Prefrontal dopamine circuits are required for active avoidance learning but not for fear learning

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Abstract

The medial prefrontal cortex (mPFC) resolves approach-avoidance conflicts and mediates associative processes required for learning to avoid threats. Dopamine (DA) projections from the ventral tegmental area (VTA) to the mPFC carry information about aversive outcomes that may inform prefrontal computations. However, the role of prefrontal DA in learning based on aversive outcomes remains poorly understood. Here, we used platform mediated avoidance (PMA) to study the role of mPFC DA in threat avoidance learning in mice. We show that activity within dopaminergic VTA terminals in the mPFC is required for signaled avoidance learning, but not for escape, conditioned fear, or to recall a previously learned avoidance strategy. Taken together, these data indicate that mPFC DA is necessary to rapidly form associations between predictive cues and actions that preempt aversive outcomes but not for cue-outcome learning.

Introduction

To thrive in a complex environment, animals must seek beneficial outcomes while avoiding threats. Natural environments are highly dynamic, requiring individuals to continuously update predictive relationships between stimuli, actions, and outcomes. With respect to threats, this learning must occur rapidly and reliably to avoid injury or death. Excessive risk taking enhances mortality rates. Conversely, excessive avoidance can be maladaptive by limiting opportunities to engage in beneficial pursuits. Disorders featuring maladaptive avoidance are observed with both excessive and insufficient avoidance[1–4]. Thus, it is critical to understand the neurobiological mechanisms that mediate rapid threat avoidance learning.

The medial prefrontal cortex (mPFC) is essential for integrating learned information about the environment to guide actions, in particular when resolving approach-avoidance conflicts and when establishing action-outcome relationships[5–7]. Activity in mPFC is likewise critical for both learning to avoid threats and for driving avoidance behavior, especially in situations that involve conflicting motivational drives[8–16]. While much progress has been made in understanding the mPFC mechanisms that drive avoidance behavior, less is known about the prefrontal mechanisms underlying avoidance learning. In particular, the neural signals that drive mPFC circuit changes underlying avoidance learning remain largely unknown.

Mesocortical DA is poorly studied compared to mesolimbic DA circuits due to challenges associated with the lower density of cortical dopaminergic fibers as well as the lack of tools to monitor DA with high temporal resolution in isolation from other catecholamines[17]. Most VTA DA neurons targeting mPFC do not collateralize and represent a subpopulation with distinct molecular, cellular and functional properties[18–24]. In contrast to the reward-related functions of mesolimbic DA projections, VTA-mPFC projecting DA neurons preferentially respond to and orient behavior toward aversive stimuli[24–29]. VTA-mPFC dopaminergic axons exhibit minimal reward response but a substantial shock response[30]. Similarly, activating mPFC DA terminals during a competitive appetitive-aversive stimulus assay biased behavior toward aversive responding[31]. Phasic stimulation of VTA-mPFC DA terminals promoted deviation from previously

52 learned rules in a set-shifting task[32], highlighting a key role for mPFC DA in behavioral flexibility.
53 Altogether, there is a compelling role for mPFC DA in processing aversive stimuli and in
54 behavioral flexibility. But the role of mPFC DA in enabling the flexible behavior required to learn
55 appropriate behavioral responses to aversive stimuli remains unknown.

56 In this study, we utilized platform mediated avoidance[14,15] (PMA) to investigate the
57 hypothesis that activity within VTA-mPFC dopaminergic projections is critical for learning to
58 preemptively avoid aversive outcomes. We further hypothesized that these processes are distinct
59 from associative learning between a stimulus and an aversive outcome that lacks an action-
60 outcome contingency. Using a combination of terminal-specific optogenetic inhibition of VTA-
61 mPFC DA projections and distinct behavioral assays, we outline a critical role for mPFC DA
62 projections in threat avoidance learning that is independent of aversive associative learning.
63 These results suggest that DA modulates mPFC activity to rapidly and adaptively utilize predictive
64 cues to drive behaviors that preempt aversive outcomes.

65

66 **Results**

67 **Platform mediated avoidance learning**

68 Deciding to avoid potential threats often comes at the cost of rewarding behaviors and requires
69 mPFC activity[8,9,14]. To model threat avoidance under conditions of conflict, we trained mice in
70 platform mediated avoidance (PMA), in which a shock-conditioned tone prompts mice to stop
71 collecting rewards and navigate to a safety platform. We first trained thirsty mice that sweetened
72 condensed milk is available in the PMA chamber and then performed three days of PMA training
73 under these conditions. In this assay, the reward area was located on the opposite side of the
74 chamber from the safety platform (Figure 1A). On each day, nine 30-second tones each co-
75 terminated with a 2-second mild foot shock (Figure 1B) and we video recorded mouse behavior
76 from above, using supervised deep learning to track eight points on their body[33] (Figure 1C).
77 Successful trials were classified as trials in which mice accessed the safety platform before shock

