Prefrontal dopamine activity is critical for rapid threat avoidance learning

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Abstract

The medial prefrontal cortex (mPFC) is required for learning associations that determine whether animals approach or avoid potential threats in the environment. Dopaminergic (DA) projections from the ventral tegmental area (VTA) to the mPFC carry information, particularly about aversive outcomes, that may inform prefrontal computations. But 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 in VTA-mPFC dopaminergic terminals is required for avoidance learning, but not for escape, conditioned fear, or to recall a previously learned avoidance strategy. mPFC DA is most dynamic in the early stages of learning, and encodes aversive outcomes, their omissions, and threat-induced behaviors. Computational models of PMA behavior and DA activity revealed that mPFC DA influences learning rates and encodes the predictive relationships between cues and adaptive behaviors. Taken together, these data indicate that mPFC DA is necessary to rapidly learn behaviors required to avoid signaled threats, but not for learning cue-threat associations.

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. Threat learning must occur rapidly and reliably to avoid injury or death. But excessively avoiding threats can also limit opportunities to engage in beneficial pursuits. Thus, appropriately balancing approach and avoidance in a threatening context is critical. Moreover, disturbances in this balance can lead to maladaptive behaviors characteristic of psychiatric disorders. Thus, it is critical to understand the neurobiological mechanisms mediating 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

encoding contextual associations with threat and safety[1–7]. 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 the process of learning these avoidance learning.

Dopamine (DA) is a potent modulator of mPFC function yet is poorly understood in comparison with the mesolimbic DA system[17]. Prefrontal DA has been difficult to study because of the lower density of dopaminergic fibers as well as the lack of tools to monitor DA with high temporal resolution in isolation from other catecholamines[18]. DA axons targeting mPFC arise from the ventral tegmental area (VTA)[19–22]. VTA-mPFC DA neurons do not collateralize and represent a subpopulation with distinct molecular, cellular, and functional properties[23–29]. In contrast to the reward-related functions of mesolimbic DA projections, VTA-mPFC projecting DA neurons and their terminals are preferentially engaged by aversive stimuli[17,20,29–34]. DA is known to play a critical role in aversive learning but has mostly been studied without regional specificity and/or in the context of conditioned fear[35–38]. Some studies suggest that DA plays a key role in threat avoidance, but most used midbrain lesions, global pharmacological inhibition of DA signaling, or microdialysis[39–42]. These approaches lack the spatiotemporal precision required to associate mPFC DA transients with distinct epochs of learning or cannot provide causal information, leaving substantial gaps in our understanding of the role of mPFC DA in learning active strategies to avoid threats.

In this study, we utilized platform mediated avoidance (PMA)[14,15] to investigate the hypothesis that the activity of VTA-mPFC dopaminergic projections is critical for learning to avoid aversive outcomes. We further hypothesized that this relationship is distinct from stimulus-outcome associative learning that lacks a learned behavioral response. To test this, we combined terminal-specific optogenetic inhibition of VTA-mPFC DA projections, fiber photometry recordings of mPFC DA release, and computational modeling of mPFC DA dynamics and behavior. Our

78 findings reveal a critical role for mPFC DA in signaled avoidance learning but not in retrieval of
79 learned avoidance strategies or cue-shock associative learning. To understand the specific
80 patterns of mPFC DA activity underlying threat avoidance learning, we also recorded mPFC DA
81 activity during cued fear conditioning, where avoidance is not possible, and during a yoked control
82 assay, in which shocks are unavoidable and the cue-shock relationship is unpredictable. Using a
83 temporal difference model of mPFC DA activity, we identified unique mPFC DA dynamics specific
84 to avoidance learning, contrasting with cued and yoked fear conditioning assays. Together, our
85 results demonstrate that VTA-mPFC DA circuit activity is necessary to link predictive cues to
86 adaptive behaviors that preempt an aversive outcome.
87
88 **Results**
89 **Mice learn tone-shock relationships and avoidance behaviors during PMA**
90 To investigate the role of mPFC DA projections in avoidance learning, we trained mice using a
91 platform-mediated avoidance (PMA) assay. In this assay, mice learn that a tone predicts a foot
92 shock, and then that they can navigate to a safety platform to avoid this shock. A reward port is
93 located on the opposite side of the chamber such that mice must choose between safety and
94 obtaining reward. Mice were first habituated to the chamber and reward port, followed by three
95 days of PMA training (Figure 1A). Each training day consisted of nine pseudorandomly timed 30-
96 second tones that co-terminated with a 2-second mild foot shock (Figure 1B). Mice were free to
97 move throughout the arena, obtain rewards, or access the safety platform. Overhead video
98 recordings were collected, and supervised deep learning was used to track eight anatomical
99 points on their body (Figure 1C).
100 Successful avoidance trials were defined as those in which mice moved to the safety
101 platform before the shock onset and remained there until the shock was over. Mice demonstrated
102 a steady increase in successful threat avoidance across the training days (Figure 1D).
103 Correspondingly, mice spent a progressively greater proportion of time on the platform during

