Decreased ventral tegmental area CB1R signaling reduces sign-tracking and shifts cue-outcome dynamics in rat nucleus accumbens

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Abbreviated Title: Endocannabinoids decrease US dopamine in sign-trackers

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Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Ethics Statement

The animal study was reviewed and approved by University of Maryland, School of Medicine Institutional Animal Care and Use Committee.

Author Contributions

DC, SB and JC conceived, developed and supervised the project. SB acquired the data. SB, DM and CS analyzed the data. SB and DC designed the experiments, interpreted the data, and wrote the manuscript. FS and YL produced and provided the dopamine sensor, $GRAB_{DA}$, used for fiber photometry. All authors contributed to manuscript revision and approved the submitted version.

ABSTRACT

Sign-tracking rats show enhanced cue sensitivity before drug experience that predicts greater discrete cue-induced drug-seeking compared to goal-tracking or intermediate-rats. Cue-evoked dopamine in the nucleus Accumbens (NAc) is a neurobiological signature of sign-tracking behaviors. Here, we examine a critical regulator of the dopamine system; endocannabinoids, which bind the cannabinoid receptor-1 (CB1R) in the Ventral Tegmental Area (VTA) to control cue-evoked striatal dopamine levels. We use cell-type specific optogenetics, intra-VTA pharmacology and fiber photometry to test the hypothesis that VTA CB1R receptor signaling regulates NAc dopamine levels to control sign-tracking. We trained rats in a Pavlovian lever autoshaping task (PLA) to determine their tracking groups before testing the effect of VTA→NAc dopamine inhibition. We found this circuit is critical for mediating the vigor of the ST response. Upstream of this circuit, intra-VTA infusions of rimonabant, a CB1R inverse agonist, during PLA decrease lever and increase foodcup approach in sign-trackers. Using fiber photometry to measure fluorescent signals from dopamine sensor, $GRAB_{DA}$, we tested the effects of intra-VTA rimonabant on NAc dopamine dynamics during autoshaping. We found that intra-VTA rimonabant decreased sign-tracking behaviors, which was associated with increases NAc shell, but not core, dopamine levels during reward delivery (US). We also observed a relationship between cue (CS)-evoked NAc dopamine activity and rigidity of behavior between rimonabant treatment sessions. Our results suggest that CB1R signaling in the VTA influences the balance between the CS- and US-evoked dopamine responses in the NAc and biases behavioral responding to cues in sign-tracking rats.

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84 **SIGNIFICANCE STATEMENT**

85 Substance Use Disorder is a chronically relapsing neurobiological disorder that affects a
86 subset of individuals that engage in drug use. Recent research suggests that there are
87 individual behavioral and neurobiological differences prior to drug experience that
88 predict addiction and relapse vulnerabilities. Here, we investigate how midbrain
89 endocannabinoids regulate a brain pathway that is exclusively involved in driving cue-
90 motivated behaviors of sign-tracking rats. This work contributes to our mechanistic
91 understanding of individual vulnerabilities to cue-triggered natural reward seeking that
92 have relevance for drug motivated behaviors.

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105 **INTRODUCTION**
106
107 Cues that reliably predict outcomes in the environment powerfully regulate
108 behavior across conditioning. In addition to their predictive value, some cues gain
109 enhanced motivational value that can lead to, or be associated with, maladaptive
110 behavior. Training in a Pavlovian lever autoshaping (PLA) task reveals distinct
111 conditioned responding phenotypes: Sign-tracking (ST) rats that engage predominantly
112 with an insertable lever cue show enhanced discrete-cue induced relapse after cocaine
113 experience. This stands in contrast to goal-tracking (GT) and intermediate (INT) rats
114 that engage more with the foodcup and show lower levels of discrete-cue induced
115 relapse after cocaine experience (Hearst & Jenkins, 1974; Tomie, 1996; Flagel et al.,
116 2007; Meyer et al., 2012). Prior studies establish a role for NAc dopamine and
117 endocannabinoid signaling in driving sign-tracking (Flagel et al., 2011; Bacharach et al.,
118 2018). Here we investigate the extent to which midbrain endocannabinoid signaling and
119 downstream Nucleus Accumbens dopamine (DA) dynamics interact to drive sign-
120 tracking behaviors.
121 Cue-evoked dopamine release in the nucleus Accumbens (NAc) core
122 distinguishes ST from GT phenotypes (Flagel et al., 2011). While pharmacological
123 studies indicate that dopamine signaling drives the acquisition and expression of both
124 sign- and goal-tracking behaviors (Danna & Elmer, 2010; Lopez et al., 2015; Fraser et
125 al., 2016), sub-second cue-evoked NAc core dopamine is strongly correlated with and
126 necessary for sign-tracking, but not goal-tracking behavior (Flagel et al., 2011;
127 Saunders & Robinson, 2012; Clark et al., 2013; Fraser & Janak, 2017). Uncovering
128 factors controlling this tracking-related difference in striatal dopamine signaling is crucial

129 to understand the neurobiological mechanisms driving individual differences in
130 motivation towards reward predictive cues.

131 Because of their role in regulating the dopamine system, we hypothesize that
132 midbrain endocannabinoid signaling drives differences in cue-evoked striatal dopamine
133 in sign- and goal-tracking phenotypes. Endocannabinoids are critical regulators of the
134 dopamine system and blocking CB1R decreases striatal dopamine release (Cheer et
135 al., 2000; Lupica et al., 2004; Cheer et al., 2004; Lupica & Riegel, 2005; Cheer et al.,
136 2007; Oleson et al., 2012; Wenzel et al., 2018). The Ventral Tegmental Area (VTA)
137 provides dense dopaminergic projections to the NAc, which are regulated by
138 endocannabinoids (eCBs) acting at pre-synaptic CB1R to influence dopamine neuron
139 firing and striatal dopamine release (Cheer et al., 2004; Lupica & Riegel, 2005).
140 Blocking CB1R signaling decreases natural and drug reward self-administration, cue-
141 induced reinstatement, and sign-tracking (McLaughlin et al., 2003; De Vries &
142 Schoffelmeer, 2005; McLaughlin et al., 2006; Justinova et al., 2008; de Bruin et al.,
143 2011; Oleson et al., 2012; Schindler et al., 2016; Bacharach et al., 2018). Blocking
144 CB1R signaling in the VTA decreases both cue-evoked NAc dopamine and associated
145 reward seeking (Oleson et al., 2012; Wenzel et al., 2018). We previously demonstrated
146 that systemically blocking CB1R receptors decreases the enhanced attractive and
147 reinforcing properties of lever cues in sign-tracking rats (Bacharach et al., 2018). We
148 posit the locus of CB1R effects on sign-tracking is via VTA CB1R suppression of NAc
149 dopamine release.

150 We hypothesize that CB1R receptor signaling in the VTA regulates cue-evoked
151 NAc dopamine levels to control sign-tracking in rats. To test this, we use an optogenetic

152 approach to examine the role of VTA→NAc dopamine projections in the expression of
153 Pavlovian conditioned approach in ST, GT, and INT rats. We use intracranial
154 pharmacology to examine the effect of intra-VTA infusion of the CB1R reverse agonist,
155 rimonabant, on Pavlovian conditioned approach. Finally, combining intracranial
156 pharmacology with fiber photometry we determine the extent to which VTA rimonabant
157 affects cue- and reward-evoked NAc core and shell dopamine dynamics during
158 Pavlovian conditioned approach. Altogether, we conclude from our data that CB1R
159 signaling in the VTA maintains the conditioning-dependent behavioral and NAc DA bias
160 towards cues in sign-tracking rats.
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162 **MATERIALS AND METHODS**

163 **Experimental subjects:** We used female (n= 52) and male (n= 6) transgenic TH::Cre
164 Sprague-Dawley rats (Envigo, Boyertown, PA; run in four cohorts) for the optogenetics
165 experiment, female (n = 22) Long-Evans rats (Charles River Laboratories, Wilmington,
166 MA; run in two cohorts) for the pharmacology experiment, and female (n=32) Long-
167 Evans rats (Charles River Laboratories, Wilmington, MA; run in three cohorts) for the
168 photometry experiments. All rats weighed between 215-350g at experimental onset. All
169 rats were single-housed and maintained on a 12:12h light-dark cycle (ZT0 at 0730). All
170 rats had ad libitum access to standard laboratory chow and tap water before food
171 deprivation to 90% of their baseline weight, which was maintained for all experimental
172 phases. Chow was provided after daily behavioral sessions. All procedures were
173 performed in accordance with the “Guide for the care and use of laboratory animals”

174 (8th edition, 2011, US National Research Council) and were approved by the University
175 of Maryland School of Medicine Institutional Animal Care and Use Committee (IACUC).
176 **Surgical Procedures:** For all surgeries, we anesthetized rats with 3
177 5% isoflurane (VetOne) and gave a subcutaneous injection of the
178 analgesic, carprofen (5mg/kg). Prior to the first skull incision we gave rats a subdermal
179 injection of 10 mg/mL lidocaine at the incision site. After lowering cannula or fiber optics
180 into place, we secured them to the skull using jeweler's screws and dental cement
181 (Dentsply Caulk, Dentsply, York, PA). All coordinates given are distance from bregma
182 according to the Paxinos and Watson rat brain atlas (6th edition, 2007).
183 *Optogenetics:* We infused 500n*L of* the Cre-dependent inhibitory chloride pump,
184 halorhodopsin, AAV5-ef1a-DIO-eNphr3.0-eYFP (UNC vector Core, Chapel Hill, NC), or
185 the control virus AAV5-ef1a-DIO-eYFP (UNC vector Core, Chapel Hill, NC) bilaterally in
186 to the VTA (coordinates from bregma: -5.4 mm AP, ±2.15 mm ML, and −8.2 mm DV) at
187 a rate of 100 nL/min using a microinfusion pump (UltraMicroPump III, World Precision
188 Instruments, Sarasota, FL, USA) and a 10μL Hamilton syringe (Hamilton, Reno, NV,
189 USA). The needle tip was left in place for 5 minutes after infusion, raised 0.1mm and left
190 another 5 minutes before the final raising. We then implanted two 200 μm core, 0.67 NA
191 fiber optics with ceramic zirconia ferrules (Prizmatix, Holon, Israel) targeting the NAc
192 core, bilaterally (coordinates from bregma: +1.8 mm AP, +2.15 mm ML, -6.6 mm DV).
193 Fiber optics were secured with Denmat (Denmat, Lompoc, CA) then a thin layer of
194 dental cement. For two of four cohorts we trained and tested rats in Pavlovian lever
195 autoshaping six to eight weeks after surgery. In two of four cohorts, we gave rats (n =
196 14 Halo, n = 19 eYFP) three days of training before surgically injecting virus and

implanting fiber optics, followed by a 6-week recovery/viral expression period, then a fourth training session before testing.

Pharmacology. We implanted guide cannulae (23G; Plastics One INC., Roanoke, VA) bilaterally into the VTA at a 10-degree angle (coordinates from bregma: -5.4 mm AP, ±2.2 mm ML, and −7.33 mm DV). Cannula were secured with jewelers screws and dental cement. At the end of surgery, we inserted dummy cannula into the guide cannula, which were kept in the guide cannula and were only removed during infusion habituation and infusion test procedures. Animals were given 2-3 weeks recovery before testing.

Fiber photometry: Guide cannulae were implanted in the VTA identical to *Pharmacology* experiments. In addition, we infused 1μL of AAV9-hSyn-DA2m ($GRAB_{DA}$) into the Nucleus Accumbens unilaterally at a 6-degree angle (coordinates from bregma: +1.8 mm AP, +2.15 mm ML, -6.6 mm DV). Following virus infusion, we implanted one 400 μm core, 0.67 NA fiber optic (Thor labs) in the NAc, 0.1mm dorsal to the virus injection coordinates. Fiber optics were first cemented in place with Metabond (Parkell Inc., New York, New York), then with Denmat. The entire headcap was covered in a thin layer of dental cement. Rats were given 4 weeks for recovery before testing.

Histology: At the end of experiments, we deeply anesthetized rats with isoflurane and transcardially perfused them with 100 ml of 0.1M sodium phosphate buffer (PBS), followed by 400 ml of 4% paraformaldehyde (PFA) in PBS. We removed brains and postfixed them in 4% PFA for two hours before we transferred them to 30% sucrose in PBS for 48 -72h at 4 °C. We subsequently froze brains and stored them at −20 °C until

219 sectioning. Coronal sections (50 µm) containing NAc and VTA were collected using
220 a cryostat (Leica Microsystems).

221 To verify cannula placements, we stained brain sections with cresyl violet and
222 coverslipped with Permount (Fischer Scientific, Waltham, MA). To verify viral expression
223 and fiber placements we mounted and coverslipped all brain sections with Vectashield
224 DAPI (Vector Laboratories, Burlingame, CA) and the mounting medium, Mowiol (Sigma-
225 Aldrich, St. Louis, MO). We used a spinning disk confocal microscope (Leica SP8) to
226 verify viral expression and fiber optic/cannula placement. For optogenetic experiments,
227 rats were excluded from analysis if cell body labeling of Halo or eYFP was observed
228 outside of the VTA (in SNc), if there was no terminal expression in the NAc core, and/or
229 fiber optics were not targeting the NAc core. For pharmacology experiments rats were
230 excluded if cannula were not targeting the VTA. For photometry experiments rats were
231 excluded if there was no virus expression below the fiber optic in the NAc, or if the CS-
232 evoked dopamine signal was less than 2 Zs above baseline during the Vehicle testing
233 day.

