in DMS calcium responses to cue-reinforced compared to unreinforced lever presses through the late phases of training, which were greater in FR-trained, but not SO-trained rats. Importantly, rats that would later be SO-trained did show increased DMS calcium responses during the early phase of training, which suggests that SO-training led to a loss of the DMS calcium response to cue-reinforced lever presses. Because calcium activity primarily reflects dendritic calcium, the reduction in calcium activity likely reflects reduced excitatory or enhanced inhibitory input into DMS neurons after SO training [72]. This reduction in overall excitatory input to the DMS may be important for habitual control of behavior. Future studies should determine the contribution of different DMS inputs to the DMS calcium response to cue-reinforced behavior to uncover which circuits are responsible. There is

evidence that mPFC projections to the DMS are disengaged as a motor skill is refined, so it is possible that a reduction in excitatory mPFC input could occur in SO-trained rats [89]. Additionally, the orbitofrontal cortex (OFC) is important for goal-directed behavior and modulates the DMS via both direct projections and indirectly through the other cortical areas and the amygdala, and could also be involved in this decrease in DMS calcium response in SO-trained rats [45,90]. Recently, the balance of activity in putatively excitatory inputs from the BLA and inhibitory inputs from the CeA to the DMS have been implicated in the influence of stress on habitual behavior, so these projections could also be involved [91]. In addition to projections from the cortex and the amygdala, the DMS also receives thalamic inputs, which may also have a role in DMS calcium activity [92].

Next, we evaluated dorsal striatal calcium and dopamine responses to the non-contingent presentation of cocaine-paired cues during a Pavlovian cue extinction paradigm. Interestingly, DMS and DLS calcium responses to passive cue presentations were of a lower magnitude than those observed when the cue was presented after a lever press during drug seeking tests, and the response to cues did not change throughout the cue extinction session. Additionally, we did not observe a dorsal striatal dopamine response to noncontingent cues during cue extinction. These findings agree with those of another study showing that noncontingent cue presentations do not result in increased DLS dopamine release, as measured by in vivo microdialysis, after SO training to self-administer cocaine [52]. Taken together, these results suggest that DLS dopamine release during habit-like drug seeking is dependent upon both the lever press action as well as contingent cue presentation, further supporting the role of DLS dopamine in connecting cues (stimuli) with the lever press behavior.

Finally, we conducted an additional drug seeking test after cue extinction and compared dorsal striatal calcium activity to activity during the late phase of training, prior to cue extinction. In doing this comparison, we found that FR-trained rats had reduced DMS calcium responses to lever presses after cue extinction, but there was no effect in SO-trained rats or in either group in the DLS. These data suggest that the learned lack of association between cocaine and the drug-associated cue that occurs during cue extinction results in reduced DMS calcium activity during subsequent drug seeking, despite no change in the smaller DMS response to noncontingent cues throughout cue extinction. Additionally, the later reduction in DMS calcium activity after cue extinction learning selectively occurs in rats trained on a schedule that promotes goal-directed behavior. Inputs to the DMS that contribute to this reduction in DMS calcium activity after cue extinction are not currently known. Our lab has previously shown that cue extinction results in reduced synaptic strength of thalamo-amygdala synapses, and opto-genetic depotentiation of these synapses mimics the effects of cue extinction [32]. Therefore, it is possible that cue extinction affects DMS calcium activity by reducing input to the DMS either directly from the BLA or through the BLA's interaction with the OFC, which could be the topic of future investigations [45,90].

We have previously shown that cue extinction reduces cue-induced drug seeking in FR-trained, but not SO-trained rats [38]. In our previous study, the effect of cue extinction in FR-trained rats was apparent when animals that underwent cue extinction were compared to those that did not. Therefore, the lack of difference in response ratio after cue extinction in

FR- compared to SO-trained rats in the current study is consistent with our previous findings given that we did not compare to 0-cue control groups, although this would be interesting to examine in future studies [38]. Interestingly, cue extinction affected DMS calcium activity in FR-trained rats, which suggests that cue extinction may inhibit circuitry that facilitates goal-directed action towards seeking cocaine under extinction conditions when cocaine is expected but not provided.