104 tone periods as they learned to preemptively avoid the cued shocks (Figure 1E). Freezing
105 behavior remained consistent and did not change significantly across days (Figure 1F). Thus,
106 mice successfully learned the tone-shock relationship and avoidance behavior required to
107 improve their PMA performance across days.
108
109 **VTA-mPFC DA terminal activity is required for avoidance learning but not for associating**
110 **a cue with an aversive outcome**
111 To investigate whether DA inputs to mPFC are required for avoidance learning, we
112 optogenetically inhibited VTA-mPFC DA terminals during tone-shock presentations across three
113 days of platform-mediated avoidance (PMA) training. First, we injected an adeno-associated virus
114 (AAV) encoding the Cre-dependent inhibitory opsin Jaws or a control fluorophore (GFP) into the
115 VTA of TH-Cre mice. In these mice, Cre recombinase is expressed under the control of the
116 tyrosine hydroxylase (TH) promoter, restricting Jaws expression to DAergic neurons. Bilateral
117 optic fibers were then implanted above the mPFC to allow optogenetic inhibition of VTA-mPFC
118 DA axon terminals (Figure 2A). Fluorescence imaging revealed GFP-labeled neurons in the VTA
119 and GFP-labeled axons in the mPFC, confirming successful targeting of the circuit (Figure 2B).
120 During training, we inhibited VTA-mPFC DA terminals using constant 635 nm laser light delivered
121 via the implanted fibers during each tone, including the shock period (Figure 2C).
122 We assessed whether inhibiting VTA-mPFC DA activity affected signaled avoidance
123 behavior and conditioned freezing (which reflects the strength of the tone-shock association)
124 across learning. Jaws-expressing mice had significantly fewer successful avoidance trials
125 compared to GFP controls, particularly on the first day and early in the second day of training
126 (Figure 2D,E). Instead, Jaws mice exhibited a higher proportion of escape trials, where they leapt
127 to the platform after being shocked, with the largest differences evident early in learning (Figure
128 2D,F). Importantly, VTA-mPFC DA inhibition did not affect freezing behavior to the tone (Figure

129 2G), nor did it induce real-time place preference or aversion (Figure S1), indicating that activity in
130 this pathway is not inherently rewarding or aversive.

131 Although mice with VTA-mPFC DA projections inhibited eventually learned the PMA task,
132 we hypothesized that there might be qualitative differences in their avoidance behavior compared
133 to control mice. To test this, we analyzed interactions with the safety platform and reward port
134 during tone presentations, when threat levels are high and avoidance behavior is adaptive, and
135 during the inter-trial intervals (ITI), when threat levels are low and avoidance behavior is
136 maladaptive. During tone periods, Jaws mice displayed shorter average platform bouts, especially
137 on the second day of training (Figure 2H). In contrast, during the inter-trial interval (ITI), Jaws and
138 GFP controls had similarly low platform bout durations (Figure 2I). Both groups showed minimal
139 reward-seeking behavior during the tone (Figure 2J). Yet during the ITI, Jaws mice interacted with
140 the reward port more than controls (Figure 2K), suggesting that inhibiting VTA-mPFC DA
141 terminals did not generally lead to reduced motivation to seek rewards. These findings indicate
142 that VTA-mPFC DA projections are essential for platform mediated avoidance learning,
143 particularly during the early stages, but are not required for tone-shock learning or to successfully
144 escape shocks by leaping to safety.