78 onset and remained there for the duration of the shock. Mice steadily increased the percent of
79 successful trials across three days of PMA training (Figure 1D). They also steadily increased the
80 percent time spent on the safety platform during each tone (Figure 1E). Freezing behavior during
81 the tones was low and did not change significantly across days (Figure 1F). Thus, mice first learn
82 to associate a cue with a foot shock and subsequently learn to preemptively avoid footshocks in
83 the context of a competing opportunity to receive rewards.
84
85 **Activity in dopaminergic terminals in mPFC is required for rapid avoidance learning**
86 To determine if activity in VTA-mPFC dopaminergic axon terminals is required for avoidance
87 learning, we employed an optogenetic approach to inhibit axon terminals during tone+shock
88 presentations across three days of PMA training. First, we injected an AAV encoding the Cre-
89 conditional inhibitory opsin Jaws[34] or a control fluorophore (GFP) into the VTA of TH-Cre mice. In
90 these mice, the bacterial recombinase Cre is expressed under the control of the promoter for
91 tyrosine hydroxylase – an enzyme necessary for dopamine synthesis – so Jaws expression is
92 restricted to dopaminergic VTA neurons. Then we placed bilateral optic fibers over mPFC (Figure
93 2A). In these mice, we observed a subset of brightly labeled GFP+ neurons in VTA and GFP+
94 axons in mPFC (Figure 2B). We then trained mice in PMA as described above. During each tone
95 (including during the shock period), we inhibited VTA-mPFC dopaminergic axon terminals using
96 constant 635 nm laser light delivered through bilateral head mounted optic fibers (Figure 2C).
97 We analyzed mouse behavior across the three days of PMA to determine whether
98 disrupting activity in VTA-mPFC DA terminals affected conditioned freezing behavior, a measure
99 of the tone-shock association, and platform mediated avoidance behavior, a measure of the
100 action-outcome association. First, we analyzed the fraction of successful trials when mice
101 preemptively avoided foot shocks (Figure 2D). Inhibiting activity in VTA-mPFC DA terminals
102 caused a significant slowing of avoidance learning. Mice expressing Jaws had significantly fewer

successful trials than GFP control mice, especially on the first day and the beginning of the second day of PMA (Figure 2E).

Interestingly, Jaws mice had a significantly higher proportion of escape trials (Figure 2F) when they leaped to the platform once the shock began. The biggest effects were apparent early in learning, at the end of day 1 and the beginning of day 2. VTA-mPFC DA terminal inhibition had no effect on freezing behavior in the context of PMA (Figure 2G). In the same mice, we showed that inhibiting VTA-mPFC DA terminals did not induce conditioned place preference or aversion (Figure S1). Together, these data reveal that VTA-mPFC activity is most important in the early stages of learning. Instead of preventing learning, our findings suggest that inhibiting mPFC DA activity slows the formation of the predictive relationship between the avoidance action and the aversive outcome without affecting the formation of the association between the tone and the shock.

Even though Jaws mice could eventually learn PMA, we wondered if they developed qualitative differences in approach and avoidance behaviors as a result of inhibiting VTA-mPFC DA activity during learning. To examine this, we quantified the patterns of behavior during tone presentations, including interactions with the platform and with the reward port. Jaws animals exhibited fractured avoidance behavior, spending significantly shorter bouts of time on the platform overall and in particular during the tone period (Figure 2H,I). Both groups of mice exhibited a behavioral strategy in which they infrequently triggered the reward during the tone period and but did so more frequently during the inter-tone intervals, when threat levels were lower. We observed no differences in reward port interactions during the tone period (Figure 2K), but Jaws mice had significantly more interactions with the reward port than controls during the inter-tone interval (ITI) (Figure 2J). Together, these data indicate that inhibition of VTA-mPFC DA terminals does not alter cue-shock associative learning, nor does it prevent learning about the location of the safety platform. Instead, the data indicate that VTA-mPFC inhibition impairs the ability to use that information to guide preemptive avoidance behavior.

129

130 **VTA-mPFC activity is required for rapid avoidance learning even without motivational**
131 **conflict**

132 To determine if the effects we observed were specific to 1) avoidance under conflict and 2) threat
133 avoidance learning vs. memory retrieval, we performed additional optogenetics experiments in
134 separate groups of mice. First, we tested whether VTA-mPFC activity was necessary during a
135 PMA assay without a reward conflict. As before, we injected an AAV expressing Cre-dependent
136 Jaws or GFP into the VTA of TH-Cre mice and implanted bilateral optic fibers above mPFC (Figure
137 3A). Because we observed the largest effects of inhibition during the first day of PMA, we
138 performed a single day of PMA training during which 12 tone-shock trials were paired with delivery
139 constant 635 nm light to mPFC and no reward was available. As before, we observed that Jaws
140 mice had a significant decrease in the fraction of successful trials, a significant increase in escape
141 trials, and no change in the percent time freezing during the tone (Figure 3C), suggesting that
142 mPFC DA activity is still required for learning to avoid threats even without a motivational conflict
143 .