Overall, the present study expands upon previous literature examining the differential roles of the DMS and DLS in goal-directed and habitual behavior, respectively. We also present novel results showing that cue extinction reduces DMS activity during later drug seeking but has no effects on the DLS, which indicates that extinction of the cocaine-cue association impacts the circuitry involved in goal-directed, but not DLS-dependent, habit-like cocaine seeking. Future experiments should examine specific projections to the DMS and how they are impacted by SO-schedule training and cue extinction. Together, these results provide novel insights into how cue extinction may reduce drug seeking that is goal-directed but not affect habitual drug seeking and indicate that future treatments for SUDs may need to address these aspects of drug-seeking behavior using different methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

All data, code, and materials used in the analyses will be made available upon request.

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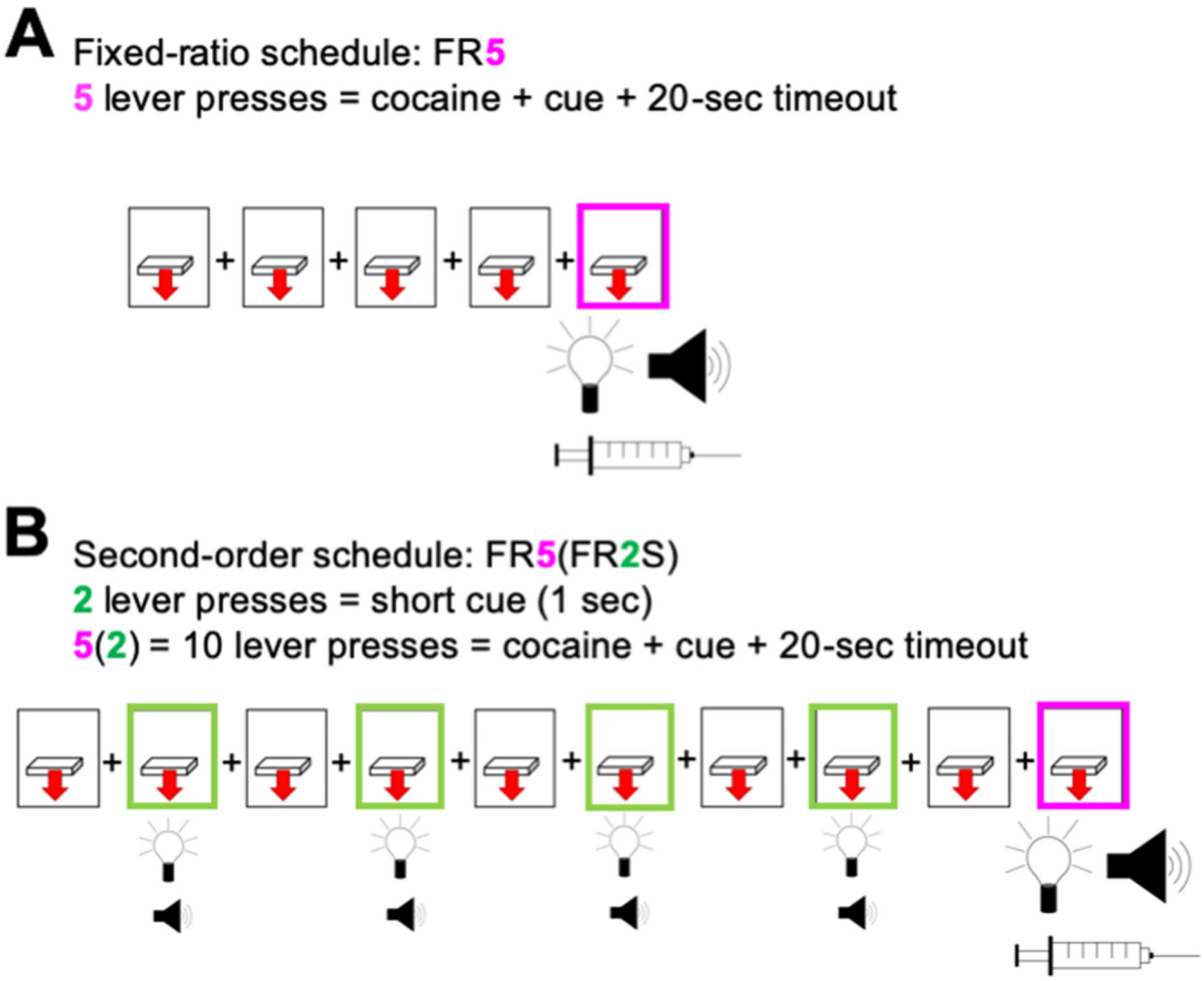

Fig. 1.