145

146 **Prefrontal DA terminals are required for avoidance learning without motivational conflict,**
147 **but not retrieval of a previously learned avoidance strategy**

148 To better understand the role of VTA-mPFC DA projections in learning and enacting adaptive
149 avoidance strategies, we conducted additional optogenetic experiments in separate groups of
150 mice. First, we tested whether VTA-mPFC DA activity was necessary for learning in a 1-day
151 version of our PMA task that lacked an explicit approach-avoidance conflict (Figure S2A,B).
152 During training, Jaws-expressing mice exhibited fewer successful avoidance trials and more
153 shock-triggered escapes, but no change in tone-elicited freezing (Figure S2C). During a retrieval
154 test the following day (without shocks or optogenetic inhibition), Jaws mice exhibited less time on

155 the platform with no difference in freezing in response to the tone (Figure S2D). These results are
156 consistent with a selective role for VTA-mPFC DA terminals for linking predictive cues with
157 behaviors that preempt aversive outcomes, even in the absence of motivational conflict.

158 We next investigated whether inhibiting VTA-mPFC DA terminals were specifically
159 required for avoidance learning, or if they were also required for retrieval of a previously learned
160 avoidance strategy. To test this, we trained a separate cohort of mice in our PMA task, then
161 interleaved trials with or without optogenetic inhibition during retrieval the next day (Figure S2E).
162 Inhibiting VTA-mPFC DA terminals had no effect on either time on platform or freezing to tones
163 during retrieval (Figure S2F,G). Thus, VTA-mPFC DA terminal activity is specifically required to
164 link predictive cues with avoidance behaviors, but not for retrieving a previously learned
165 avoidance strategy.

166

167 **Inhibiting prefrontal DA terminal activity alters PMA learning rates**

168 To further dissect the role of VTA-mPFC DA terminal activity in avoidance learning, we applied a
169 Rescorla-Wagner learning model[43,44] to our optogenetic behavioral data (Figure 2). This model
170 assumes that the value of the safety platform is updated based on learning rates from shock trials
171 (α). This model successfully captured the learning trajectories of both laser-only control mice
172 (GFP-expressing) and those with inhibited VTA-mPFC DA terminals (Jaws-expressing) (Figure
173 3B-F), but we observed differences in the learning rates of the GFP and Jaws groups. In GFP-
174 but not Jaws-expressing mice, α tended to decrease across days, suggesting that optogenetic
175 inhibition of VTA-mPFC DA terminals causes a failure to form stable representations of the
176 platform value.

177 We also considered a different model in which the learning rate was informed by both
178 success ($\alpha_{success}$) and failure ($\alpha_{failure}$) trials. By incorporating value updates driven by both shocks
179 and shock omissions, the model reveals the relative contribution of different trial outcomes to
180 avoidance learning. In control mice, $\alpha_{failure}$ was highest on day 1, indicating that aversive outcomes

181 primarily drove behavioral adjustments during early learning (Figure S3). But by the second day
182 of training, $\alpha_{success}$ became dominant, suggesting that successful avoidance outcomes guided the
183 majority of behavioral changes during later learning stages (Figure S3). In contrast, Jaws-
184 expressing mice did not exhibit this transition from high $\alpha_{failure}$ to high $\alpha_{success}$. Instead, aversive
185 outcomes continued to drive behavioral changes throughout training (Figure S3). These results
186 suggest that VTA-mPFC DA terminal activity is critical for how aversive outcomes shape future
187 behavior.
188

189 **Prefrontal DA is most dynamic early in PMA learning and reflects trial outcomes,**
190 **avoidance behaviors, and threatening locations.**

191 Our optogenetic and modeling data suggested that VTA-mPFC DA terminal activity was most
192 important in the early stages of avoidance learning. However, it was unclear how VTA-mPFC DA
193 terminal activity relates to DA levels in mPFC. To examine this, we expressed the fluorescent DA
194 sensor $GRAB_{DA2m}$[45] (GRABDA) in mPFC and recorded its fluorescence using fiber photometry
195 during PMA training (Figure 4A). Consistent with the critical role of VTA-mPFC DA terminal activity
196 early in learning, we observed the largest mPFC DA dynamics on Day 1 of PMA training (Figure
197 4B).