234 **Behavioral apparatus:** Experiments were conducted in individual sound-isolated
235 standard experimental chambers (25 × 27× 30cm; Med Associates). For Pavlovian lever
236 autoshaping each chamber had one red house light (6 W) located at the top of a wall
237 that was illuminated for the duration of each session. During PLA, the opposite wall in
238 the chamber had a recessed food cup (with photo beam detectors) located 2 cm above
239 the floor grid. The food cup was attached to a programmed pellet dispenser that
240 delivered 45 mg food pellets (catalog#1811155; Test Diet 5TUL; protein 20.6%, fat

12.7%, carbohydrate 66.7%). One retractable lever was positioned on either side of the food cup, counterbalanced, 6cm above the floor.

Optogenetics: Each Med Associates chamber was equipped with a green LED (525 nm, max 300mW, Prizmatix) to deliver light to the NAc core. A TTL pulse was generated by MedPC 1s prior to lever insertion and was sent to an Arduino minicontroller (Somerville, MA) which drove an LED for 11s total, (terminating with the retraction of the lever). The complete light path is MED TTL→Arduino→LED→Patch cord (1000μm core, Prizmatix)→commutator (Prizmatix)→ bifurcated (2x500μm core, Prizmatix) fiber optic patch cord → 2 implanted fiber optics ferrules targeting the NAc core. A ceramic sleeve covered in black heat shrink tightly joined the patch cord and fiber optic and prevented light loss. We used a light meter (PM100D, Thorlabs) to calibrate light output in each box before and after each session.

Fiber photometry: We used LEDs (ThorLabs) to deliver 465 nm light to measure $GRAB_{DA}$ fluorescence signals and 405 nm light as an isosbestic control. The two wavelengths of light were sinusoidally modulated at 210 and 337Hz respectively. The LEDs connected to a fluorescence mini cube (Doric Lenses). The combined LED output passed through a fiber optic cable (1 m long; 400 μm core; 0.48 NA; Doric) which was connected to implanted fiber optics (400 μm core, Thor Labs). We maintained the light intensity at the tip of the fiber optic cable at 10-15 μW across behavioral sessions. LED light collected from the $GRAB_{DA}$ and isosbestic channels was focused onto a femtowatt photoreceiver (Newport). We low-pass filtered and digitized the emission light at 3Hz and 5 KHz respectively by a digital processor controlled by Synapse software suite (RZ5P, Tucker Davis Technologies (TDT)). We time-stamped the behavioral events

including lever insertion, pellet delivery, lever press, and food cup entry through TTL pulses in Synapse software.

Training in Pavlovian lever autoshaping: Across all experiments, we gave rats a single 38-minute magazine training session during which one food pellet was delivered into the food cup on a variable interval (VI) 90 s schedule (60-120 s) for 25 trials. We trained rats in four or five daily Pavlovian lever autoshaping (PLA) sessions, which consisted of 25 reinforced lever conditioned stimulus (CS+) presentations occurring on a VI 90 s schedule (60-120 s). CS+ trials consisted of the insertion of a retractable lever for 10 s, after which the lever was retracted and two food pellets were delivered to the food cup regardless of whether a lever or food cup response was made. For two-lever PLA experiments, on the opposite side of the wall from the CS+, we included a CS- lever for which the extension and retraction had no programmed consequences. Sessions consisted of 25 CS+ pairings and 25 CS- trials with a VI90s schedule between rewarded trials. For experiments in the unpaired condition, lever extension/retraction did not produce food pellets. Instead, two food pellets were delivered pseudorandomly during the ITI (at least 20s after/ 20s before lever retraction/extension, respectively).

Measurements and difference scores: Behavioral measurements were collected during the 20s pre-CS period (ITI), 10s CS period, and the 5s post-CS reward delivery period. An automated measurement of the latency to first contact the lever and/or food cup during the cue for each trial was recorded. On trials in which a contact did not occur, a latency of 10 s was recorded. For each session the lever or food cup probabilities were calculated by determining the number of trials that the lever or food cup response was made, divided by total number of trials in the session.

We used a Pavlovian Conditioned Approach (PavCA) analysis (Meyer et al., 2012) to determine sign- goal- and intermediate tracking groups. The PavCA score quantifies the difference between lever-directed and food cup-directed behaviors, and ranges from -1.0 to +1.0. An individual rat's PavCA score is the average of three difference score measures (each ranging from -1.0 to 1.0) including: (1) preference score, (2) latency score and (3) probability score. The preference score is the total number of lever presses during the CS, minus the total number of food cup responses during the CS, divided by the sum of these two measures. The latency score is the session averaged latency to make a food cup response during the CS, minus the session averaged latency to lever press during the CS, divided by the duration of the CS (10s). The probability score is the probability of lever press minus the probability of food cup response observed across the session. ST PavCA scores range from +0.33 to +1.00, INT PavCA scores range from +0.32 to -0.32, and GT PavCA scores range from -0.33 to -1.00.

Testing in Pavlovian lever autoshaping:

Optogenetics: We habituated rats to the optogenetic patch cable after D4 of training. On subsequent sessions, rats were tethered to the patch cable during the session. During the OFF test, rats were tethered but no light was delivered. For the ON Test, light was delivered to the NAc at 4-6mW from the fiber tip. During the ON test, LED light delivery began 1s prior to lever extension and remained on for the duration of the 10 s lever cue (11s total). Lever retraction, termination of the LED light, and delivery of food occurred simultaneously at the end of each of the 25 trials.

Pharmacology: We habituated rats to handling and infusion procedures throughout training. After the last training day, we inserted injectors in to the cannulae and the infusion pump was turned on, but nothing was infused. Before test sessions in PLA, we gave each rat an infusion of rimonabant or vehicle in two separate counterbalanced test sessions that occurred 48 hours apart. We removed dummy cannulae and inserted 30G injector cannulae (Plastics One) extending 1.0 mm beyond the end of the guide cannulae. We connected each injector cannula using polyethylene-50 tubing, which was attached to a 5 μL Hamilton syringe (Hamilton, Reno, NV) that was placed in an infusion pump (CMA syringe pump 4004; Harvard apparatus). We infused rimonabant (2 μg/μL) or vehicle bilaterally into the VTA at a rate of 0.25 μL/min for a total of 2 minutes, or 0.5 μL total volume per hemisphere. We kept the injectors in place for an additional minute before slowly removing them and replacing dummy cannulae for behavioral testing. We tested rats in PLA 10 minutes after the completion of the infusion.

Drug solutions were prepared immediately before each test session. Rimonabant (SR141716A, 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, NIDA Drug Supply Program) was dissolved in a 1:1:18 solution of ethyl alcohol (Sigma), emulphor (Alkamuls EL-620, Solvay Chemicals, Princeton, NJ), and saline (Hospira) and sonicated for 15 min. The vehicle solution consisted of the 1:1:18 solution of ethyl alcohol, emulphor, and saline.

Photometry: We habituated rats to patch cables during magazine training and performed recordings during all of training and testing phases. Testing and drug infusions procedures were identical to pharmacology experiments.

Data and Statistical analyses: Behavioral data was analyzed using SPSS statistical software (IBM) with mixed-design repeated-measures ANOVA. Significant main and interaction effects ($p < 0.05$) were followed by post-hoc within-subject, repeated-measures ANOVA or t-tests. For significant post-hoc t-tests we report Cohen's d effect size.

Optogenetics: For training data, we used mixed repeated measures ANOVA including within-subject factor of Session (4) and between-subject factors of Tracking (GT, INT, ST) and Virus (Halo, eYFP). For Test data, we used mixed repeated measures ANOVA including within-subject factor of Light (OFF, ON) and between-subject factors of Tracking (GT, INT, ST) and Virus (Halo, eYFP). In cases with a significant Treatment x Tracking interaction, we then probed individually Response x Treatment interactions within each tracking group.

Pharmacology: For PLA training data, we used mixed repeated measures ANOVA of lever and food cup measures (contact, latency and probability), using between-subject factors of Tracking group (ST, INT) and within-subject factor of Session to analyze lever- and food cup-directed behaviors. For Test data, we used mixed repeated measures ANOVA including within-subject factor of Treatment (Veh, Rimo) and between-subject factors of Tracking (INT, ST). In cases with a significant Treatment x Tracking interaction, we then probed individually Response x Treatment interactions within each tracking group.

Photometry: For training data, we used mixed repeated measures ANOVA including within-subject factor of Session (5). For Test data, we used mixed repeated

measures ANOVA including within-subject factor of Treatment (Veh, Rimo) and Epoch (Cue, Reward).

Photometry signal analysis: To calculate ΔF/F, a least-squares linear fit was applied to the 405 nm signal. ΔF/F = (490 nm signal − fitted 405 nm signal)/fitted 405 nm signal. To calculate Z-scores on each trial, we took the average of the ΔF/F signal over a 10s baseline period before the CS insertion and divided the total trial measurements by that average. All 25 trials per session for each animal were averaged in to one average per animal, which was used for subsequent analysis. To screen for reliable photometry signal, we defined significant transients in a behavioral window (5s post lever insertion) as having a maximum CS-evoked peak amplitude ≥ 2 Z-score (p=0.05) above baseline. We excluded rats whose vehicle day CS-evoked signals did not meet this criterion. Peak height was calculated using the "findpeaks" function in Matlab. We took the maximum peak height above Z = 0 in the 2.5s following the event of interest (either CS (lever extension) or US (lever retraction and pellet delivery)). In the case there were no peaks above Z=0, the peak height measure was recorded as 0 for that subject. The latency at which the maximum peak occurred was used for the peak latency calculation. We used the "trapz" function in Matlab to examine area under the curve for 5s post event. For training data in F**ig. 4**, of n=22 rats, four were only recorded on D1 and D5. Thus, In **Fig. 4D inset** and **4F** data are from n=18 animals, while data in **4D main figure, 4E,** and **4G** are from n=22 animals. When examining differences in signal between NAc Core and Shell in figures **4H-J**, we analyze data from 20 of 22 rats because 2 fiber placements were exactly on core/shell border.

377 **RESULTS**
378
379 **Individual differences in the acquisition of conditioned approach in a Pavlovian**
380 **lever autoshaping task**

381 We first examined the role of the VTA→NAc dopamine projection in regulating
382 individual differences in Pavlovian approach. We infused a Cre-dependent control virus
383 (AAV5-ef1a-DIO-eYFP) or a Cre-dependent Halorhodopsin (Halo) (AAV5-ef1a-DIO-
384 eNphr3.0-eYFP) in to the VTA of TH::Cre rats and implanted bilateral optical fibers
385 targeting the NAc core (**Fig. 1A, B**). After a recovery period, we trained rats (Halo, n =
386 31, eYFP, n = 27) in four Pavlovian lever autoshaping (PLA) sessions where the
387 extension and retraction of a lever for 10s predicted the delivery of food (**Fig. 2A**). We
388 classified rats as Sign-Trackers (ST), Goal-Trackers (GT), or Intermediates (INT) based
389 on the Pavlovian conditioned approach (PavCA) score, which reflects an animals'
390 tendency to approach the lever relative to the food cup. Over acquisition we observed
391 main effects of Session ($F(3,156) = 34.921$, $p < 0.001$) and Tracking ($F(2,52) = 67.568$,
392 $p < 0.001$), and a Session x Tracking interaction ($F(6,156) = 24.923$, $p < 0.001$),
393 confirming individual differences in conditioned approach (**Fig. 2B)**. Main effects and
394 interactions of all behavioral measures collected in PLA are presented in **Table 1**. There
395 were no main effects or interactions with Virus (Max F = 3.061, min $p = 0.086$),
396 demonstrating that the virus infused had no effect on the acquisition of behaviors that
397 characterize sign, goal- and intermediate rats.

398 **VTA-NAc dopamine terminal inhibition selectively reduces approach in sign-**
399 **trackers**

400 Prior pharmacology studies establish a role for NAc core dopamine in specifically
401 driving sign-tracking (Clark et al., 2013; Fraser et al., 2016; Fraser & Janak, 2017;
402 Saunders & Robinson, 2012). Here we test the role of VTA-NAc core projections by
403 inhibiting VTA dopaminergic axons projecting to the NAc core during the 10s lever cue
404 in sign-, goal- and intermediate tracking groups. We gave two test sessions; one in
405 which the rat were tethered to the patch cable but light was not delivered (OFF
406 condition) and a second session in which rats were tethered and LED light was
407 delivered to the NAc core (ON condition) (**Fig. 2A**).