Schedules of reinforcement. In this example of a fixed-ratio (FR5) schedule of reinforcement, the fifth lever press results in cocaine infusion and a 20-s timeout period during with an audiovisual cue is presented (A). In this example of an FR5(FR2S) second-order (SO) schedule of reinforcement, a brief, 1-s audiovisual stimulus (S) is presented on an FR2 schedule, and upon the fifth completion of the FR2S schedule (after 10 total lever presses), cocaine infusion and the 20-s timeout with audiovisual cue presentation occurs.

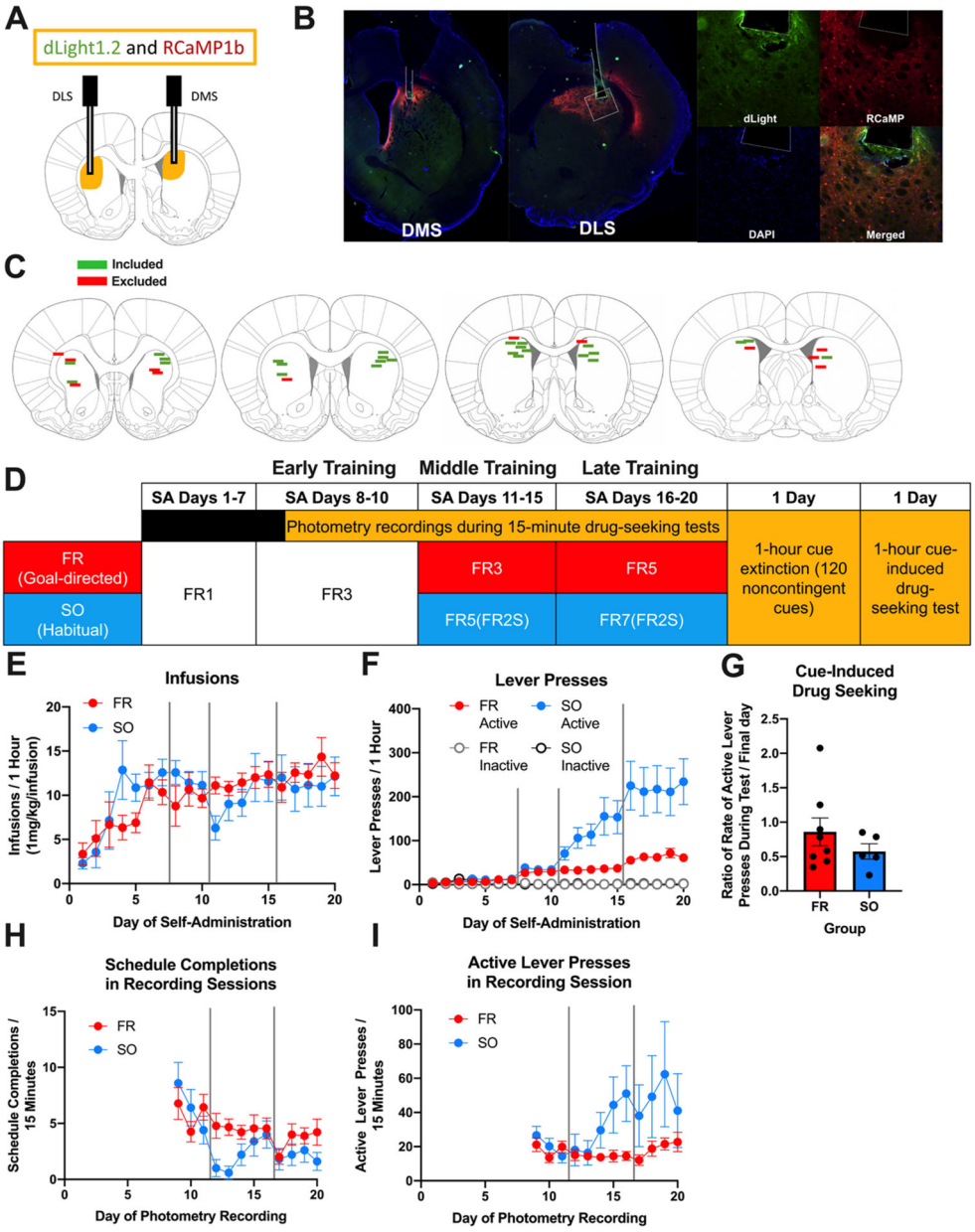

Fig. 2.