144 To determine to what extent VTA-mPFC dopaminergic projection activity is necessary for
145 avoidance behavior versus for learning associations between conditioned stimuli and adaptive
146 behaviors, we inhibited VTA-mPFC DA terminals during PMA learning and then examined
147 avoidance behavior during a PMA memory retrieval session the following day. During retrieval,
148 mice were presented with 10 tones without foot shocks or laser inhibition. Jaws mice spent
149 significantly less time on the safety platform during tones compared to controls (Figure 3D),
150 indicating the VTA-mPFC DA terminal inhibition impaired learning as opposed to preventing the
151 expression of avoidance behavior. We observed no group differences in conditioned freezing
152 during the tones, indicating further that VTA-mPC DA terminal activity is not needed to learn the
153 tone-shock association.

154

155 **VTA-mPFC activity is not required to maintain a learned avoidance strategy**

156 We next investigated whether inhibiting VTA-mPFC axon terminals would affect PMA retrieval
157 after learning had occurred (Figure 3E). We trained mice in PMA (without conflict) without laser
158 inhibition (Figure 3F). The next day, mice had a PMA retrieval session. In this case, half the tones
159 were paired with laser inhibition and laser on and off trials were randomly interleaved. This
160 manipulation had no effect on the percent time on the platform or percent time spent freezing
161 during the tones (Figure 3G), supporting the idea that VTA-mPFC activity is only required during
162 the early stages of avoidance learning to enable the mPFC to associate action-outcome
163 contingencies.

164

165 **Activity in dopaminergic terminals in mPFC is not required for cued fear conditioning**

166 We next sought to determine which aspects of VTA-mPFC DA terminal activity are specific to
167 PMA, which requires animals to first learn that a tone predicts a shock (cue-outcome contingency)
168 and then to learn that during the tone, they can enter the platform to avoid the foot shock (action-
169 outcome contingencies). To do so, we examined the role of mPFC DA terminals in cued fear
170 conditioning, which features similar tone-shock associative learning without any action-outcome
171 contingency. To test the role of VTA-mPFC DA terminals in cued fear conditioning, we injected
172 an AAV expressing cre-dependent Jaws or a fluorophore control (GFP) into VTA of TH-Cre mice
173 and implanted optic fibers bilaterally above mPFC (Figure 4A). On the training day, we paired
174 tones with continuous delivery of far-red light through the optic fibers in mPFC. After three
175 baseline tones, 12 subsequent tones were paired with a 2-second footshock (identical to PMA).
176 On the test day, mice were presented with six tones without shocks or laser illumination (Figure
177 4B). On the training day, mice in both groups significantly increased their freezing across the
178 session, but we observed no difference between the GFP and Jaws groups (Figure 4C). On the
179 test day, there was no difference in the time spent freezing during tone presentation (Figure 4D).

180 Together, these results indicate that VTA-mPFC activity is not required to learn the association
181 between cues and shocks.
182
183 **Discussion**
184 Here we discovered that VTA-mPFC DA activity is critical for rapidly learning to avoid aversive
185 outcomes in PMA. On the other hand, activity in the same projection is not required for developing
186 a conditioned freezing response during auditory fear conditioning, indicating that activity in this
187 pathway is not required for establishing cue-outcome relationships. These findings extend our
188 understanding of the role of VTA-mPFC DA projections beyond aversive processing and
189 behavioral flexibility to include a causal function in learning the predictive relationships between
190 cues, actions and aversive outcomes.
191 The mPFC is heavily implicated in mediating approach-avoidance conflicts[8,9,9,11,15] and in
192 the recall and expression of cued fear[35–40], but the mechanisms that drive the circuit plasticity
193 required for learning to avoid aversive outcomes are poorly understood. DA signaling plays a
194 critical role in aversive learning but has mostly been studied without regional specificity and/or in
195 the context of fear conditioning[41]. While fear conditioning paradigms involve associative learning
196 between a stimulus and an aversive outcome, they lack the possibility of taking action to avoid
197 the shock, i.e. action-outcome contingencies, which require mPFC activity[42–44]. Studies that used
198 midbrain lesions, global pharmacological inhibition of DA signaling and microdialysis identified a
199 key role for DA in threat avoidance behaviors[45–47]. While these studies built a critical foundation
200 of knowledge, the approaches lack the spatiotemporal precision to reveal the causal role of mPFC
201 DA in avoidance behavior. Previous research using microdialysis lacked the temporal resolution
202 to align mPFC DA with cues and behavior, while other research using voltammetry could not
203 separate DA from fluctuations in other catecholamines (e.g., norepinephrine)[17]. Therefore, the
204 nuanced relationship between VTA-mPFC DA activity, threatening cues, and threat-induced
205 behaviors, and how these relationships may change across learning was not understood. Using

206 temporally precise manipulations, we showed that inhibiting VTA-mPFC DA terminals delayed the
207 acquisition of avoidance behavior without affecting the learning or recall of the cue-shock
208 relationship.