408 We examined the effect of dopamine inhibition on the number of lever contacts in
409 the ST group (**Fig. 2C**) and found a main effect of Light ($F(1,14) = 8.735$, $p = 0.010$) and
410 a Light x Virus interaction ($F(1,14) = 67.602$, $p = 0.015$). This interaction was driven by a
411 decrease in pressing by the Halo group ($t_{(9)} = 3.884$, $p = 0.004$, Cohen's d = 0.71) but
412 not the eYFP group ($t_{(5)} = 0.277$, $p = 0.793$). We additionally examined foodcup
413 contacts and found no significant main effects or interactions (Max F = 3.35, Min p =
414 0.089 (ME light)) (**Fig. 2D**). In addition to lever and foodcup contacts, we analyzed the
415 latency and probability to lever and food cup contact and found no significant
416 interactions with virus (Max F = 2.07, Min $p = 0.172$). Collectively, these results
417 demonstrate that dopamine inhibition in ST rats doesn't affect the latency or probability
418 to make a response, but selectively reduces the vigor with which the animals engage
419 with and press the lever. Consistent with pharmacological manipulations in the NAc
420 core (Saunders & Robinson, 2012; Clark et al., 2013; Fraser & Janak, 2017), inhibition
421 of the VTA→NAc dopamine projection is necessary for the vigor of lever responding in
422 the sign-tracking rats.

423 We next examined the effect of dopamine terminal inhibition in INT rats. Even
424 though the INT rats had substantial levels of lever pressing by the end of training, there
425 was no Light x Virus interaction ($F(1,23) = 0.208$, $p = 0.653$) (**Fig. 2E**). Poking behavior
426 remained unaffected by dopamine inhibition as well. Although there was a main effect of
427 Light ($F(1,23) = 19.504$, $p < 0.001$), there was no Light x Virus interaction ($F(1,14) =$
428 0.848, $p = 0.367$) (**Fig. 2F**). Intermediate rats display similar amount of both lever and
429 foodcup directed behaviors, and our results demonstrate that NAc dopamine inhibition
430 does not affect the levels of either response. The preferred response of GT rats is
431 foodcup directed behavior. There was no significant interaction with Light x Virus
432 ($F(1,15) = 0.125$, $p = 0.729$) (**Fig. 2G**). We found a main effect of Light when examining
433 pressing behavior ($F(1,15) = 5.547$, $p = 0.033$) (**Fig. 2H**), but no Light x Virus interaction
434 ($F(1,15) = 0.922$, $p = 0.352$).

435 We performed an analysis of continuous data showing the relationship between
436 PavCA score and the difference in lever contacts between treatments (ΔLever contacts
437 = contacts light ON - contacts light OFF). We found a significant negative correlation (R^2
438 $= 0.3452$, $p = 0.0005$ **Fig. 2I**), indicating the greater the PavCA score (ST) the larger the
439 decrement in lever presses induced by dopamine inhibition. We did not see a significant
440 correlation in the eYFP control group ($R^2 = 0.03746$, $p = 0.33$, **Fig. 2J**), nor did we
441 observe any relationship between PavCA score and difference in food cup responses
442 between treatments ($R^2 = 0.0012$, $p = 0.8554$; eYFP $R^2 = 0.0014$, $p = 0.853$) (data not
443 shown). To further confirm that inhibiting VTA dopaminergic axons in the NAc was
444 specific to lever pressing of the ST, we analyzed data only from the rats expressing
445 Halorhodopsin using within between-subjects factor of Tracking (ST, INT, GT) and

446 within subjects factors of Light (OFF, ON) and Response (press, poke). We found a 3-
447 way interaction ($F(2,28) = 7.98$, $p = 0.002$) that was driven by a decrease in pressing by
448 the sign-tracking rats. This analysis further demonstrates that VTA dopamine axon
449 inhibition specifically reduces lever pressing in sign-tracking rats.

450 In conclusion, inhibiting dopaminergic terminals in the NAc specifically reduced
451 the amount of lever pressing in ST animals and had no effect on the responding in INT
452 or GT rats. Here we have demonstrated this VTA→NAc pathway is important for
453 regulating sign-tracking behavior, we next manipulate CB1R in the VTA where we
454 predict CB1R regulates cue-evoked NAc DA levels during sign-tracking.

455

456 **Intra-VTA CB1R inhibition reduces sign-tracking**

457 Given the specific effects of VTA→NAc core dopamine inhibition in sign-tracking
458 rats, we next examine how VTA CB1R regulate cue attraction in sign-trackers. As a
459 comparison group, we include INT rats who also display lever directed behaviors, but do
460 not require NAc dopamine to execute these behaviors (**Fig. 2E,F)**. All rats were
461 implanted with cannulae targeting the VTA (**Fig. 3A).** First, we trained 19 rats (11 ST, 8
462 INT) for 5 PLA sessions to determine tracking groups. For the PavCA score we observe
463 main effects of Session ($F(4,68) = 21.98$, $p < 0.001$) and Tracking ($F(1,17) = 22.77$, $p <$
464 0.001) and a Session x Tracking interaction ($F(4,68) = 3.45$, $p = 0.013$) (**Fig. 3B**). On
465 the fifth training session INT rats showed similar levels of lever directed and food cup
466 directed behaviors (PavCA = 0.13) whereas ST rats showed predominantly lever-
467 directed behaviors (PavCA = 0.62) further confirming these two groups of rats show
468 different patterns of conditioned responding in response to Pavlovian reward-predictive

469 cues by the end of training. We present main effects and interactions of all PLA training
470 measures in **Table 2**.

471 Prior to the 6th and 7th reinforced PLA sessions, we gave rats counterbalanced
472 intra-VTA infusions of rimonabant or vehicle. Based on previous findings (Bacharach,
473 2018), we predicted that intra-VTA rimonabant would reduce measures of sign-tracking
474 behavior. Indeed, we found that blocking VTA CB1R decreased the PavCA score
475 selectively for ST, but not INT rats (**Fig. 3C**). We found a main effect of Tracking
476 ($F(1,17) = 16.94$, $p = 0.001$) and a Treatment x Tracking interaction ($F(1,17) = 10.67$, p
477 $= 0.005$). This interaction was driven by a significant decrease in PavCA in the sign-
478 trackers ($t_{(10)} = 3.272$, $p = 0.008$, Cohen's d = 0.78) and a non-significant increase in
479 PavCA in INT rats ($t_{(7)} = -1.544$, $p = 0.167$, Cohen's d = 0.43).

480 Out of the three scores that comprise the PavCA index, the probability score
481 was most affected by intra-VTA rimonabant injections (**Fig. 3D**). We found a main
482 effect of Tracking ($F(1,17) = 19.63$, $p < 0.001$) and a Treatment x Tracking interaction
483 ($F(1,17) = 24.40$, $p < 0.001$). Compared to vehicle, ST rats showed a significant
484 decrease ($t_{(10)} = 4.509$, $p = 0.001$, Cohen's d = 0.76) whereas INT showed a significant
485 increase ($t_{(7)} = -2.646$, $p = 0.033$, Cohen's d = 0.61) in the probability score when VTA
486 CB1R signaling was decreased. To further understand the change in probability score,
487 we compared the probability to poke vs. the probability to press across treatment
488 conditions within each tracking group (**Fig. 3E**). Sign-trackers showed a main effect of
489 Response ($F(1,10) = 48.31$, $p < 0.001$) and a Treatment x Response interaction ($F(1,10)$
490 $= 20.332$ $p = 0.001$), suggesting that rimonabant differentially affected pressing vs.
491 poking behavior. Sign-trackers showed a significant reduction in the probability to lever

492 press ($t_{(10)}$ = 4.734, p < 0.001, Cohen's d = 0.58) and an increase in the probability to
493 poke, though the latter wasn't significant ($t_{(10)}$ = -1.573, p = 0.147, Cohen's d = 0.44).
494 Intermediates showed a main effect of Treatment (F(1,7) = 15.16, p = 0.006) and
495 Treatment x Response interaction (F(1,7) = 7.00, p = 0.033). Intermediates showed a
496 reduction in the probability to press ($t_{(7)}$ = 2.950, p = 0.021, Cohen's d = 0.91) and a
497 strong reduction in the probability to poke ($t_{(7)}$ = 4.194, p = 0.004, Cohen's d = 1.49). At
498 first pass, these data suggest that CB1R blockade reduces all behavior in intermediate
499 rats, this treatment instead shifts sign-tracking rats away from lever directed and
500 towards food cup directed behaviors.

501 As a measure of general task engagement and motivation to consume a reward
502 we measured the latency to collect the pellet once it was delivered for each trial (**Fig.**
503 **3F**). We found a main effect of Treatment (F(1,17) = 12.65, p = 0.002) and a Treatment
504 x Tracking interaction (F(1,17) = 8.73, p = 0.009). Rimonabant had no effect on the
505 latency to collect the pellet in ST rats ($t_{(10)}$ = -0.686, p = 0.51, Cohen's d = 0.13),
506 suggesting that the changes in ST rat's lever and food cup approach reported above
507 were not due to decreased task engagement. In contrast, intra-VTA rimonabant
508 increased the latency to collect the pellet in INT rats ($t_{(7)}$ = -3.212, p = 0.015, Cohen's d
509 = 1.21), suggesting that their motivation to consume the pellets may have In part
510 contributed to overall reduced lever and food cup approach during the cue. Regardless
511 of latency to collect the pellet, all rats tested still ate 100% of the pellets delivered during
512 the task, confirming there were no deficits in consummatory behavior arising from VTA
513 CB1R manipulation.

514 To further examine this possibility, we next determined whether rimonabant
515 decreased overall task engagement or motivation to consume food reward. We
516 examined total behavior (lever contacts + foodcup contacts) during testing and found a
517 main effect of Treatment ($F(1,17) = 19.16$, $p < 0.001$) and a Treatment x Tracking
518 interaction ($F(1,17) = 6.82$, $p = 0.018$) (**Fig. 3G**). This interaction was driven by a
519 significant decrease in contacts in INT rats ($t_{(7)} = 4.741$, $p = 0.002$, Cohen's d = 1.22).
520 These data indicate that intra-VTA rimonabant injections did not affect overall levels of
521 conditioned responding in ST rats, but this treatment blunted behavior overall in INT
522 rats**.** We further probed this interaction by investigating lever vs. foodcup contacts in
523 each tracking group (**Fig. 3H**). Similar to the probability data, ST rats showed a main
524 effect of Response ($F(1,10) = 24.732$, $p = 0.001$) and a Treatment x Response
525 interaction ($F(1,10) = 10.661$, $p = 0.0009$). This interaction was driven by a decrease in
526 lever contacts ($t_{(10)} = 3.167$, $p = 0.01$, Cohen's d = 0.50) and an increase in foodcup
527 pokes ($t_{(10)} = -1.273$, $p = 0.116$, Cohen's d = 0.44). In contrast, INT rats showed a
528 reduction in both responses; main effect of Treatment ($F(1,7) = 22.473$, $p < 0.002$). In
529 summary, ST had similar amounts of total approach behavior in which a reduction in
530 lever-directed behavior was compensated for by an increase in foodcup-directed
531 behavior. INT rats still engaged in both types of responding, but both responses were
532 decreased.

533 As a control, we determined whether locomotor activity changed with intra-VTA
534 rimonabant injections. We gave an open-field test to a subset of ST and INT rats and
535 found no differences in locomotor activity when rats were given intra-VTA vehicle or
536 rimonabant infusions (distance travelled in meters: Veh mean ± SEM = 19.65, 1.44;

537 Rimo mean ± SEM = 18.48, 0.86) . There were no main effects of Tracking, Treatment,
538 nor a Tracking x Treatment interaction (Max F = 1.147, Min p = 0.307).
539 Taken together, CB1R inhibition in the VTA causes divergent behavioral
540 response profiles across ST and INT rats. Rimonabant in the VTA caused a decrease in
541 all appetitive motivated behaviors measured in the INT rats. In contrast, rimonabant only
542 decreased behavior directed towards the lever cue in ST rats, while increasing the
543 amount of cue-induced food cup behaviors. Thus, CB1R receptor blockade decreases
544 sign-tracking behavior leading to more balanced levels of lever- and foodcup-directed
545 behaviors.
546
547 **CS-evoked dopamine increases with sign-tracking behavior across Pavlovian**
548 **training**
549 In order to understand rimonabant's effect on cue-evoked dopamine in the sign-
550 trackers, we measured $GRAB_{DA}$ signals in the NAc core and shell while manipulating
551 CB1R in the VTA. We infused the fluorescent dopamine sensor, $GRAB_{DA}$, and
552 implanted fiber optic cannula targeting the NAc in separate group of 22 sign-tracking
553 rats (**Fig. 4A, B**). Rats acquired a sign-tracking response as previously observed
554 (PavCA: main effect of Session ($F(4,84) = 33.630$, $p < 0.001$) (**Fig. 4C**). Further, when
555 examining pressing and poking contacts, ST rats showed main of effects of Session
556 ($F(4,84) = 5.229$, $p < 0.001$) and Response ($F(1,84) = 48.689$, $p = 0.003$), and a
557 Session x Response interaction ($F(4,84) = 19.172$, $p < 0.001$) where pressing behavior
558 was significantly greater than poking behavior on D2-D5 of training (*p's* < .0001) (**Fig.**
559 **4C inset**).