FR- and SO-trained rats do not differ in daily cocaine self-administration or cue-induced drug-seeking after cue extinction. Schematic for fiber placement and virus expression in the DLS and DMS (A). Representative images of fiber placement and virus expression in the DMS and DLS (left) and at higher magnification in the DLS (right) with fluorescent channels shown individually and merged and fiber locations outlined in dotted white lines (B). For all rats, fiber placement and virus expression were evaluated via fluorescent microscopy, and green bars represent fibers appropriately placed with confirmed virus expression at the base of the fiber, while red bars indicate fibers excluded from analysis due to either fiber misplacement and/or lack of virus expression (C). Rats (n = 16) were trained to self-administer cocaine for 20 days on different schedules of reinforcement (FR or

SO) before undergoing cue extinction and a subsequent cue-induced drug-seeking test, and after acquisition, photometry recordings occurred in 15-min drug-seeking tests that preceded daily self-administration, during cue extinction, and during the subsequent cue-induced drug-seeking test (D). During self-administration, there was a main effect of training day on the number of daily cocaine infusions, where infusions increased for both groups as training progressed (E). For lever presses during daily self-administration, there was a 3-way training day × training schedule × lever interaction (F). There was no difference between groups in the ratio of active lever presses during the post-cue extinction cue-induced drug-seeking test compared to the final day of self-administration (G). During photometry recording sessions, there was a main effect of test day and a test day × training schedule interaction on the number of schedule completions (H), and there was a main effect of training schedule on the number of active lever presses (I). Graphs show group means ± SEM and individual data points where possible, and gray vertical lines separate different reinforcement schedules.