209 While VTA-mPFC DA activity supports behavioral flexibility in some scenarios[27,32,48–52], we
210 do not find evidence that inhibiting VTA-mPFC DA activity slows avoidance learning by impairing
211 behavioral flexibility. In support of this idea, Jaws mice did not exhibit elevations in competing
212 behaviors - including freezing and collecting rewards - during conditioned tones. Instead, Jaws
213 mice exhibited fractured avoidance behavior - once they entered the safety platform, they left
214 more quickly than controls. These data suggest that reducing mPFC DA does not impair
215 deviations from existing behavioral schemes. Instead, mPFC DA likely biases prefrontal
216 computations to rapidly adopt behavioral strategies that prioritize actions that preempt aversive
217 outcomes, including entering and then staying on the platform until the end of the shock-
218 conditioned tone.

219 Interestingly, Jaws mice collected more rewards than GFP mice during the ITI even though
220 no laser manipulation was present. Over the course of learning, control mice had slightly longer
221 platform bouts during the ITI, suggesting they prioritized safety seeking over reward seeking even
222 when no overt threats were present. One interpretation of these findings is that Jaws mice have
223 less generalized fear than controls. If true, this would suggest that while mPFC DA activity is
224 critical for learning the predictive relationships between cues, actions and outcomes, it also
225 promotes generalization of conditioned fear outside the cue periods.

226 Our study causally manipulated the activity of VTA-mPFC terminals during presentations
227 of tones, foot shocks, and foot shock omissions on successful trials. Since we performed these
228 experiments in TH-Cre mice, the effects we observed were likely related to DA activity. However,
229 recent studies showed that a subset of TH+ VTA cells also co-express the vesicular glutamate
230 transporter VGLUT2[53] and mesocortical DA neurons may co-release glutamate[54]. A more
231 complete understanding of how these projections drive plasticity will require temporally precise

232 manipulations during isolated events (e.g. only during shocks), examination of how activity arising
233 from VTA-mPFC terminals influences mPFC microcircuit activity, and investigation of to what
234 extent observed effects arise from DA vs. other neurotransmitters.

235 Protracted disturbances in the balance of reward seeking vs. avoidance is a hallmark of
236 numerous psychiatric disorders[3,55,56]. Environmental factors (e.g., stress or trauma) can promote
237 maladaptive learning that precipitates pathological avoidance[57–60]. Primary treatments include
238 cognitive behavioral therapy and drugs whose targets cluster in neuromodulatory systems,
239 including in the dopamine system. But once instantiated, excessive avoidance can interfere with
240 therapy and exacerbate features of psychiatric illness[61]. Establishing a mechanistically detailed
241 and temporally specific understanding of the role of mPFC DA in specific types of learning
242 promises to enable more precise interventions that combine pharmacologic, brain stimulation
243 and/or psychotherapeutic modalities during precise windows of vulnerability or capacity for
244 change. Therefore, it is critical to understand the neurobiology of avoidance learning if we are to
245 effectively intervene on psychiatric disorders.

246

247 **Materials and Methods**

248 All experiments were conducted in accordance with procedures established by the administrative
249 panels on laboratory animal care at the University of California, Los Angeles.

250

251 **Animals** Male and female C57Bl6/J mice (JAX stock #000664) or tyrosine hydroxylase Cre (TH-
252 Cre; line FI12; www.gensat.org) mice were group housed (two to five per cage) and kept on a
253 12/12 h light/dark cycle (lights on 7AM to 7PM). Mice were sexed by examination of external
254 genitalia at P21. Animals received *ad libitum* food and water.

255

256 **Surgery** Mice were induced in 5% isoflurane in oxygen until loss of righting reflex and transferred
257 to a stereotaxic apparatus where they were maintained under 2% isoflurane in oxygen. Mice were

258 warmed with a circulating water heating pad throughout surgery and eye gel was applied to the
259 eyes. The head was shaved and prepped with three scrubs of alternating betadine and then 70%
260 ethanol. Following a small skin incision, a dental drill was used to drill through the skull above the
261 injection targets. A syringe pump (Kopf, 693A) with a Hamilton syringe was used for injections.
262 Injections were delivered at a rate of 100 nl/min and the syringe was left in the brain for 10 min
263 following injection. For optogenetics experiments, animals were injected with 500nL of AAV-CAG-
264 FLEX- JAWS-KGC-GFP-ER2 or AAV5-CAG-FLEX-EGFP-WPRE at a titer of $5x10^{12}$ vg/mL
265 bilaterally into the VTA (AP: -2.9 mm, ML: +/- 0.45 mm, DV: -4.5 mm from bregma). Bilateral optic
266 fibers (200 µm core, Newdoon) were implanted over the prelimbic (PL) area of the mPFC (AP:
267 +1.8, ML: +/- 0.35, DV: -1.8 mm from bregma). For pain management mice received 5 mg/kg
268 carprofen diluted in 0.9% saline subcutaneously. Mice received one injection during surgery and
269 daily injections for 2 d following surgery. Optogenetic experiments waited at least four weeks after
270 surgery before experimentation.
271
272 **Behavior**
273 *Platform mediated avoidance*
274 Three days prior to starting PMA, citric acid (2% w/v) was added to animals' drinking water. This
275 self-initiates a reduction in water consumption and increases performance on liquid reward tasks
276 similar to forced water restriction[62]. Animals were then placed into a 20x22cm arena with metal
277 rod flooring (Lafeyette Instruments) and a plexiglass platform covering one-quarter of the floor
278 space. Opposite the platform was a reward port and reward pump (Campden Instruments). The
279 reward port featured an infrared beam that triggered 25 µL of 10% sweetened condensed milk
280 (Nestle) reward on a variable interval schedule (mean interval: 30 seconds). Once a day for two
281 days, animals were placed in the arena and allowed to freely explore and consume reward for 45
282 minutes. The following day, PMA training began. For three days, animals were placed into the
283 arena with a 180 second baseline period followed by delivery of a 30 second, 75 dB, 4kHz tone