198 To understand how mPFC DA encodes trial outcomes, we examined GRABDA signals
199 during shocks and shock omissions during PMA. Unsuccessful trials that resulted in shocks
200 evoked large initial DA responses that diminished with repeated exposure to the aversive outcome
201 (Figure 4C). Conversely, successful avoidance trials elicited negative DA responses that also
202 diminished with repeated avoids (Figure 4D). Unlike these outcome-specific signals, tone onset
203 responses were smaller and did not consistently vary in magnitude across learning (Figure 4E).
204 GRABDA signals were unrelated to overall movement velocity (Figure 4F), indicating that the
205 observed dynamics did not simply reflect motor activity. Additional controls confirmed that

206 GRABDA fluorescence reflected DA dynamics rather than movement artifacts: fiber photometry
207 recordings in GFP-expressing animals showed no shock or avoidance responses (Figure S4),
208 and administration of the D2 receptor antagonist eticlopride diminished shock-evoked GRABDA
209 responses (Figure S5).

210 We observed only small increases in mPFC DA following tone onset, but all animals had
211 fluctuations in mPFC DA throughout the 30-second tone, suggesting some of the activity may
212 relate to specific behaviors. We therefore investigated whether mPFC DA encoded avoidance
213 and freezing behaviors. mPFC DA ramped up during platform entries and returned to baseline
214 upon reaching safety (Figure 4G). Similarly, DA signals increased during platform exits onto the
215 shock grid and remained elevated (Figure 4H). We observed a nonsignificant trend for DA signals
216 to increase before freezing onset (Figure 4I) and decrease before freezing offset (Figure 4J).
217 Together, our findings indicate that mPFC DA encodes trial outcomes, threat-induced behaviors,
218 and threatening locations. Moreover, mPFC DA dynamics are strongest during the early stages
219 of learning.

220 **Removing shock avoidability from PMA alters mPFC DA encoding of trial outcomes and**
221 **cues.**

222 We found that mPFC DA is dynamically increased in response to an aversive outcome, but is
223 modulated in the opposing direction when mice successfully avoid it, and that learning modulates
224 the amplitude of these signals. This led us to ask whether it was the ability to avoid the shock on
225 a platform or the particular pattern of shocked and non-shocked outcomes that drove these
226 learning-related changes in mPFC DA. To investigate this, we removed the safety platform from
227 the arena, eliminating the ability to intentionally avoid shocks, and then for each mouse in this
228 cohort, we yoked the trials associated with shocks to mice in the PMA group. Thus, both PMA
229 and yoked groups experienced identical patterns of shocked and non-shocked trials. So, in PMA,

230 shock omissions were associated with platform entries whereas in the yoked controls, there was
231 no discernable relationship between actions and shock omissions. We then used GRABDA to
232 record DA dynamics during this yoked paradigm (Figure 5A,B).

233 Mice exposed to yoked conditioning exhibited increased freezing to the tone on Day 1
234 which plateaued on Days 2 and 3 (Figure 5C). We observed DA responses to shocks and tone
235 onsets (Figure 5D) but found that prefrontal DA dynamics were distinct from those seen in PMA.
236 Unlike in PMA, DA response to shocks remained elevated across the session (Figure 5E).
237 Moreover, we did not observe a decrease in mPFC DA during the early shock omissions, but
238 instead saw low DA signals trending towards an increase across the session (Figure 5F).
239 Furthermore, in contrast to PMA, DA levels during the tone onset increased significantly over time
240 (Figure 5G). These findings indicate that the shock omission by nature of platform avoidance, not
241 the pattern of cue-shock pairings, drove the pattern of mPFC DA dynamics we observed. They
242 also suggest that mPFC DA levels are sensitive to both the predictability and to the avoidability
243 of shocks.