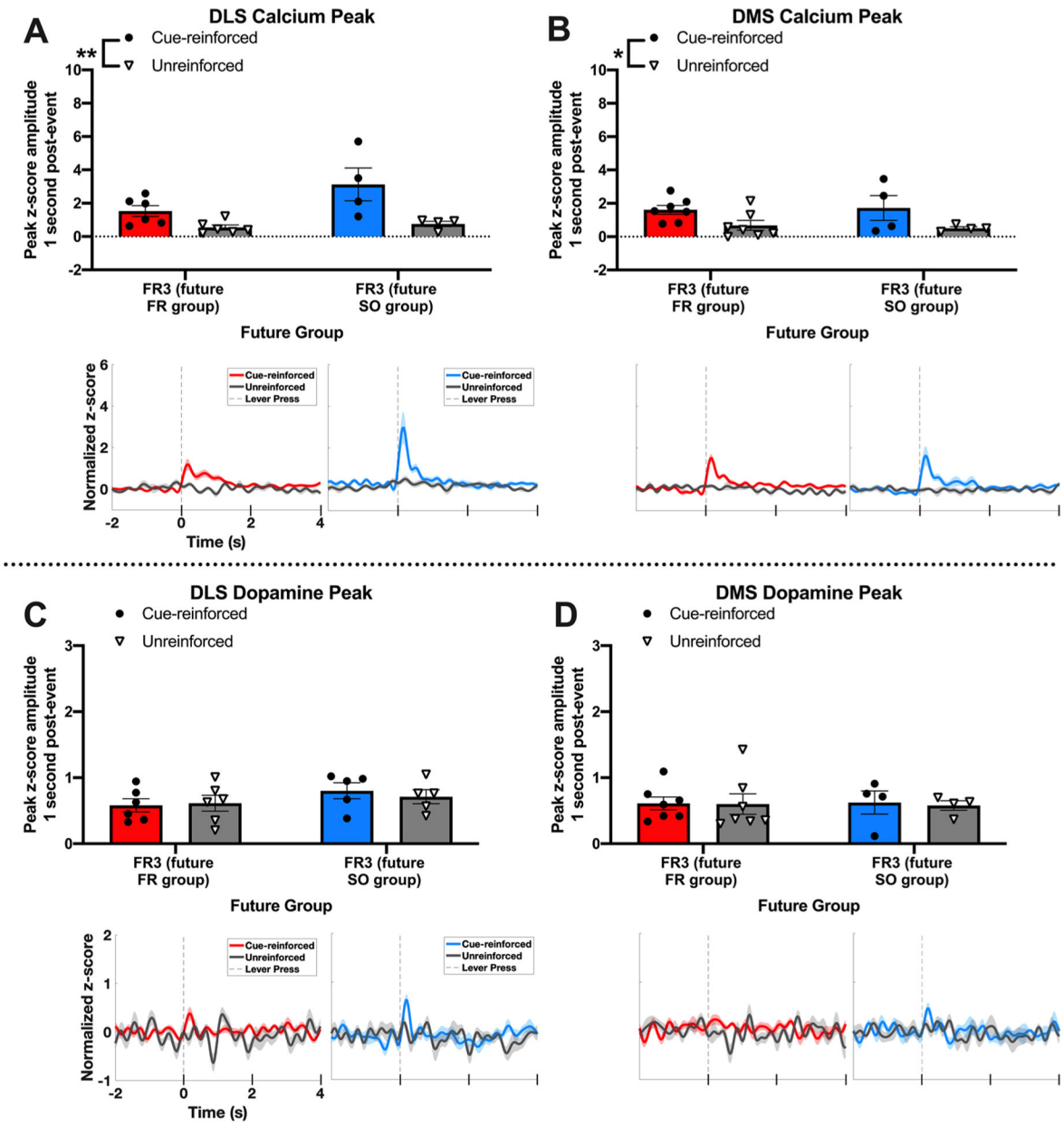

Fig. 3.

After acquisition, dorsal striatal calcium responses are greater for cue-reinforced than unreinforced active lever presses. After acquisition and prior to splitting rats into FR- and SO-trained groups (n = 16), fiber photometry recordings occurred during 15-min drug-seeking tests prior to daily self-administration, during which some active lever presses had no consequence (unreinforced) and an active lever press that completed the FR3 schedule resulted in cue presentation (cue-reinforced) and timeout (levers retracted, houselight extinguished). For DLS (A) DMS (B) calcium, there was a main effect of cue reinforcement on peak z-score amplitude during the 1 s after lever press, but there was no effect of future training schedule or interaction. For DLS (C) and DMS (D) dopamine peak z-score amplitude, there was no effect of cue reinforcement, future training group, or interaction.

Graphs show group means ± SEM and individual data points. Traces show overall average trace for each event for each future group aligned to behavioral events with SEM shown with shading and dashed vertical lines indicating time of lever press. Note that the average peak for each animal does not necessarily correspond visually with the peak of the average trace. This is because the peak for each trial can occur at any point during the 1 s after each behavioral event, whereas the average trace is the average of each animal's average response. *$p < 0.05$; **$p < 0.01$.