that co-terminated with a 2 second, 0.15 mA shock delivered to the floor (Lafeyette Instruments). Eight subsequent tone-shock pairings were delivered at randomly chosen intervals between 80 and 160 seconds. Training on PMA continued for two more days for a total of three days. Data presented in tone tone blocks are the average performance over three tones.

Cued fear conditioning

Experimentally naïve animals were placed in the same arena as PMA without the platform or reward port. On training day, animals received three, 10 second 4 kHz tones to assess baseline freezing. The subsequent twelve tones were paired with a footshock identical to PMA. The following day (recall), animals were exposed to six, 30 second 4 kHz tones unpaired with shock.

Optogenetics

JAWS activation was achieved via continuous red light (635 nm, Shanghai Laser and Optics Century) delivered through the entirety of the tone-shock periods. For PMA, laser illumination was concurrent with all the tones. For fear conditioning, this occurred on the first day (training).

Real-time place preference

After PMA, a subset of animals were placed in a real-time place preference (RTPP) assay. Animals were placed in a 68 x 23 cm chamber for 20 minutes. During the first ten minutes, the animals freely explored the arena to establish a baseline side preference. During the subsequent ten minutes, a closed loop video monitoring system (Bioviewer) tracked the location of the animal and triggered a 635 nm laser when the animal entered the “laser” side of the arena. The ratio of time spent in the “non-laser” side of the arena to the “laser” side of the arena was calculated for both baseline (laser off) and test (laser on) periods.

Histology Animals were deeply anesthetized with isoflurane and transcardially perfused with phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Brains were removed and post-fixed overnight in 4% PFA before being moved to PBS. Brains were then sectioned via vibratome at 60 microns. To detect VTA axons expressing JAWS-GFP in the mPFC, anti-GFP immunohistochemistry was performed. Sections were incubated with chicken anti-GFP polyclonal antibody (1:2000, Aves Labs) overnight at 4℃. Sections were next rinsed and incubated with donkey anti-chicken AlexaFluor 488 conjugate (1:500; Jackson ImmunoResearch) at room temperature for 2 hr. Sections were washed before being mounted with DAPI. Images of fluorescent expression and implant targeting were taken using a Leica DM6 scanning microscope (Leica Microsystems) using a 10x objective.

Data Analysis

Behavioral Analysis

High resolution, overhead videos of PMA and fear conditioning were collected at 50 Hz (Teledyne FLIR) and animal positioning extracted via supervised deep learning networks (cite DLC). Animal position information was transformed into freezing and ROI features using BehaviorDEPOT software[63].

Statistical analysis

Modeling was performed with custom Python code. All other statistical testing was performed in Graphpad Prism.

Data and code availability

Custom MATLAB code available upon request.

Acknowledgements

We thank Dr. Peter Balsam for helpful discussions of this work. This work was supported by a Klingenstein-Simons Fellowship in Neuroscience, a Whitehall Foundation Research Grant and 1R01MH127214-01A1 (LAD), a Whitehall Foundation Research Grant and 1R01MH131858-01A1 (SAW), T32NS115753 and F31MH133387 (ZEZ) and T32NS115753 (TG).

Author Contributions

LAD, SAW, ZEZ, MFG and TG conceptualized the experiments. ZEZ, MFG and MS performed experiments. TG analyzed behavioral data. LAD, SAW and ZEZ wrote the manuscript. All authors edited the manuscript.

Declaration of Interests

The authors declare no competing interests.