244

245 **Prefrontal DA is not required for cued fear conditioning**

246 While our yoked task design maintained the tone-outcome sequence of PMA, it also introduced
247 uncertainty to the tone-shock relationship. In PMA, the tone-shock relationship is predictable yet
248 avoidable. To examine mPFC DA dynamics when mice learn about shocks that are predictable
249 but unavoidable, we contrasted our previous results with a standard cued fear conditioning assay.
250 We recorded DA dynamics during one day of fear conditioning, in which every tone terminates
251 with an unavoidable shock, and during fear memory retrieval, in which conditioned tones are
252 presented without shocks (Figure 6A,B). Prefrontal DA dynamics during both fear conditioning
253 and retrieval were distinct from those observed in PMA and yoked conditioning (Figure 6C,D).
254 During training, we observed a large increase in mPFC DA during the first shock that steadily
255 declined in amplitude with subsequent shocks (Figure 6E). On the retrieval day, there was a large

256 increase in DA when the shock was first unexpectedly omitted but that response rapidly
257 diminished with repeated tone presentations (Figure 6F). Tone onset responses during training
258 showed no consistent change (Figure 6G), but during retrieval, they significantly decreased
259 across repeated tone presentations (Figure 6H).

260 Given that VTA-mPFC DA terminal inhibition only affected avoidance learning and not
261 conditioned freezing in PMA, we hypothesized that VTA-mPFC DA terminals are not required for
262 tone-shock learning. To test this, we suppressed VTA-mPFC DA terminal activity during fear
263 conditioning (Figure 6I). On the training day, both Jaws-expressing and GFP control mice showed
264 increased freezing to the tone, with no differences between groups (Figure 6K). Similarly, on the
265 retrieval day, when tones were presented without shocks or optogenetic inhibition, freezing levels
266 during tone presentations were unaffected (Figure 6L). These results indicate that mPFC DA
267 activity is not essential for learning tone-shock associations, regardless of the availability of an
268 avoidance contingency, but reflects important aspects of cue-outcome learning (e.g. unexpected
269 shocks, expected shocks, and unexpected shock omissions).

270

271 **Temporal difference model captures assay-specific dynamics governing mPFC DA**

272 To investigate how mPFC DA dynamics support learning across PMA, yoked conditioning,
273 and cued fear conditioning, we applied a temporal difference (TD) learning model to capture
274 DA activity. We hypothesized that similar computational principles might underlie mPFC DA
275 signals during associative aversive learning in general, but that assay-specific features (e.g.
276 predictability of tone-shock relationships and avoidability of shock outcomes) would modulate
277 the temporal dynamics of mPFC DA signals. To examine this, we augmented a standard TD
278 model with an uncertainty parameter (β) as a temporal scaling factor. This parameter allows
279 for flexible temporal dynamics in the modeled DA signal, which may vary with assay-specific
280 demands (Figure 7A). Since our Rescorla-Wagner model of behavior revealed that mPFC DA

activity affects learning based on shocks vs. omissions (Figure S6), we separated trials depending on whether mice experienced a shock or not, and assessed model parameters for each trial case.

The model performed similarly well at predicting mPFC DA activity across PMA, yoked conditioning, and cued fear conditioning (Figure 7B). However, the parameter values required to accurately predict mPFC DA differed significantly across the three assays. In particular, the *uncertainty* factor (β) and *discounting* factor (γ) during shock trials were significantly different (Figure 7C,D). In PMA, β initially increased and then decreased in late learning, while it remained relatively stable in yoked conditioning and sharply decreased across learning in cued fear conditioning (Figure 7C). In contrast, γ initially decreased and then increased in late learning on shocked trials in PMA, while both yoked and cued fear conditioning exhibited an increase in γ that remained stable (Figure 7D). While there were interesting trends across learning in the non-shocked trials, these were not significantly different across assays. Trends in *b* (representing the base level of DA) and α (learning rate) were apparent across learning in both shocked and non-shocked trials, but were also not significantly different between assays (Figure 7E,F). Altogether, these findings suggest that the distinct learning demands of each task – such as the potential to preemptively avoid – shape when and how specific computational principles are implemented, especially during aversive outcomes.