560 Voltammetry studies establish that ST, but not GT, show a transfer of DA
561 transients from the US to CS over Pavlovian training (Clark et al., 2013; Flagel et al.,
562 2011; Saddoris et al., 2016) which is interpreted as signal supporting enhanced
563 motivational value of the CS in ST rats. We examined the NAc $GRAB_{DA}$ signal across
564 training (**Fig. 4D, inset**) and found main effects of Session ($F(4,68) = 5.044$, $p = 0.001$)
565 and Epoch (CS,US; $F(1,17) = 36.414$, $p < 0.001$) and a Session x Epoch interaction
566 ($F(4,68) = 19.022$, $p < 0.001$). CS evoked NAc $GRAB_{DA}$ signal was greater than US
567 evoked $GRAB_{DA}$ signal on D2-D5 of training (*p's* < 0.0007). This data reflect the classic
568 signature of a dopamine response to transfer from the US to the CS across conditioning
569 (Day et al., 2007; Schultz et al., 1997).

570 We next compared the relationship of CS- and US-evoked on $GRAB_{DA}$ signals for
571 each rat across D1 and D5. We calculated the peak height difference score (peak
572 height: CS-US/CS) on D1 versus D5 (**Fig. 4E**). We found that there was a significant
573 increase in this score on D5 compared to D1 of training ($t_{(21)} = -5.771$, $p < 0.001$,
574 Cohen's d = 1.53), demonstrating that on $GRAB_{DA}$ signals to the US were transferred to
575 the CS in ST over the course of training.

576 On a session-by-session basis we observe the magnitude of sign-tracking (lever
577 contact) positively correlates with CS-evoked NAc DA peak heights across training
578 (sessions 1-5, n is rat average per session; $R^{2=}0.155$, $p = 0.0001$, **Fig. 4F**).
579 Furthermore, the increase in sign-tracking across training (D5 minus D1 lever contacts)
580 correlates positively with the change in peak height difference scores (CS-US/CS; D1-
581 D5) ($R^2 = 0.2061$, $p = 0.0338$) (**Fig. 4G**). Together these data suggest that greater

582 escalation of sign-tracking is associated with greater CS relative to US DA signals
583 across conditioning.

584 Due to known differences in dopamine release and dynamics in NAc core vs.
585 shell (Cacciapaglia et al., 2012; Saddoris et al., 2013; West & Carelli, 2016; Zahm,
586 1999), we examine region-specific NAc $GRAB_{DA}$ signals across conditioning (**Fig 4H, I**).
587 We examined the area under the curve between two time epochs (Cue (CS): 5s post
588 lever insertion, Reward (US): 5s post lever retraction), which reveals main effect of
589 Epoch (CS > US, $F(1,18) = 12.97$, $p = 0.002$) and an Epoch x Region interaction
590 ($F(1,18) = 12.76$, $p = 0.002$, **Fig. 4J**), which is driven by greater NAc core CS vs US
591 responses ($t_{(11)} = 5.220$, $p < 0.001$, Cohen's d = 1.26) and greater US $GRAB_{DA}$
592 responses in NAc shell than NAc core ($t_{(18)} = 2.184$, $p = 0.042$, Cohen's d = 0.94).
593 These results confirm that cue-evoked NAc $GRAB_{DA}$ signal increases to and correlated
594 lever directed behavior in sign-tracking rats, consistent with prior reports (Day et al.,
595 2007; Flagel et al., 2011; Clark et al., 2013; Saddoris et al., 2016). We also provide
596 evidence that dopamine is released differentially in the core versus shell during training.
597

598 **Intra-VTA rimonabant primarily affects dopamine encoding of outcomes rather**
599 **than cues**

600 We used thirteen ST rats in our analysis of test data. Nine rats were excluded
601 from test analysis due to off target cannula placement in the VTA infusion site (n=4),
602 excessive damage at VTA infusion site (n=2), or a loss of signal during testing (n=3).
603 Similar to our prior behavioral findings, we found intra-VTA rimonabant significantly
604 decreased the PavCA score of ST rats ($t_{(12)} = 3.476$, $p = 0.005$, Cohen's d =0.82) (**Fig.**

605 **5A**). The decrease in PavCA was accompanied by similar reductions in the preference,
606 latency, and probability scores (vehicle vs. rimonabant; *p's* = 0.019, 0.005, 0.002,
607 respectively).

608 Due to known differences in dopamine release and dynamics in NAc core vs.
609 shell in training and consistent with prior reports (Cacciapaglia et al., 2012; Saddoris et
610 al., 2013; West & Carelli, 2016; Zahm, 1999), we include NAc Subregion as a statistical
611 factor in addition to Epoch and Treatment (Rimonabant, Vehicle). We first examined the
612 AUC during the two epochs and found a significant NAc Subregion x Epoch x Treatment
613 interaction ($F(1,11) = 5.311$, $p = 0.042$) (**Fig. 5B**). To follow up, we analyzed the total
614 $GRAB_{DA}$ signal during the US period and found a main effect of Treatment ($F(1,11) =$
615 7.919, $p = 0.017$), and a Treatment x Subregion interaction ($F(1,11) = 9.996$, $p = 0.009$).
616 This interaction was driven by increases in $GRAB_{DA}$ signals specifically in the NAc shell
617 during the US reward period ($t_{(5)} = -3.257$, $p = 0.023$, Cohen's d = 0.66). We present
618 NAc subregion specific traces and heatmaps in **Fig. 5C and 5D**.

619 We next probed if the rimonabant-induced NAc DA signal changes were
620 associated with behavioral changes in Pavlovian lever autoshaping. We correlated
621 changes in dopamine signals during the CS and US periods (AUC^{Rimo} - AUC^{Veh}) with
622 changes in PavCA score ($PavCA^{Rimo}$ -$PavCA^{Veh}$). We found that treatment-induced
623 changes in NAc $GRAB_{DA}$ signals during the CS period did not correlate with changes in
624 PavCA score ($R^2 = 0.01$, $p = 0.734$) (**Fig. 5E**). However, treatment-induced changes in
625 NAc $GRAB_{DA}$ signals during the US period were negatively correlated with changes in
626 the PavCA score ($R^2 = 0.433$, $p = 0.015$) (**Fig. 5F**). This suggests that the more intra-
627 VTA rimonabant shifted behavior towards goal-tracking PavCAs (more negative

628 scores), the greater the rimonabant-induced NAc DA signaling during the US period
629 relative to vehicle. We found similar negative correlations between rimonabant-induced
630 changes in US-AUC and the Preference, Latency, and Probability scores (*p's* = 0.006,
631 0.100, 0.059, respectively).

632 Consistent with this, US peak heights were increased with rimonabant treatment
633 (main effect of Treatment: ($F(1,11) = 10.228$, $p = 0.008$) and varied by NAc subregion
634 (Treatment x Subregion interaction ($F(1,11) = 5.77$, $p = 0.035$) **Fig. 5G**). This was driven
635 by increases in US peak height in the NAc shell ($t_{(5)} = -2.735$, $p = 0.041$, Cohen's d =
636 0.78). However, treatment-induced changes in US peak height did not predict variability
637 in PavCA or other behavioral measures, suggesting that the sustained US NAc DA
638 signaling that is captured by the AUC analysis (above) relates more to behavior than the
639 US peak.

640 We next examined dopamine dynamics surround the CS. On a population level,
641 the CS peak height was not affected by intra-VTA rimonabant treatment and we
642 observed a large amount of variability in CS peaks across NAc (**Fig. 6A**). Thus, we
643 examined treatment-related changes in CS-evoked peak ($CSpeak^{Rimo}$ - $CSpeak^{Veh}$) as a
644 function of changes in PavCA score ($PavCA^{Rimo}$ -$PavCA^{Veh}$), which were positively
645 correlated (**Fig. 6B**). This suggests that the more intra-VTA rimonabant shifted behavior
646 towards goal-tracking PavCAs (more negative PavCA difference score), the smaller the
647 rimonabant-induced NAc DA CS peak relative to vehicle. Notably, rats that showed less
648 of a behavioral shift (PavCA difference scores near zero) tended to have greater CS-
649 evoked DA peaks with intra-VTA rimonabant relative to vehicle. This suggests the more
650 rigid the behavior was between treatment sessions, the greater the CS-evoked NAc

651 dopamine signaling when VTA CB1R signaling was disrupted. Placements of VTA
652 infusions and NAc recording sites are given in **Fig. 6C,D.** Taken together, with inhibition
653 of CB1R signaling in the VTA we find that shifts in behavior away from sign-tracking and
654 towards goal-tracking (negative PavCA shifts) are associated with smaller NAc DA CS
655 peaks (**Fig. 6B**) and greater NAc DA US processing (**Fig. 5F**).
656

657 **CS-evoked dopamine is specific to rewarded lever**

658 Lastly, as a control in separate groups of rats, we confirmed that the $GRAB_{DA}$
659 signals in the NAc are specific to the learned association between the CS and the US.
660 We trained a separate group of fiber implanted, NAc $GRAB_{DA}$ expressing rats (**Fig. 7A**)
661 in a 2-lever Pavlovian autoshaping procedure. Here, a CS+ lever predicted food reward
662 and a separate CS- lever predicted no reward. We examined total behavior (lever and
663 food cup contact) during the CS+ versus CS- periods across training (**Fig. 7B**) and
664 found a main effect of Cue ($F(1,5) = 11.939$, $p = 0.018$) and a Cue x Session interaction
665 ($F(4,20) = 11.325$, $p < 0.001$). Rats showed more approach during the CS+, compared
666 to the CS-presentations on D5 of training ($t_{(5)} = 3.598$, $p = 0.016$, Cohen's d = 1.88).
667 This confirms that by D5 of training, the rats could behaviorally discriminate between a
668 reinforced from nonreinforced cue. On D5 of training, we found the CS evoked peak
669 height was greater for the CS+ lever than the CS ($t_{(5)} = 2.947$, $p = 0.032$, Cohen's d =
670 0.79; **Fig. 7 C,D**). We also trained a sperate group of fiber implanted, NAc $GRAB_{DA}$
671 expressing rats (n=4) rats in an unpaired control condition in which we delivered the
672 same number of food pellets, but lever extension was delivered pseudorandomly during
673 the ITI period and was never explicitly paired with pellet delivery. Unpaired rats

displayed low levels of lever and foodcup approach during lever extension periods throughout training **(Fig. 7E**). Rats did not develop conditioned responses to either the lever or foodcup (D1 vs. D5 ($t_{(3)}$ = 1.023, p = 0.382) (**Fig. 7E inset left**)**.** Despite not showing conditioned lever or foodcup approach, rats were still engaged in the task and collected the pellet faster on D5 than D1 of training ($t_{(3)}$ = 3.696, p = 0.034, Cohen's d = 2.56) (**Fig. 7E inset right**)**.** In this unpaired condition, we observed very low levels of lever-associated NAc $GRAB_{DA}$ signals which diminished, though not significantly, as training progressed (D1 vs. D5 $t_{(3)}$ = 2.425, p = 0.119) (**Fig. 7F**). In the unpaired rats, NAc $GRAB_{DA}$ signals to the CS on D1 likely represent a salience or novelty signal (Horvitz et al., 1997; Mirenowicz & Schultz, 1994), which goes away over time as rats become familiar with the lever extension and retraction. There was reliable NAc $GRAB_{DA}$ signal to the US food delivery (**Fig. 7G**)**,** that remained unchanged from D1 to D5 of training ($t_{(3)}$ = -1.594, p = 0.209) (**Fig. 7G inset**)**,** confirming the unpredictable delivery of the US in unpaired rats. Our results indicate that the NAc $GRAB_{DA}$ signal observed to the CS and US during the paired, single-lever condition arise due to rats learning and maintaining the CS-US relationship.

DISCUSSION

Here, we demonstrate that optogenetic inhibition of VTA-NAc core dopamine projections reduces lever approach, specifically in sign-tracking rats. While intermediate rats also display lever approach, VTA-NAc core DA terminal inhibition did not affect this behavior, nor did it affect the preferred food cup response in goal-tracking rats. Hence, VTA→NAc core dopamine release during the cue period is necessary for the enhanced

697 cue attraction specifically seen in sign-tracking rats. Having confirmed the importance of
698 VTA-NAc core DA projections in ST rats, we next examined whether disrupting VTA
699 CB1R signaling similarly decreased lever approach in sign-tracking rats. We compared
700 the effects of intra-VTA rimonabant injections in ST and intermediate rats because both
701 display lever directed approach but differently engage VTA-NAc core. We found that
702 intra-VTA infusions of the CB1R inverse agonist, rimonabant, resulted in different
703 response profiles between ST and INT rats. In INT rats, decreasing CB1R signaling
704 decreased all appetitive motivated behaviors that we measured. Both lever and food
705 cup responses decreased and the latency to collect the reward increased. In ST rats,
706 intra-VTA rimonabant selectively reduced lever approach while increasing food-cup
707 approach, shifting the rats away from sign-tracking (lever directed) and towards goal-
708 tracking (food-cup behaviors). These results suggest that intact VTA CB1R signaling
709 biases behavior towards the lever and away from the foodcup in ST rats. We predicted
710 that the VTA rimonabant-induced decrease in cue attraction would be associated with
711 disrupted cue-triggered dopamine signals downstream in the NAc. While we did not
712 observe overall effects of VTA CB1R inhibition on CS evoked NAc DA signaling, VTA
713 rimonabant-induced behavioral shifts away from sign-tracking were positively correlated
714 with changes to cue-evoked NAc core $GRAB_{DA}$ signals. Rats that showed the greatest
715 changes in behavior with intra-VTA rimonabant (away from ST) tended to have
716 decreased NAc DA CS peaks during rimonabant relative to vehicle sessions. In
717 contrast, rats that showed less of a behavioral shift (i.e., more rigid behavior between
718 sessions) tended to have greater CS-evoked DA peaks during rimonabant relative to
719 vehicle sessions.