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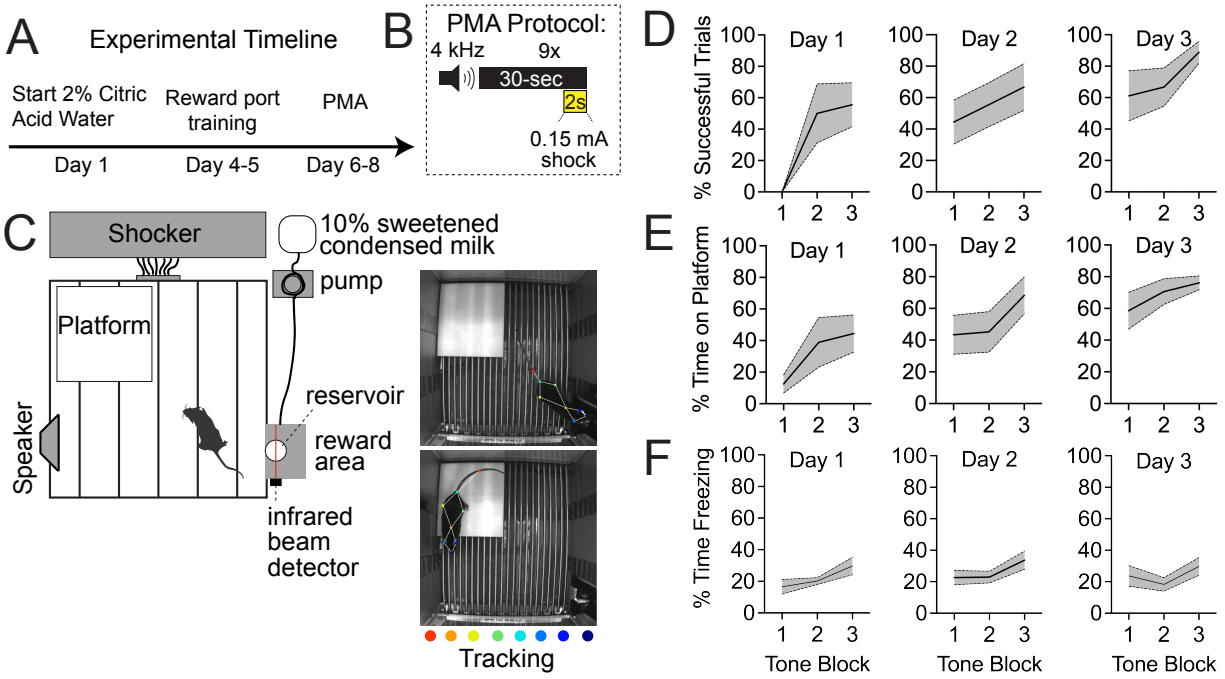

Figure 1. Mice learn to perform a platform mediated avoidance assay.
A. Experimental timeline.
B. Behavioral training protocol.
C. Schematic of PMA chamber (left) and frames from videos (right).
D-F. Behavior across 3 days of PMA. On each day, data is analyzed in bins of 3 tones.
D. Successful trials ($F_{Trial}(1.27,6.34)=17.99$, $p=0.004$; $F_{Day}(1.96,9.79)=7.64$, $p=0.01$; n=6 mice).
E. Time on platform during tones ($F_{Trial}(1.37,6.82)=21.03$, $p=0.002$; $F_{Day}(1.74,8.71)=8.24$, $p=0.01$; n=6 mice).
F. Freezing during tones ($F_{Trial}(1.72,8.61)=1.31$, $p=0.31$; $F_{Day}(1.38,6.9)=1.83$, $p=0.22$; n=6 mice).