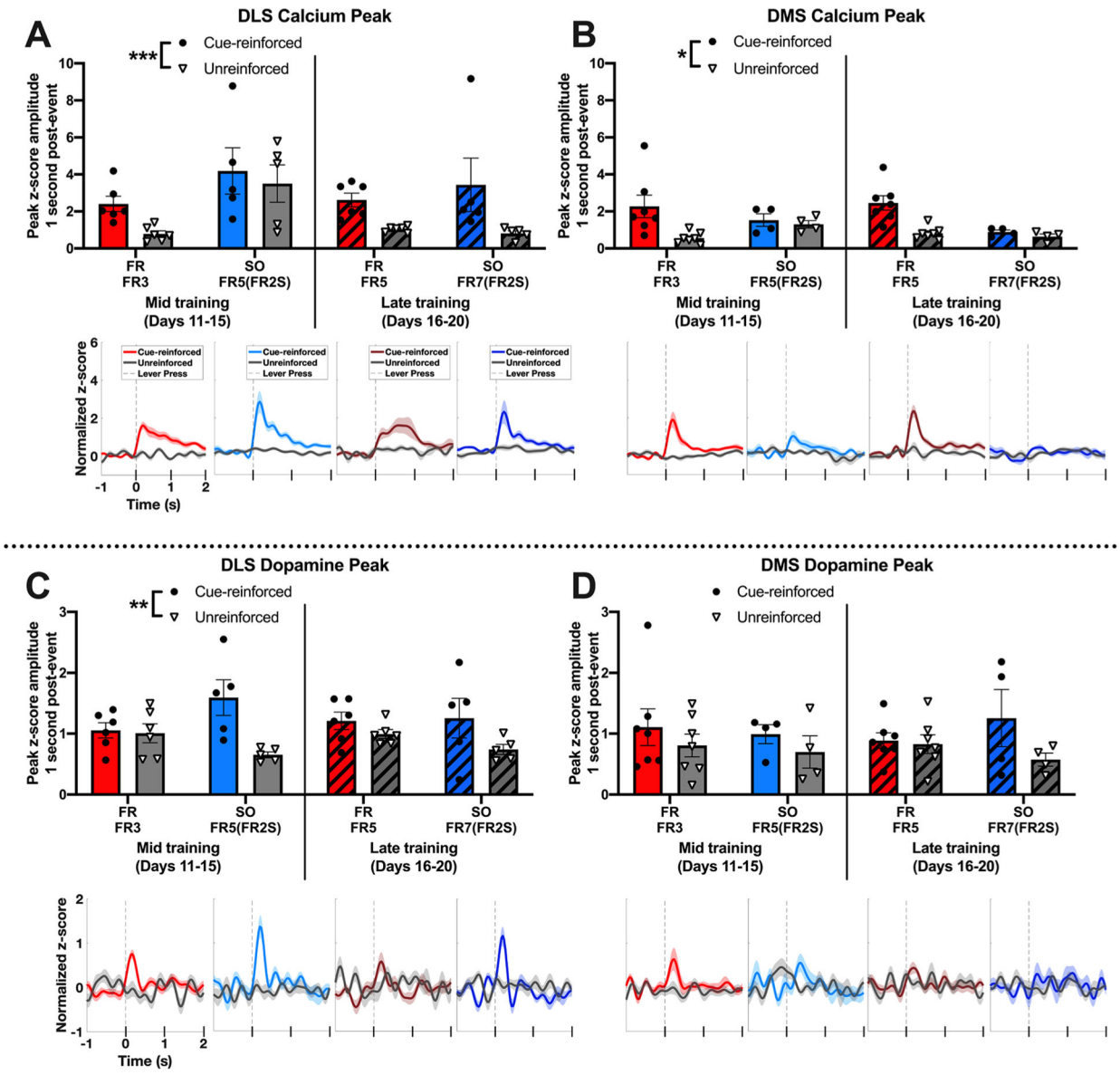

Fig. 4.

FR- and SO-trained rats have different patterns of dorsal striatal calcium and dopamine activity during drug seeking. Rats were separated into FR-trained (n = 9; 5 male and 4 female) and SO-trained (n = 7; 4 male and 3 female) groups for the remaining 10 days of self-administration and trained on different schedules of reinforcement accordingly for the middle and late phases of training. Dorsal striatal calcium and dopamine responses to cue-reinforced and unreinforced lever presses were compared for each training schedule and phase of training. There was a main effect of cue reinforcement on DLS calcium peak amplitude, but no main effects of training schedule or phase of training or interactions (A). For DMS calcium peak amplitude, there was a main effect of cue reinforcement and a cue reinforcement × training schedule interaction, but no other main effects or interactions (B). There was a main effect of cue reinforcement and a cue reinforcement × training schedule interaction for DLS dopamine peak amplitude (C), but there were no main effects

or interactions for DMS dopamine peak amplitude (D). Graphs show group means ± SEM and individual data points. Traces show overall average trace for each event for each group aligned to behavioral events with SEM shown with shading and dashed vertical lines indicating time of lever press. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