Discussion

We demonstrate a critical role for mPFC DA terminal activity in signaled threat avoidance learning and show that mPFC DA itself is linked to specific aspects of the learning process. During threat avoidance learning, DA encodes predictive cues, aversive outcomes and their omission, and threat-related behaviors. Trial-by-trial mPFC DA, measured during avoidance learning, exhibited distinct dynamics in response to conditioned cues, foot shocks and shock omissions compared with assays lacking an avoidance contingency. These data reveal that prefrontal DA dynamics

306 are sensitive to both the predictability and avoidability of aversive outcomes. Consistent with these
307 observations, inhibiting VTA-mPFC DA terminal activity significantly slowed avoidance learning
308 in PMA. In contrast, VTA-mPFC DA terminal activity was not required to link a predictive cue to a
309 passive freezing response, regardless of the avoidability of the shock. This causal dissociation
310 underscores a specialized role for prefrontal DA inputs in rapidly linking predictive cues with
311 behaviors required to actively avoid aversive outcomes.

312 The DA system has a well-established role in associative learning, but most studies have
313 examined the role of striatal dopamine in reward-based learning. However, more recent studies
314 have begun to elucidate a key role for DA in aversive learning as well[22,32,46]. In particular, VTA DA
315 neurons projecting to mPFC appear to be specialized for aversive processing[28–30,47,48] and
316 aversive stimuli drive increases in mPFC DA release[32,46,49,50]. Optogenetic stimulation of prefrontal
317 DA terminals biases behavior towards aversive rather than appetitive behavioral responses[31].
318 Prefrontal DA is also increased during fear conditioning, and prefrontal DA is implicated in the
319 acquisition of contextual fear, and in the expression but not in the acquisition of a tone-shock
320 association[36–38,51–55]. These findings are in agreement with ours and highlight a key role for mPFC
321 DA in learned fear, and suggest prefrontal DA plays different roles depending on the behavioral
322 demands of aversive tasks.

323 Previous studies have also suggested a role for mPFC DA in threat avoidance. However,
324 most of these studies focused on already fully-learned avoidance, not its acquisition. Thus the
325 dopaminergic circuit mechanisms supporting rapid avoidance learning have remained poorly
326 understood. Moreover, previous studies have primarily used lesions and systemic pharmacology
327 as causal manipulations, or microdialysis to measure DA responses. These approaches lack the
328 spatiotemporal precision required to associate DA activity with distinct epochs of learning. Studies
329 that used optogenetics to examine the role of VTA-mPFC DA circuit activity in real-time or
330 conditioned place avoidance yielded mixed results, some finding that stimulation is sufficient to
331 enhance anxiety-like behaviors and promote conditioned place preference[47], and others finding

332 no effect[17,60]. So, while these studies indicated that mPFC DA activity may have a key role in
333 threat avoidance, the role of mPFC DA in learning to preemptively avoid threats remains poorly
334 understood.

335 We addressed this important question by using optogenetics and fiber photometry to
336 precisely manipulate and record mPFC DA activity during PMA. Consistent with previous
337 microdialysis studies that examined prefrontal dopamine in a shuttle-box assay[42], we found that
338 during both PMA and fear conditioning, mPFC DA levels were highest early in learning. While
339 aversive foot shocks initially elicited large positive DA responses across all assays, in line with
340 previous studies[20,30,46,61], learning-related changes in the amplitude of these signals depended on
341 the behavioral context. Predictable shocks (during PMA and FC) elicited responses that
342 diminished across learning, while unpredictable shocks (during the yoked assay) did not.
343 Relatively, the increases in mPFC DA seen during conditioned tones and threat-induced
344 behaviors were small. During shock omissions, mPFC DA initially decreased in PMA but
345 increased during fear memory retrieval before diminishing. This builds on previous work showing
346 that VTA-mPFC projections regulate fear extinction learning[62–66], revealing the rapid adaptation
347 of prefrontal DA responses during extinction trials. Together, these findings suggest that, rather
348 than driving specific behaviors, large fluctuations in mPFC DA may enable learning-related circuit
349 plasticity that is required for individuals to flexibly link predictive cues with adaptive actions
350 required for avoidance.