720 We also found that intra-VTA rimonabant increased NAc $GRAB_{DA}$ signals during
721 the reward period and that this effect was driven by increased US DA signaling in the
722 NAc shell. VTA rimonabant-induced behavioral shifts away from sign-tracking were
723 negatively correlated with changes to US-evoked NAc core $GRAB_{DA}$ signals. That is,
724 rats that showed the greatest changes in behavior (away from ST) with intra-VTA
725 rimonabant tended to have increased NAc DA US peaks during rimonabant relative to
726 vehicle sessions. In contrast, rats that showed less of a behavioral shift with intra-VTA
727 rimonabant tended to have lower US-evoked DA peaks during rimonabant relative to
728 vehicle sessions. Our results demonstrate decreasing VTA CB1R signaling decreases
729 sign-tracking and increases goal-tracking approach, potentially by suppressing cue-
730 evoked, and enhancing reward-evoked NAc dopamine signals.

731 Prior pharmacology and voltammetry tracking studies have established NAc core
732 DA as necessary for sign-tracking (Flagel et al., 2011; Saunders & Robinson, 2012;
733 Clark et al., 2013; Fraser & Janak, 2017). Consistent with manipulations of NAc
734 dopamine receptors, we show that optogenetic inhibition of VTA-NAc core dopamine
735 projections during lever presentation similarly reduces lever approach in ST rats, but not
736 in intermediate rats that also display lever approach. Our results using Pavlovian
737 conditioning are consistent with a substantial instrumental literature showing that CB1R
738 signaling maintains both cued food-seeking and drug-seeking behaviors (McLaughlin et
739 al., 2003; De Vries & Schoffelmeer, 2005; Ward & Dykstra, 2005; Economidou et al.,
740 2006; Salamone et al., 2007; Ward et al., 2007; Justinova et al., 2008; de Bruin et al.,
741 2011; Oleson et al., 2012; Schindler et al., 2016). Consistently, sign-trackers and
742 intermediates show decreases in lever approach when we disrupt VTA CB1R signaling.

743 However, in intermediate rats this attenuating effect was not specific to the lever, as
744 food cup responding and task engagement were generally reduced, consistent with
745 prior rimonabant manipulations (McLaughlin et al., 2003; Ward & Dykstra, 2005;
746 McLaughlin et al., 2006; Salamone et al., 2007; Ward et al., 2007; Oleson et al., 2012).
747 However, this was not the case in sign-trackers that decreased lever approach but
748 showed no differences in overall task engagement and tended to increase food cup
749 responding when VTA CB1R signaling was disrupted. Thus, blocking VTA CB1R
750 signaling rebalanced lever- and foodcup-directed behavior, such that a decrease in
751 lever approach was compensated for by an increase food cup approach to result in a
752 significant decrease in the PavCA score. The eCB system is also involved in
753 maintaining Pavlovian cue-reward associations. Systemic blockade of CB1R signaling
754 disrupts cue-driven approach behaviors in Pavlovian tasks (Bacharach et al., 2018;
755 Gheidi et al., 2020). The present findings using region specific manipulation of CB1R
756 provide evidence that the VTA is a site of endocannabinoid action to promote sign-
757 tracking and confirm our prior work showing systemic rimonabant dose-dependently
758 reduces sign-tracking and the attracting and reinforcing properties of the lever cue
759 (Bacharach et al., 2018).

760 We found evidence that manipulating VTA CB1R signaling influences dopamine
761 release during the US/reward period that varies by NAc subregion. There are
762 anatomical and functional differences between the core and shell (Jones et al., 1996;
763 Zahm, 1999). In cued-instrumental and Pavlovian settings, NAc core dopamine release
764 is associated with the predictive value of a cue (Roitman et al., 2004; Cacciapaglia et
765 al., 2012; Hart et al., 2014; Saddoris et al., 2015; Stelly et al., 2021). In addition to the

766 predictive value of this dopamine signal, core dopamine also carries incentive
767 motivational value (Berridge, 1996; Flagel et al., 2011; Saunders & Robinson, 2012;
768 Saunders et al., 2013). Our photometry and optogenetic data from the NAc core
769 corroborate these findings in that NAc core is necessary for cue-approach in sign-
770 trackers and we see strong cue-induced dopamine under both vehicle on rimonabant
771 conditions. We find both NAc core DA inhibition and VTA CB1R antagonism decrease
772 sign-tracking. In our photometry recordings, the relationship between VTA CB1R
773 signaling and cue-evoked DA signaling spans recordings from both the NAc core and
774 shell. We observe consistent effects of VTA CB1R antagonism on PavCA scores across
775 our pharmacology experiment (**Fig. 3**) when rats are untethered, and our combined
776 pharmacology and photometry experiment (**Fig. 5**) when rats are tethered. This
777 suggests this technical difference of tethering rats, which does moderately slow
778 responding, did not interfere with VTA treatment effects on behavior. We do not observe
779 NAc region-specific differences in effects of VTA CB1R antagonism on cue-evoked DA
780 signaling. Overall VTA CB1R antagonism does not affect CS-evoked NAc DA, but
781 variability in the effects of rimonabant on behavior do relate to magnitude of treatment
782 effects on CS evoked NAc DA. This relationship suggests VTA CB1R signaling may
783 influence NAc DA encoding of predictive or incentive motivational properties of the CS
784 and thus bias behavior towards cues.

785 The NAc shell DA is more heavily involved in representing contextual elements
786 associated with reward seeking, tracking reinforcer value, and representing reward-
787 guided motivation (Bossert et al., 2007, 2012; Cacciapaglia et al., 2012; Cruz et al.,
788 2014; West & Carelli, 2016; Valyear et al., 2020). Additionally, NAc shell dopamine

789 release is less temporally restricted to cue onset, and differential dopamine dynamics
790 are observed during cue-onset, the entire cue period, and the US period (Saddoris et
791 al., 2015, 2016). We find that VTA CB1R blockade *increases* NAc shell dopamine
792 signaling during the US period and is associated with increased food cup exploration,
793 which may give rise to changes in US processing and/or reinforcer value representation.
794 The present study used almost exclusively female rats, and thus was not
795 designed to test for sex differences. Here we find that greater shifts in sign-tracking are
796 associated with decreased NAc CS DA during intra-VTA rimonabant vs vehicle
797 conditions. Yet the effect of rimonabant was more subtle than in our prior study using
798 voltammetry in male rats (Oleson, Beckert et al. 2012), as group effects of VTA
799 rimonabant in the present study were not evident. Instead, the decrease in CS-evoked
800 NAc DA was greatest in female rats that shifted away from sign-tracking the most. It is
801 likely differences in the sex of rats and DA recording techniques are the most likely
802 factors influencing different observations between studies. We used female rats for
803 several reasons. First, we and others see evidence for increased propensity for females
804 to sign-track (Pitchers et al., 2015; Kochli et al., 2020; Madayag et al., 2017). Second, in
805 our systemic rimonabant study, we observed greater CB1R manipulation effect sizes in
806 females compared to males on the attracting and reinforcing properties of cues
807 (Bacharach et al., 2018). Consistent with these behavioral and pharmacological
808 findings, a recent study shows higher conditioned responding in females compared to
809 males (Lefner et al., 2022). Females in that study showed lower US-evoked NAc DA
810 responses compared to males during Pavlovian conditioning. This latter result has
811 relevance for the present findings, which point towards a role for VTA CB1R regulation

812 of behavioral and dopaminergic cue bias observed in female rats. Future studies
813 designed to probe sex differences would be needed to determine if the present findings
814 are sex-specific or not.

815 Here we examine effects of VTA CB1R signaling on NAc dopamine during
816 Pavlovian behavior, while prior work examining such effects used instrumental tasks.
817 Importantly, prior instrumental studies used short, fixed inter trial intervals (10-20 s),
818 which has implications for CB1R receptor involvement in dopamine neuron activity.
819 Dopamine is intimately involved in interval timing (Buhusi & Meck, 2005; Mikhael &
820 Gershman, 2019). Oleson et al. (2012) found that there was larger cue-induced
821 dopamine release in a fixed interval, which affected more by rimonabant, versus a
822 variable interval task. Here we used a Pavlovian lever autoshaping task with a much
823 longer 90s variable intertrial interval. This long and unpredictable interval between trials
824 may limit our ability to see group differences in CS-evoked NAc DA changes with CB1R
825 manipulations. Regardless, the present study revealed the greatest rimonabant-induced
826 decrease in CS-evoked NAc DA were associated with the largest decreases in
827 Pavlovian sign-tracking. This finding corresponds with our prior work indicating VTA
828 CB1R signaling gates cue-evoked NAc DA to regulate instrumental motivational
829 processes (Oleson, Beckert et al. 2012).

830 Together, our results suggest that both VTA CB1R signaling and NAc core DA
831 support the maximal expression of sign-tracking behavior. Disrupting VTA CB1R
832 signaling rebalances behavior away from sign-tracking and towards goal-tracking.
833 Further, the intra-VTA rimonabant induced shift in behavior of ST rats is related to
834 decreases in CS- and increases in US-evoked NAc shell dopamine signaling, indicating

that VTA CB1R receptors are involved in maintaining NAc representations of the CS-US relationship. Our results suggest that CB1R signaling in the VTA maintains the conditioning-dependent behavioral and NAc DA bias towards cues relative to outcomes in sign-tracking rats, potentially by downregulating reward-related NAc shell DA signaling. Future causal role studies are needed to test the necessity of NAc shell DA for mediating the balance between CS and US directed behaviors in lever autoshaping.

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Figure Legends

Figure 1: *Histological verification of viral and optogenetic fiber placement*. ***A***, (Left) Representative VTA transduction of Halorhodopsin (green). Staining for Tyrosine Hydryoxylase (TH) is given in red, DAPI in blue. White arrows depict triple overlap. (Right) Representative image Halorhodopsin terminal expression (green) and DAPI (blue) in the Nucleus Accumbens. White dotted line indicates boundary of NAc core. ***B***, (Top) The extent of viral coronal transduction (in mm) of Halorhodopsin and eYFP in the VTA. (Bottom) Bilateral optogenetic fiber optic placement in the NAc across the three tracking groups.

Figure 2: *VTA-NAc dopamine terminal inhibition selectively reduces approach in sign-trackers.* ***A***, After training in PLA, rats were given test sessions with light turned OFF(top) or ON(bottom) where light was delivered to the NAc core during the 10s cue period. ***B***, PavCA score mean ± SEM. ST, GT, and INT acquire individual differences in conditioned responding in PLA task. * Main effect of Session; % Significant Session x Tracking interaction. ***C***, Terminal inhibition of dopamine axons in the NAc significantly reduces the amount of lever contacts and ***D,*** has no effect on foodcup contacts in Sign-trackers. * With curved bracket indicates significant Light x Virus interaction. * With straight brackets indicates significant post-hoc within-subject t-test. ***E***, ***F***, No significant effects of terminal inhibition in INT rats. ***G***, ***H***, No significant effects of terminal inhibition in GT rats. ***I,*** Correlation between the change in lever pressing behavior between light conditions as a function of Halo-expressing rats PavCA scores. ***J,*** Correlation between the change in lever pressing behavior between light conditions and eYFP-expressing rats PavCA scores.

Figure 3: *Intra-VTA CB1R receptor inhibition reduces sign-tracking.* ***A***, Coronal sections (in mm) depicting location of VTA injector tips for rimonabant infusion. ***B***, PavCA score mean ± SEM of ST and INT that acquire individual differences in conditioned responding in PLA task. * Main effect of Session; % Significant Session x Tracking interaction. ***C***, Rimonabant significantly decreases PavCA in ST, but not in INT. * with curved bracket indicates significant Tracking x Treatment interaction. * With straight bracket indicates significant effect of treatment. **D**, ST show a significant reduction in the probability score and INT show significant increase in probability score. * With curved bracket indicates significant Tracking x Treatment interaction. * With straight bracket indicates significant effect of Treatment. ***E***, In ST, the probability to press is significantly reduced while poking is increased. In INT, both pressing and poking is significantly reduced. * Indicates significant effect of Treatment. ***F***, Latency to collect the pellet after each trial is not changed in ST but is significantly increased in INT. ***G***, Total behavior (presses + pokes) was unaffected by rimonabant in ST but decreased in INT rats. ***H***, In ST, the number of lever presses is significantly reduced while poking is increased. In INT, both pressing and poking is significantly reduced.