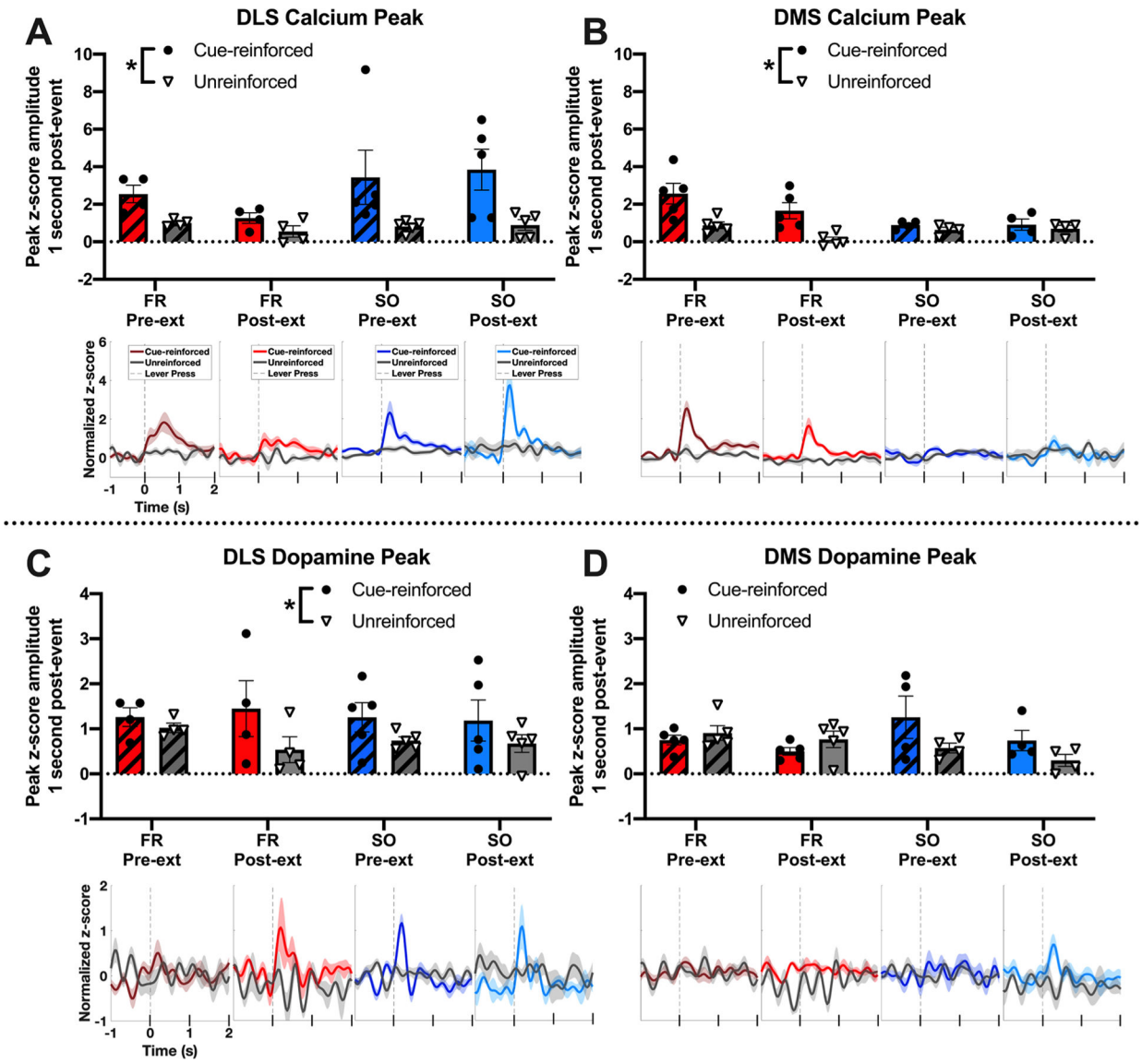

Fig. 5.

Cue extinction results in changes in DMS, but not DLS, calcium and dopamine activity during drug seeking. To examine the effects of cue extinction on dorsal striatal calcium and dopamine activity, peak amplitudes during the post-cue extinction drug-seeking test (post-ext) were compared to the late phase of training (pre-ext) (n = 13; exclusions explained in methods). There was a main effect of cue reinforcement on DLS calcium peak amplitude, but no effects of cue extinction or training schedule or interactions (A). For DMS calcium peak amplitude, there was a main effect of cue reinforcement, a main effect of cue extinction, and significant cue reinforcement × training schedule and training schedule × cue extinction interactions, but no other interactions (B). There was a main effect of cue reinforcement on DLS dopamine peak amplitude, with no other main effects or interactions (C). Finally, there was a main effect of cue extinction on DMS dopamine peak amplitude, but no other effects or interactions (D). Data from “pre-extinction” is from the late phase data in Fig. 4. Graphs show group means ± SEM and individual data points. Traces show

overall average trace for each event for each group aligned to behavioral events with SEM shown with shading and dashed verical lines indicating time of lever press. *$p < 0.05$.