351 In line with this, we show that inhibition of VTA-mPFC DA slows avoidance learning in
352 PMA. Indeed, our Rescorla-Wagner model of PMA behavior suggested that inhibiting VTA-mPFC
353 DA impeded the animals' ability to use information about aversive outcomes to update the value
354 of a safe location. Our temporal difference model of mPFC DA activity revealed that in the PMA
355 condition, *uncertainty* and *future discounting* parameters decreased across learning in shock
356 trials. In contrast, yoked and fear conditioned groups showed less change in *future discounting*

357 during shock trials, reflecting more stability in the weight of future predictions. These parameters
358 suggest that, as animals learn to avoid signaled threats, dopamine increasingly encodes the
359 predictive relationships between cues and behavioral strategies required to avoid aversive
360 outcomes. In contrast to PMA, VTA-mPFC DA activity is not required for learning cue-shock
361 associations. This behavioral distinction may reflect the general function of mPFC, which is not
362 required for tone-shock learning[67], but rather for learning to appropriately coordinate actions to
363 support goals[68–70]. Overall, our findings align with evidence that the mPFC plays a critical role in
364 context-dependent learning and application of rule-based behaviors[64–67], and suggest mPFC DA
365 to be a key mechanism guiding adaptive responses to contextual contingencies.

366 Through associative learning, mPFC circuits integrate information about environmental
367 cues and internal goals to promote adaptive action selection. Activation of DA receptors in the
368 mPFC modulates neuronal excitability and long-term synaptic plasticity[75–77], which may enable
369 rapid learning of avoidance behaviors. DA influences activity primarily via D1 and D2 receptor
370 classes, which are expressed in largely non-overlapping prefrontal cell types[78–81]. mPFC D1
371 receptor-expressing neurons have strong projections to cortical and thalamic targets[82] and recent
372 evidence shows that DA enhances the signal-to-noise ratio in classes of mPFC projection neurons
373 that encode aversive stimuli[31]. Given that classes of mPFC projection neurons play unique roles
374 in threat avoidance learning[13,83], mPFC DA likely shapes cortical network activity to enhance
375 activity in particular neuronal classes during avoidance learning.

376 Our study causally manipulated the activity of VTA-mPFC DA terminals during behavioral
377 assays. Since we performed these experiments in TH-Cre mice, the effects we observed were
378 likely related to DA activity. However, recent studies showed that a subset of TH+ VTA cells also
379 co-express the vesicular glutamate transporter VGLUT2[84] and mesocortical DA neurons may co-
380 release glutamate[76]. A more complete understanding of how these projections drive plasticity and
381 learning will require investigation of whether observed effects arise specifically from DA vs. other

382 co-transmitters, temporally precise manipulations during isolated events (e.g. only during shocks),
383 and examination of how activity arising from VTA-mPFC terminals influences mPFC microcircuit
384 activity.

385 Altogether, our findings reveal that mPFC DA plays a distinct and essential role in
386 avoidance learning by dynamically encoding trial outcomes and using them to update behavioral
387 strategies. This specialization enables animals to rapidly adapt their behavior in complex
388 environments, linking predictive cues to goal-directed avoidance strategies. By dissociating the
389 roles of mPFC DA in avoidance learning and fear conditioning, this study advances our
390 understanding of the contribution of prefrontal DA to adaptive behavior. These findings also have
391 significant implications for understanding disorders characterized by maladaptive avoidance,
392 such as anxiety, PTSD, and OCD[86,87]. Disruptions in mesocortical dopamine signaling could
393 impair the ability to link predictive cues with adaptive avoidance behaviors, contributing to
394 pathological avoidance or excessive fear responses.