Figure 4: *CS-evoked dopamine increases with sign-tracking behavior across Pavlovian training*. ***A***, Placements of fiber optics where the $GRAB_{DA}$ signal was recorded. Black and red fiber placements were used in the analysis of training data whereas only red fibers were used in analysis of Test data. ***B***, Example image showing optic fiber placement in the NAc and $GRAB_{DA}$ expression in NAc core. ***C***, PavCA mean ± SEM of sign-trackers who show significantly higher presses than pokes throughout training (inset). * Indicates significant within-subjects repeated measures t-test comparing presses vs. pokes. ***D***, Mean $GRAB_{DA}$ signal of 22 rats on D1 vs D5 of PLA. ***D inset,*** the peak height of CS-evoked dopamine is significantly higher than US dopamine on days 2-5 of training. Data from 18 rats, see methods * Significant within-subjects repeated measures t-test comparing CS vs US peak heights. **E,** We calculated the Peak height Difference Score ((CS peak height - US peak height) / CS peak height)) for each animal. A positive score indicates the CS peak is larger than the US peak. There was a significant change in this relationship over training. * Indicates main effect of Session. ***F,*** Session-by-session correlation between number of lever contacts and CS-evoked peak height for each of the five days of training. Each point represents an individual rat's number of lever contacts and average CS-evoked peak height per session. ***G,*** Correlation between the change in NAc DA peak height difference score (D5 minus D1) and the change in lever contacts (D5 minus D1) for each rat. ***H,*** Mean $GRAB_{DA}$ signal of n= 12 rats recorded from the NAc core of D1 (gray) vs. D5 (red) of training. ***I,*** Mean $GRAB_{DA}$ signal of n=10 rats recorded from the NAc shell of D1 (gray) vs. D5 (red) of training. ***J,*** Area under the curve (average of D1 and D5) analysis showing differences in region (core vs. shell) and time epoch (CS vs. US) * With curved bracket indicates significant Subregion x Epoch interaction. * With straight bracket indicates significant effect of Epoch.

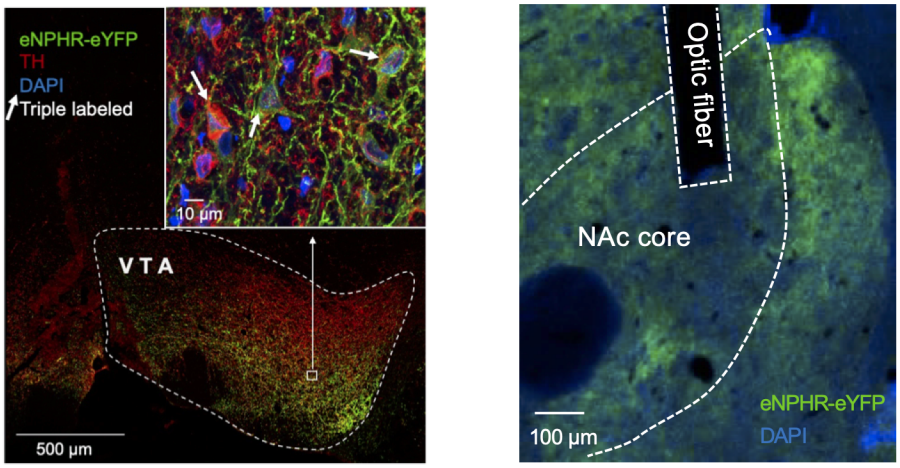
Figure 5: *Intra-VTA rimonabant primarily affects dopamine encoding of outcomes rather than cues.* ***A***, Rimonabant significantly reduces PavCA score in ST rats, as well as preference, latency, and probability scores. * Indicates main effect of Session. ***B***, Area under the curve for the 5s CS period vs. 5s US period in animals recorded in NAc core vs. shell under vehicle or rimonabant conditions. * With curved bracket indicates significant Subregion x Treatment interaction. * With straight bracket indicates significant effect of Treatment. ***C left,*** Mean -SEM shading of $GRAB_{DA}$ signal in the NAc core in vehicle (gray) vs. rimonabant (blue) conditions. ***C right*****,** trial by trial heatmap of average $GRAB_{DA}$ signal in NAc core over vehicle and rimonabant conditions. ***D left,*** Mean -SEM shading of $GRAB_{DA}$ signal in the NAc shell in vehicle (gray) vs. rimonabant (blue) conditions ***D right*****,** trial by trial heatmap of average $GRAB_{DA}$ signal in NAc shell over vehicle and rimonabant conditions. ***E,*** Correlation between the change in area under the curve for CS epoch (Rimo minus vehicle) with change in the PavCA score (rimo minus vehicle). ***F,*** Correlation of the change in area under the curve for US epoch (rimo minus vehicle) with change in the PavCA score (rimo minus vehicle). ***G,*** US peak height analysis showing differences in region (core vs. shell) and treatment (core vs. shell) * With curved bracket indicates significant Subregion x Treatment interaction. * With straight bracket indicates significant effect of Treatment.

Figure 6. *CB1R inhibition effect on CS-evoked peak dynamics predict magnitude of treatment effect on behavior.* ***A,*** Rimonabant does not change CS-induced peak height in core or shell. ***B,*** Correlation of the change in CS-evoked peak height (rimo minus vehicle) with the change in PavCA score (rimo minus vehicle). ***C***, Site of injector tip placement in the VTA for rimonabant infusion. ***D,*** $GRAB_{DA}$ expression and photometry recording location in NAc. Solid lines indicate recording location for NAc Core and dotted lines indicate location of NAc Shell fibers.

Figure 7. *CS-evoked dopamine is specific to rewarded lever.* ***A***, Histological placements of photometry fibers in the NAc. Two separate groups of rats were used for training in 2-lever PLA or Unpaired PLA. 6 rats were run in the 2-lever program and their fiber placements are indicated by the solid black line. Four rats were run in the unpaired program, placements indicated with dashed line. ***B***, Rats show behavioral discrimination (presses + pokes) between the CS+ and CS- lever. * Main effect of Session; % Significant Session x Response interaction. ***C, D*** The CS-evoked $GRAB_{DA}$ signal is significantly higher to the rewarded than the unrewarded lever by day 5 of training. * Indicates main effect of Stimulus. ***E,*** Rats in the Unpaired condition display low amounts of behavior towards both the lever or foodcup. *Left inset*: total behavior does not increase from D1 to D5 suggesting no conditioned behavior has emerged. *Right inset*: Latency to collect the pellet reduces over training, suggesting rats are still engaged and food-motivated during the task. * Indicates main effect of Session. ***F***, $GRAB_{DA}$ responses to the lever on D1 vs D5 of training: A CS-evoked excitatory response to the lever is not established. ***G***, $GRAB_{DA}$ signal to pellet delivery remains high across training.

Figure 1

A.


eNPHR-eYFP
TH
DAPI
Triple labeled
10 µm
VTA
500 µm
Optic fiber
NAc core
100 µm
eNPHR-eYFP
DAPI

B.

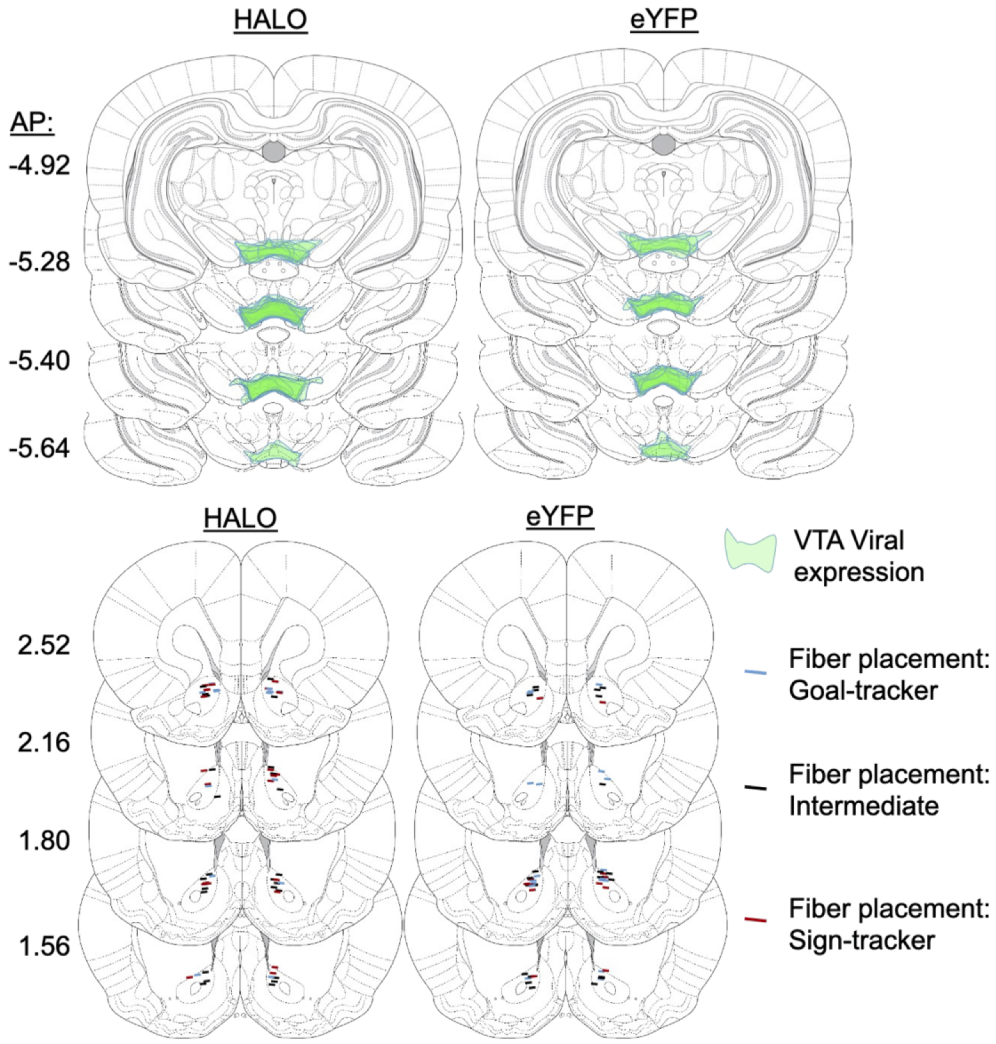

HALO
eYFP
AP:
-4.92
-5.28
-5.40
-5.64
HALO
eYFP
2.52
2.16
1.80
1.56
VTA Viral expression
Fiber placement: Goal-tracker
Fiber placement: Intermediate
Fiber placement: Sign-tracker

Figure 2

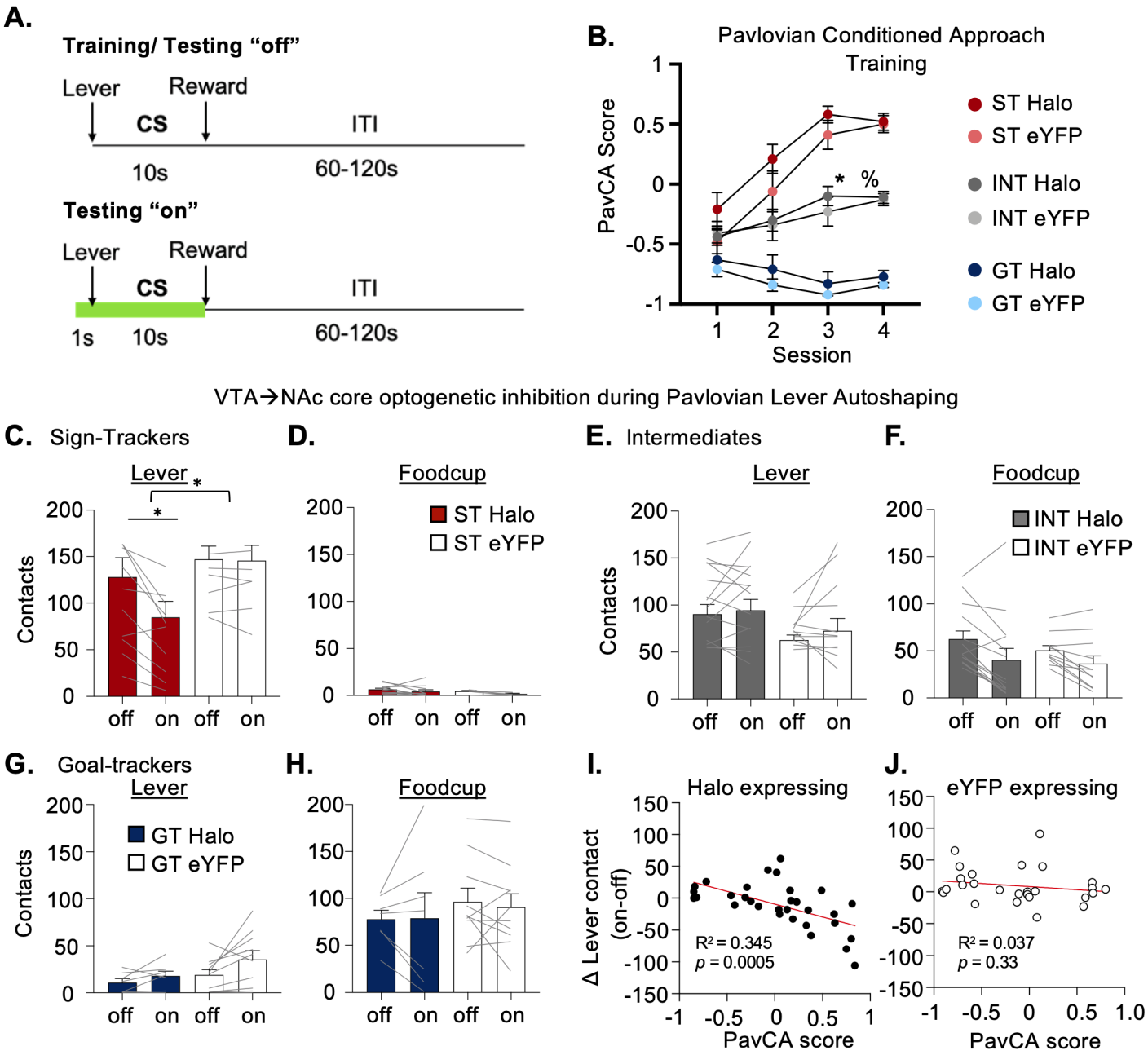

A.
Training/ Testing "off"
Lever
Reward
CS
ITI
10s
60-120s
Testing "on"
Lever
Reward
CS
ITI
1s
10s
60-120s
B.
Pavlovian Conditioned Approach Training
PavCA Score
Session
ST Halo
ST eYFP
INT Halo
INT eYFP
GT Halo
GT eYFP
VTA→NAc core optogenetic inhibition during Pavlovian Lever Autoshaping
C. Sign-Trackers
Lever
Contacts
off on off on
D.
Foodcup
ST Halo
ST eYFP
E. Intermediates
Lever
F.
Foodcup
INT Halo
INT eYFP
G. Goal-trackers
Lever
GT Halo
GT eYFP
H.
Foodcup
I.
Halo expressing
Δ Lever contact (on-off)
R² = 0.345
p = 0.0005
PavCA score
J.
eYFP expressing
R² = 0.037
p = 0.33
PavCA score