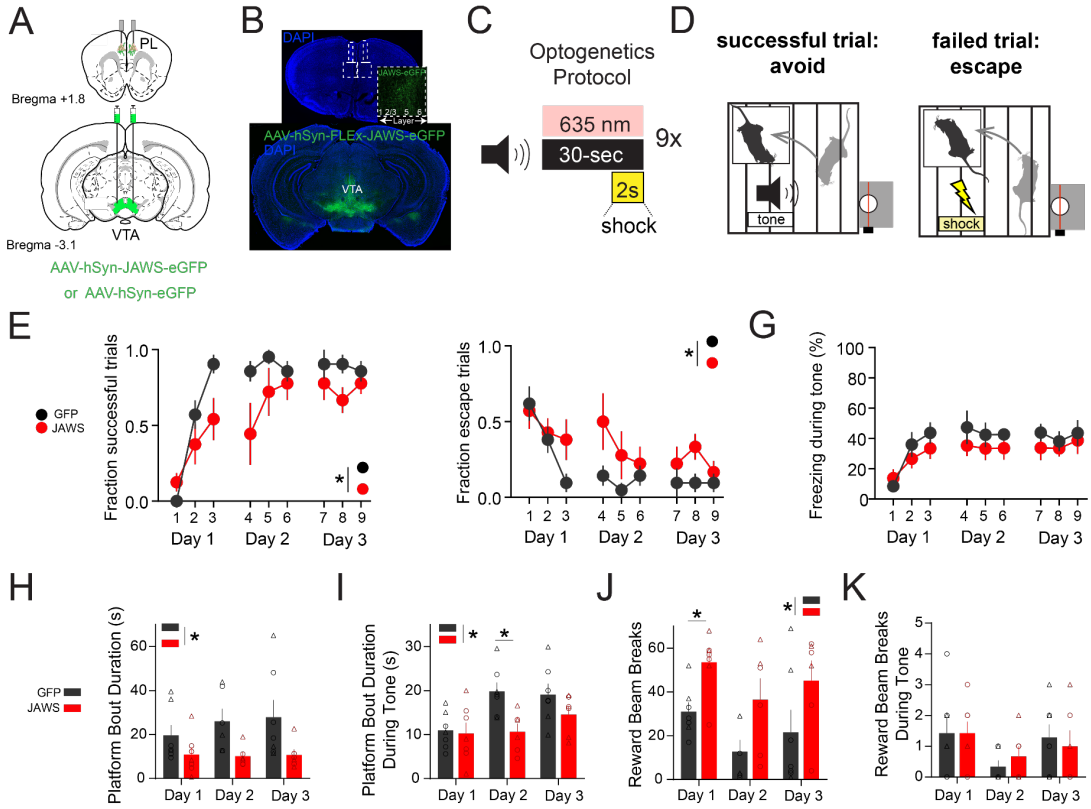

Figure 2. Optogenetic inhibition of DAergic axon terminals in mPFC impairs avoidance learning.
A. Schematic of viral and fiberoptic targeting locations.
B. Coronal sections from a representative brain showing Jaws-eGFP expression in VTA and bilateral fiber placement in PL.
C. Optogenetic inhibition during PMA. 635nm laser light was presented coincidently with a 30 second 4kHz tone that co-terminated with a 2-second footshock.
D. Schematics showing a successful trial when mice preemptively avoided the shock vs. an escape trial when mice leaped to the platform after shock begins.
E. Fraction of successful trials across days in GFP vs. Jaws mice ($F_{time}(4.26, 49) = 17$, $P<0.0001$; $F_{opsin}(1, 13) = 5.66$, $p=0.03$; $F_{interaction}(8, 92) = 2.197$, $p=0.06$).
F. Fraction of escape trials across days in GFP vs. Jaws mice (F_{time} $(4,43, 49.86) = 5.43$, $p=0.0007$; F_{opsin} $(1, 13) = 4.78$, $p=0.04$; $F_{interaction}$ $(8, 90) = 1.04$; $p=0.4$).
G. Fraction time freezing during tone across days in GFP vs. Jaws mice (F_{time} $(4.32, 46.98) = 8.41$, $P<0.0001$; F_{opsin} $(1, 12) = 1.4$, $p=0.2$; $F_{interaction}$ $(8, 87) = 1.01$; $p=0.4$).
H. Platform bout duration across days in GFP vs. Jaws mice ($F_{time}(1.88, 19.83) = 0.5$, $p=0.5$; $F_{opsin}(1, 12) = 6.8$, $p=0.02$; $F_{interaction}(2, 21) = 0.7$, $p=0.4$).
I. Platform bout duration during tone periods across days in GFP vs Jaws mice ($F_{time}(4.26, 49) = 17$, $p=0.005$; $F_{opsin}(1, 13) = 5.66$, $p=0.04$; $F_{interaction}(8, 92) = 2.197$, $p=0.06$; GFP vs. Jaws Day 1 $p = 0.9$, Day 2 $= 0.02$, Day 3 $= 0.4$.
J. Number of reward beam breaks across days in GFP and Jaws mice ($F_{time}(1.9, 19.9) = 4.8$, $p=0.01$; $F_{opsin}(1, 12) = 8$, $p=0.01$; $F_{interaction}(2, 21) = 0.02$, $p=0.97$; GFP vs Jaws Day 1 $p=0.01$; Day 2 $p=0.1$; Day 3 $p=0.3$).
K. Number of reward beam breaks during the tone period across days in GFP and Jaws mice ($F_{time}(1.9, 32.1) = 2.5$, $p=0.09$; $F_{opsin}(1, 33) = 5.66$, $p=0.9$; $F_{interaction}(2, 33) = 0.2$, $p=0.7$). E-K statistical testing performed with a mixed effects model, n=7 GFP, n=7-8 Jaws.
Values are mean +/- SEM. Posthoc testing performed with Tukey's test.