Figure 3

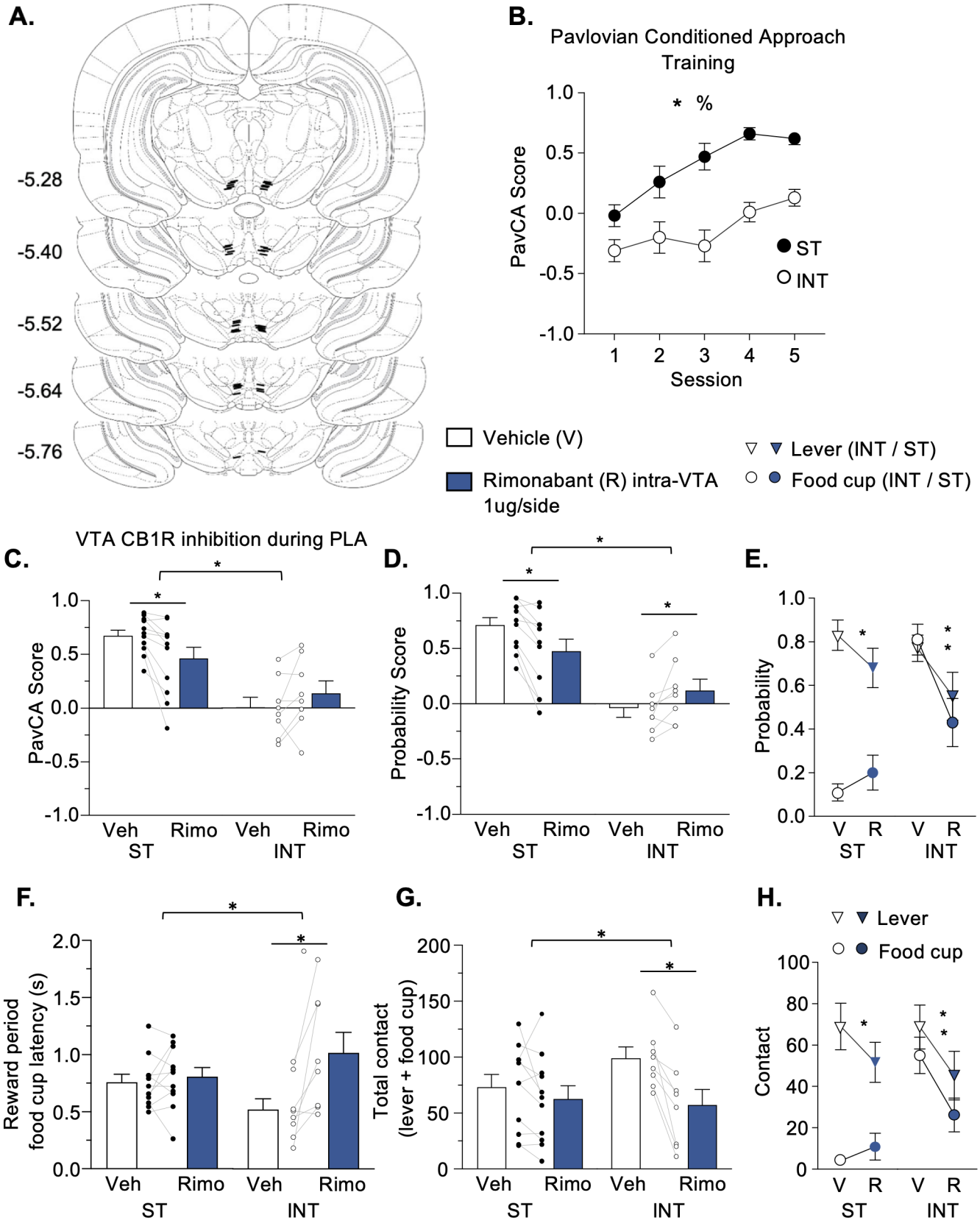

A.
-5.28
-5.40
-5.52
-5.64
-5.76
B.
Pavlovian Conditioned Approach Training
PavCA Score
Session
ST
INT
Vehicle (V)
Rimonabant (R) intra-VTA 1ug/side
Lever (INT / ST)
Food cup (INT / ST)
VTA CB1R inhibition during PLA
C.
PavCA Score
D.
Probability Score
E.
Probability
F.
Reward period food cup latency (s)
G.
Total contact (lever + food cup)
H.
Lever
Food cup
Contact
Veh
Rimo
V
R

Figure 4

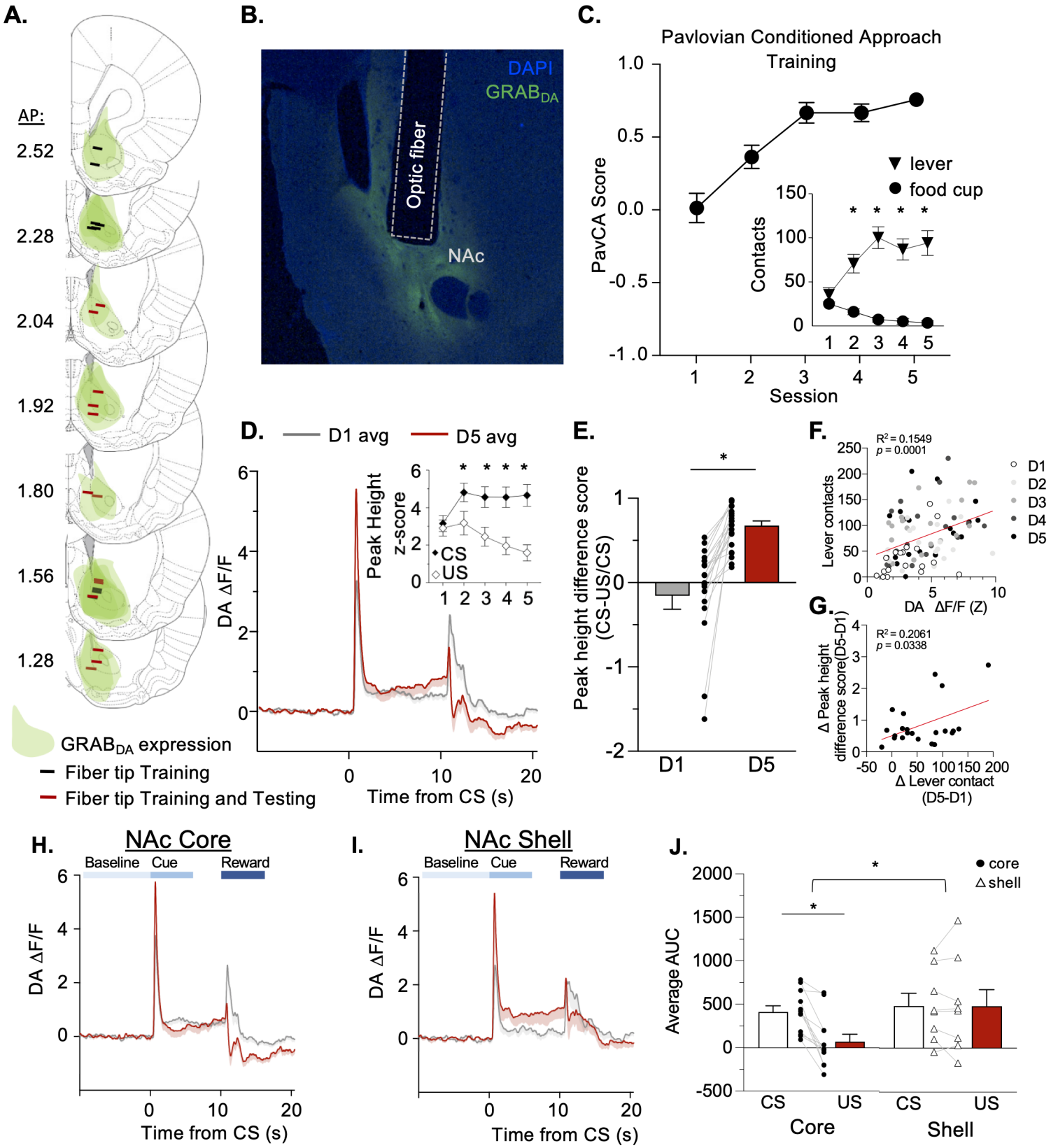

A.
AP:
2.52
2.28
2.04
1.92
1.80
1.56
1.28
GRABDA expression
Fiber tip Training
Fiber tip Training and Testing
B.
DAPI
GRABDA
Optic fiber
NAc
C.
Pavlovian Conditioned Approach Training
PavCA Score
Session
lever
food cup
Contacts
D.
D1 avg
D5 avg
DA ΔF/F
Time from CS (s)
Peak Height z-score
CS
US
E.
Peak height difference score (CS-US/CS)
D1
D5
F.
R² = 0.1549
p = 0.0001
Lever contacts
DA ΔF/F (Z)
D1
D2
D3
D4
D5
G.
R² = 0.2061
p = 0.0338
Δ Peak height difference score(D5-D1)
Δ Lever contact (D5-D1)
H.
NAc Core
Baseline
Cue
Reward
DA ΔF/F
Time from CS (s)
I.
NAc Shell
Baseline
Cue
Reward
DA ΔF/F
Time from CS (s)
J.
core
shell
Average AUC
CS
US
CS
US
Core
Shell

Figure 5

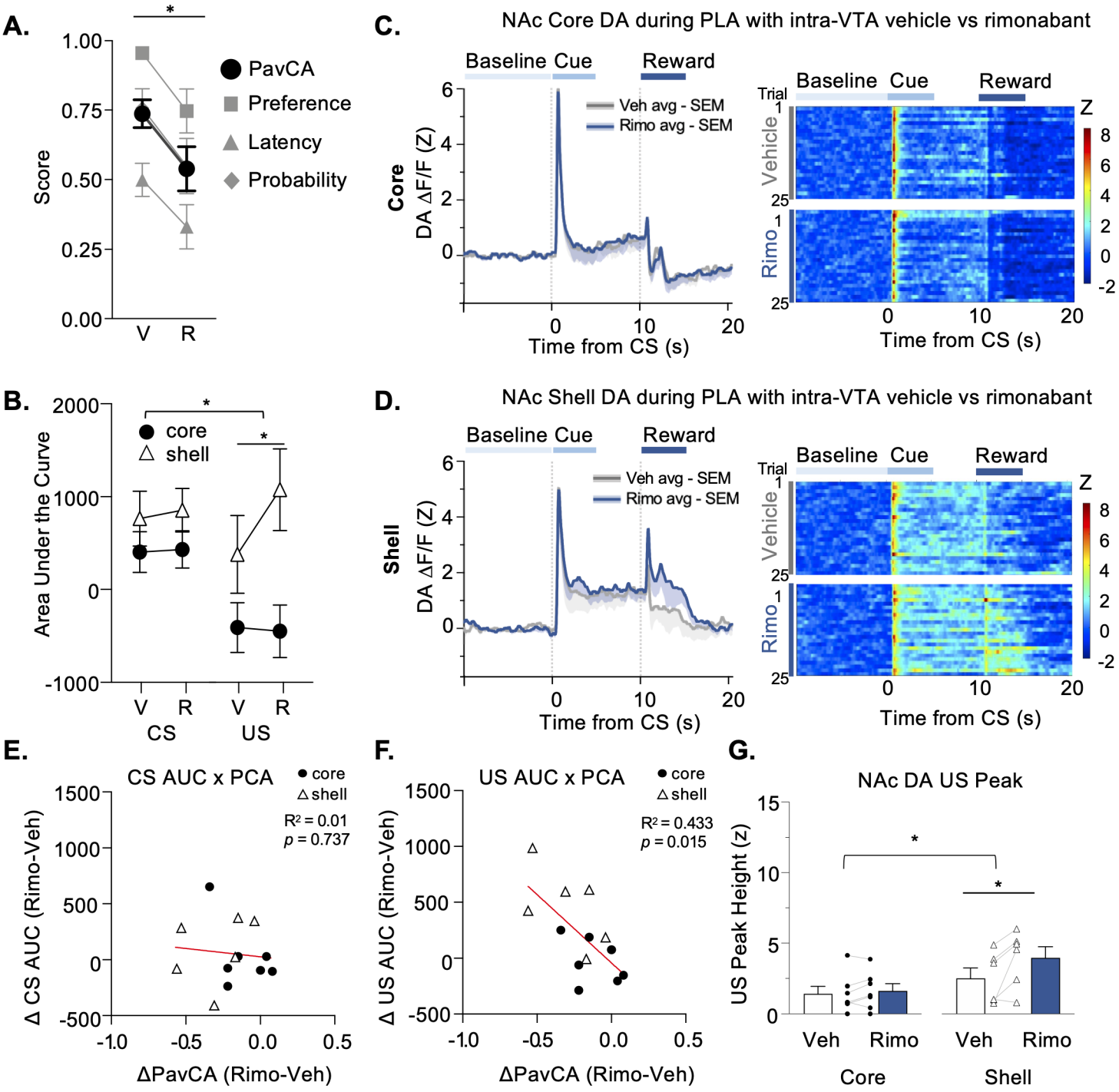

A.
*
1.00
0.75
0.50
0.25
0.00
Score
V
R
PavCA
Preference
Latency
Probability
B.
2000
1000
0
-1000
Area Under the Curve
core
shell
*
*
V
R
V
R
CS
US
C.
NAc Core DA during PLA with intra-VTA vehicle vs rimonabant
Baseline
Cue
Reward
Core
DA ΔF/F (Z)
Veh avg - SEM
Rimo avg - SEM
Time from CS (s)
Trial
Vehicle
Rimo
Z
D.
NAc Shell DA during PLA with intra-VTA vehicle vs rimonabant
Shell
E.
CS AUC x PCA
core
shell
R² = 0.01
p = 0.737
Δ CS AUC (Rimo-Veh)
ΔPavCA (Rimo-Veh)
F.
US AUC x PCA
R² = 0.433
p = 0.015
Δ US AUC (Rimo-Veh)
G.
NAc DA US Peak
US Peak Height (z)
Veh
Rimo
Core
Shell

Figure 6

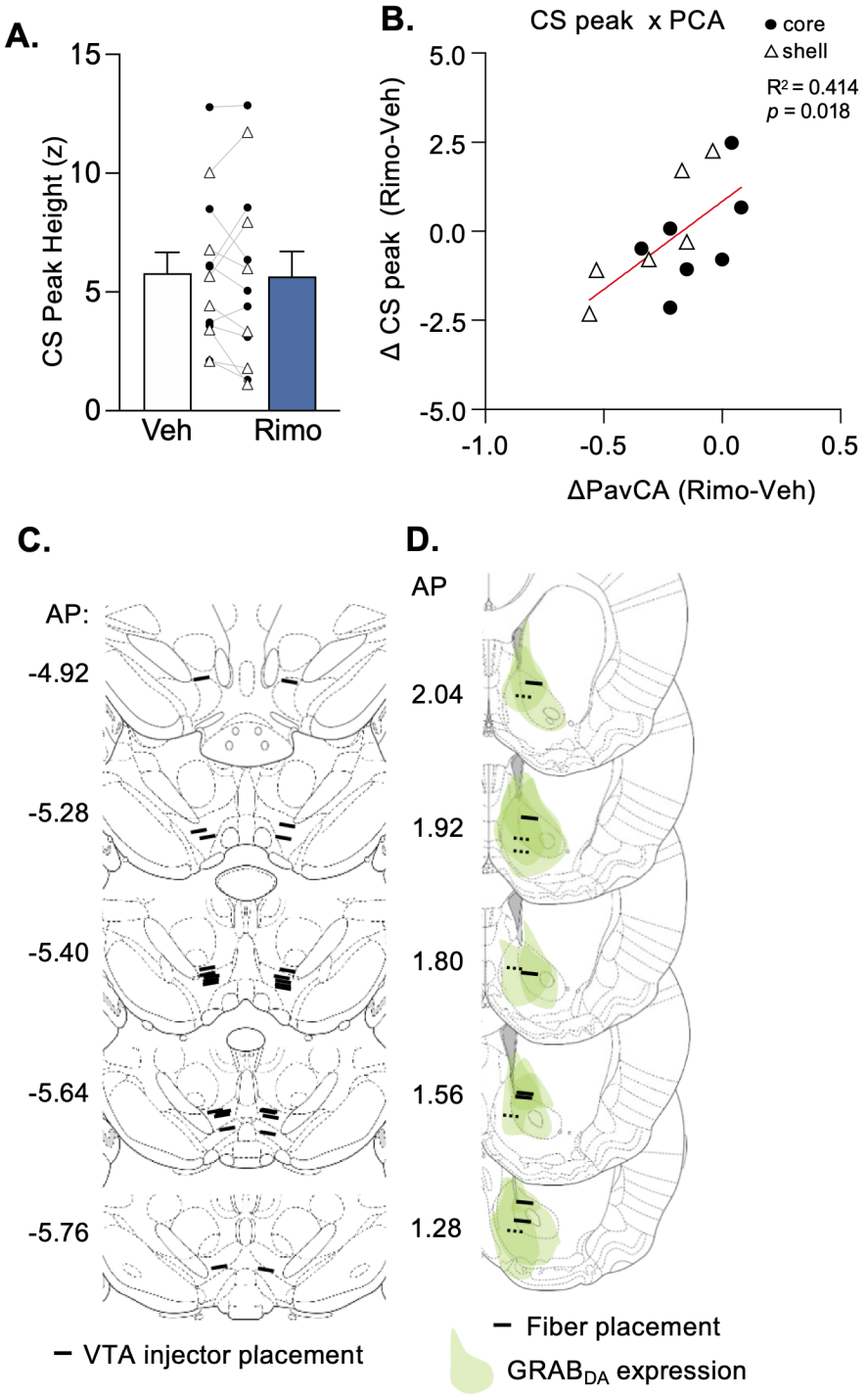

A.
CS Peak Height (z)
15
10
5
0
Veh
Rimo
B.
CS peak x PCA
core
shell
R² = 0.414
p = 0.018
Δ CS peak (Rimo-Veh)
5.0
2.5
0.0
-2.5
-5.0
-1.0
-0.5
0.0
0.5
ΔPavCA (Rimo-Veh)
C.
AP:
-4.92
-5.28
-5.40
-5.64
-5.76
VTA injector placement
D.
AP
2.04
1.92
1.80
1.56
1.28
Fiber placement
GRAB$_{DA}$ expression

Figure 7

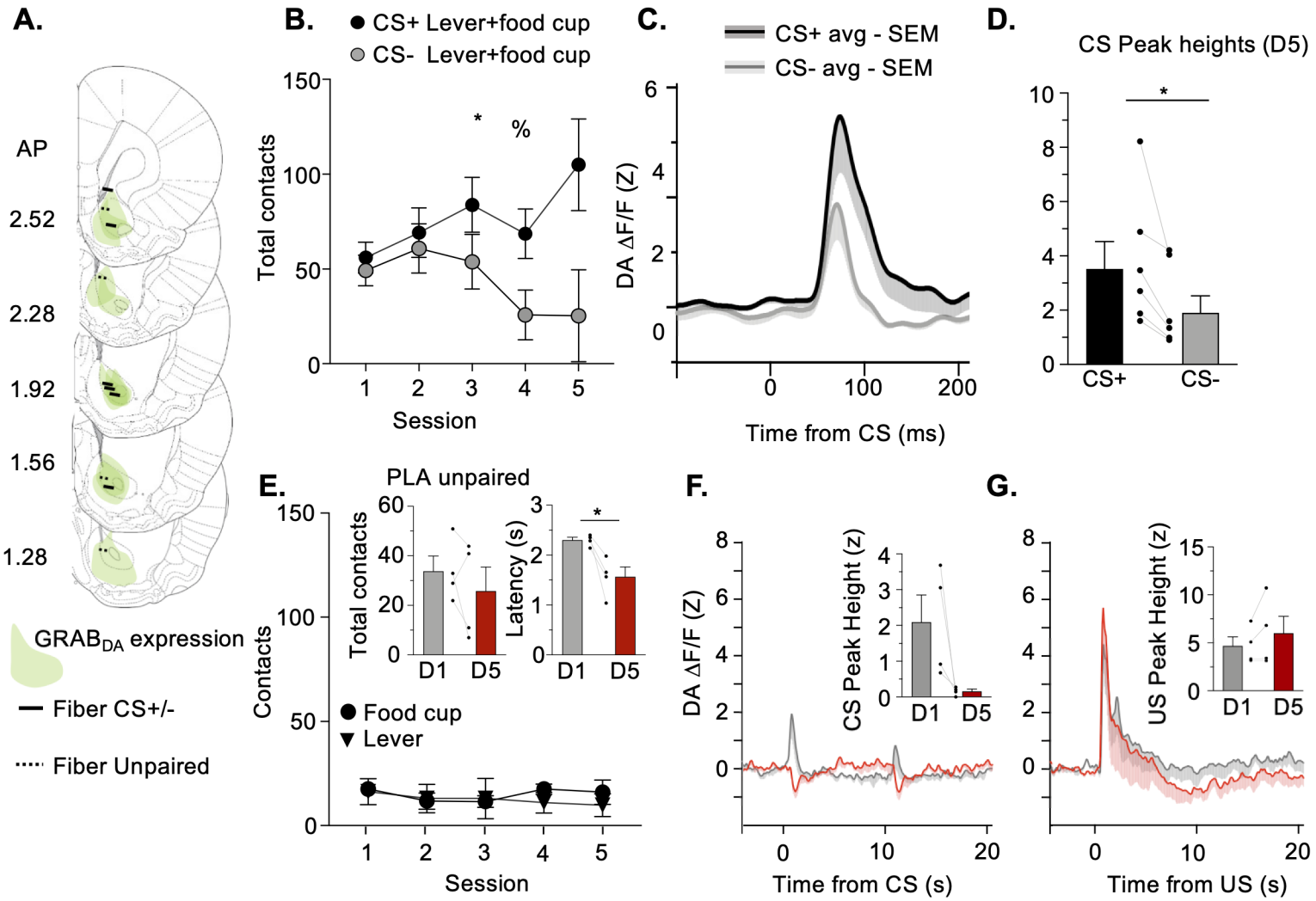

A.
AP
2.52
2.28
1.92
1.56
1.28
GRAB$_{DA}$ expression
Fiber CS+/-
Fiber Unpaired
B.
CS+ Lever+food cup
CS- Lever+food cup
Total contacts
Session
C.
CS+ avg - SEM
CS- avg - SEM
DA ΔF/F (Z)
Time from CS (ms)
D.
CS Peak heights (D5)
CS+
CS-
E.
PLA unpaired
Total contacts
Latency (s)
D1
D5
Contacts
Food cup
Lever
Session
F.
DA ΔF/F (Z)
CS Peak Height (z)
Time from CS (s)
G.
US Peak Height (z)
Time from US (s)

Table 1:

		Lever						Foodcup					
		Contact		Latency		Probability		Contact		Latency		Probability	
Factor	Degrees Of Freedom	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Session	(3,156)	59.965	<0.001	33.276	<0.001	35.963	<0.001	3.962	0.009	3.185	0.026	2.427	0.068
Tracking	(2,52)	43.241	<0.001	38.632	<0.001	80.645	<0.001	12.628	<0.001	22.588	<0.001	25.657	<0.001
Virus	(1,52)	1.537	0.221	0.125	0.726	0.875	0.354	0.383	0.539	3.198	0.08	3.831	0.056
Session x Tracking	(6,156)	23.015	<0.001	10.522	<0.001	10.116	<0.001	14.473	<0.001	13.420	<0.01	13.967	<0.01
Session x Virus	(3,156)	1.163	0.326	2.009	0.115	0.762	0.517	0.805	0.493	1.399	0.245	1.343	0.263
Tracking x Virus	(2,52)	0.299	0.743	0.236	0.791	0.182	0.834	1.081	0.347	1.694	0.194	2.306	0.110
Session x Tracking x Virus	(6,156)	0.887	0.506	1.070	0.383	1.078	0.378	0.791	0.578	0.484	0.820	0.470	0.830

Table 2

		Lever						Foodcup					
		Contact		Latency		Probability		Contact		Latency		Probability	
Effect	Degrees Of Freedom	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Session	(4,68)	9.649	<0.001	22.185	< 0.001	18.602	< 0.001	7.644	< 0.001	3.610	0.01	4.146	0.005
Tracking	(1,17)	0.763	0.395	0.475	0.500	2.052	0.170	17.49	<0.001	27.433	<0.001	25.23	<0.001
Session x Tracking	(4,68)	1.645	0.173	2.681	0.039	3.673	0.009	9.811	< 0.001	9.049	< 0.001	11.458	< 0.001