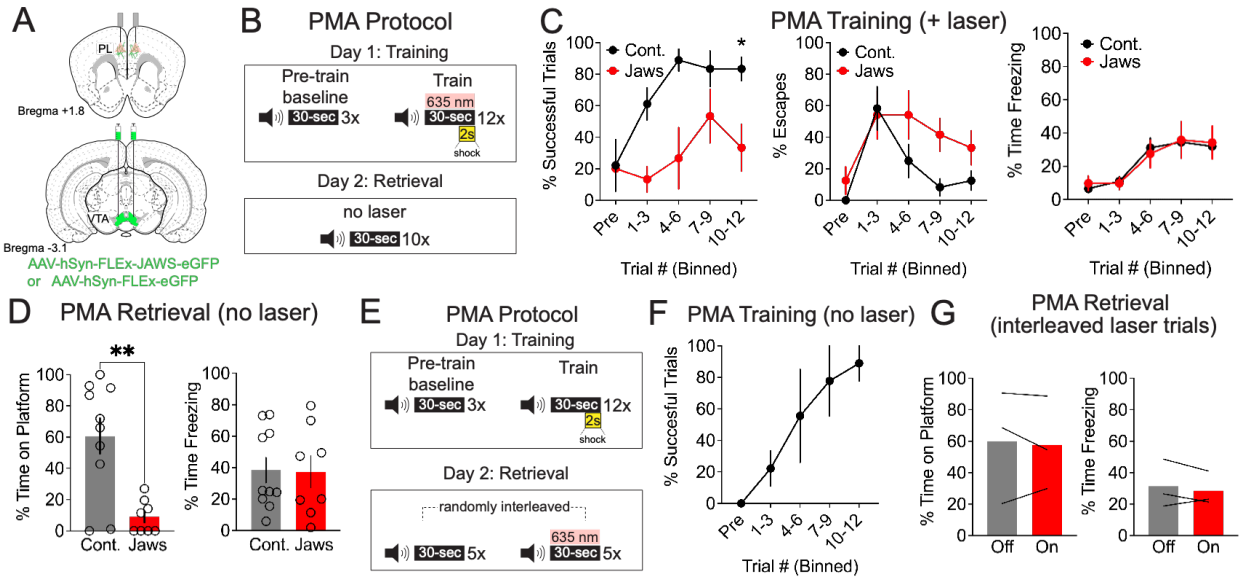

Figure 3. Optogenetic inhibition of VTA-mPFC DA terminals during PMA without motivational conflict.

A. Schematic of viral and fiber optic targeting locations.

B. Protocol for optogenetic inhibition during PMA training. On training day, 635nm laser light was presented coincidently with a 30 second 4kHz tone that co-terminated with a 2-second footshock. On retrieval day, mice were presented with 10 tones without shocks or laser.

C. Fraction of successful trials across days in control vs. Jaws mice ($F_{time}(4.323, 49.71) = 16.66$, $P<0.0001$; $F_{opsin}(1, 13) = 5.092$, $p=0.04$; $F_{interaction}(8, 92) = 2.197$, $p=0.03$; Mixed effects model, N=7 Jaws, N=8 control). Fraction of escape trials across days in control vs. Jaws mice (F_{time} $(3.975, 44.71) = 2.33$, $p=0.07$; F_{opsin} $(1, 12) = 1.68$, $p=0.22$; $F_{interaction}$ $(8, 90) = 3.09$; $p=0.004$. Mixed effects model, N=7 Jaws, N=8 control mice). Fraction time freezing during tone across days in control vs. Jaws mice (F_{time} $(4.39, 46.6) = 8.39$, $P<0.0001$; $F_{opsin}(1, 11) = 0.60$ $p=0.46$; $F_{interaction}$ $(8, 85) = 0.88$; $p=0.54$. Mixed effects model, N=7 Jaws, N=7 control mice).

D. Fraction time on platform and fraction of time freezing during tones on retrieval day (platform: $p=0.002$, freezing: $p=0.6$, two-tailed t-test, n=10 control, N=8 Jaws mice).

E. Protocol for optogenetic inhibition during PMA retrieval. Mice are trained in PMA without laser inhibition. The next day, mice are presented with 10 randomly interleaved tones, half of which are paired with constant 635 nm light.

F. Successful trials during PMA training.

G. Percent time on platform and percent time freezing during the tone on interleaved light on and light off trials.

*$p<0.05$, **$p<0.01$. Plots show mean +/- SEM.

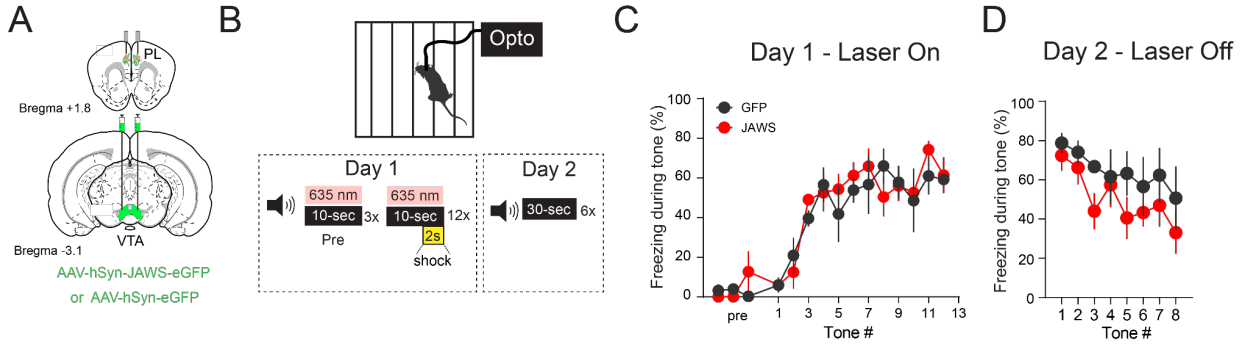

Figure 4. mPFC DA reflects but is not required for associative learning in cued fear conditioning.
A. AAV injection and fiber placement for optogenetic inhibition of VTA-mPFC axon terminals.
B. Experimental protocol.
C. Freezing during fear conditioning did not differ between GFP and Jaws groups. Two-way repeated measures ANOVA (F_{time} (4.7, 33.3)=18.2, P<0.001; F_{opsin} (1, 7)=0.6, p=0.4; $F_{interaction}$ (14, 98)=0.5, p=0.9).
D. Freezing during fear memory retrieval did not differ between GFP and Jaws groups. Two-way RM ANOVA (F_{time} (2.8, 20.2)=5.5, p=0.006; F_{opsin} (1, 7)=1.2, p=0.2; $F_{interaction}$ (5, 35)=0.8, p=0.5).
* P<0.05, ** P<0.01.