395 **Materials and Methods**

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

398

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

403

404 **Surgery** Mice were induced in 5% isoflurane in oxygen until loss of righting reflex and transferred
405 to a stereotaxic apparatus where they were maintained under 2% isoflurane in oxygen. Mice were
406 warmed with a circulating water heating pad throughout surgery and ophthalmic ointment was
407 applied to the eyes. The head was shaved and prepped with three scrubs of alternating betadine
408 and then 70% ethanol. Following a small skin incision, a dental drill was used to drill through the
409 skull above the injection targets. A syringe pump (Kopf, 693A) with a Hamilton syringe was used
410 for injections. Injections were delivered at a rate of 100 nl/min and the syringe was left in the brain
411 for 10 min following injection. For optogenetics experiments, animals were injected with 500nL of
412 AAV5-CAG-FLEX- JAWS-KGC-GFP-ER2 (Addgene #84445) or AAV5-CAG-FLEX-EGFP-WPRE
413 (Addgene #51502) at a titer of $5x10^{12}$ vg/mL bilaterally into the VTA (AP: -2.9 & -2.6 mm, ML: +/-
414 0.45 mm, DV: -4.5 mm from bregma). Bilateral optic fibers (200 µm core, Newdoon) were
415 implanted over the prelimbic (PL) area of the mPFC (AP: +1.8, ML: +/- 0.35, DV: -1.8 mm from
416 bregma). For fiber photometry experiments, AAV5-hSyn-GRAB_DA2m ($1x10^{13}$ vg/mL) was
417 injected unilaterally into PL (AP: +1.8, ML: -0.35, DV: -2 mm from bregma) with an optic fiber (400
418 µm core, Doric) implanted 200 µm above it. For pain management mice received 5 mg/kg
419 carprofen diluted in 0.9% saline subcutaneously. Mice received one injection during surgery and

420 daily injections for 2 d following surgery. For optogenetic experiments, we waited at least four
421 weeks after surgery before experimentation.
422
423 **Behavior**
424 *Platform mediated avoidance*
425 Three days prior to starting PMA, citric acid (2% w/v) was added to animals' drinking water. This
426 self-initiates a reduction in water consumption and increases performance on liquid reward tasks
427 similar to forced water restriction[88]. Animals were then placed into a 20x22cm arena with metal
428 rod flooring (Lafeyette Instruments) and a plexiglass platform covering one-quarter of the floor
429 space. Opposite the platform was a reward port and reward pump (Campden Instruments). The
430 reward port featured an infrared beam that triggered 25 µL of 10% sweetened condensed milk
431 (Nestle) reward on a variable interval schedule (mean interval: 30 seconds). Once a day for two
432 days, animals were placed in the arena and allowed to freely explore and consume reward for 45
433 minutes. The following day, PMA training began. For three days, animals were placed into the
434 arena with a 180 second baseline period followed by delivery of a 30 second, 75 dB, 4kHz tone
435 that co-terminated with a 2 second, 0.15 mA shock delivered to the floor (Lafeyette Instruments).
436 Eight subsequent tone-shock pairings were delivered at randomly chosen intervals between 80
437 and 160 seconds. Training on PMA continued for two more days for a total of three days. Data
438 presented in tone blocks are the average performance over three tones.
439
440 *Fear conditioning and yoked outcomes*
441 For cued fear conditioning, animals were placed in the same arena as PMA without the platform
442 or reward port. On training day, animals received three tones (4 kHz, 10s duration) to assess
443 baseline freezing followed by twelve tones that were paired with a coterminal footshock identical
444 to PMA. The following day (recall), animals were exposed to six tones (4 kHz, 30s duration)
445 unpaired with shock.Data are presented as individual tones. For yoked fear conditioning, the

446 same arena as cued fear conditioning was used. Trial outcomes for individual subjects were
447 matched to a PMA subject. On each of three days of training, subjects experienced nine tones (4
448 kHz, 30s duration) and based on the outcome of the matched PMA subject (avoid or shock), the
449 shock was either omitted (for avoid outcome) or delivered (for shock outcome). Data presented
450 in tone blocks are the average performance over three tones.
451
452 *Optogenetics*
453 JAWS activation was achieved via continuous red light (635 nm, 10 mW, Shanghai Laser and
454 Optics Century) delivered through the entirety of the tone-shock periods. For PMA, laser
455 illumination was concurrent with all the tones. For fear conditioning, this occurred on the first day
456 (training).
457
458 *Real-time place preference*
459 After PMA, a subset of animals were placed in a real-time place preference (RTPP) assay.
460 Animals were placed in a 68 x 23 cm chamber for 20 minutes. During the first ten minutes, the
461 animals freely explored the arena to establish a baseline side preference. During the subsequent
462 ten minutes, a closed loop video monitoring system (Bioviewer) tracked the location of the animal
463 and triggered a 635 nm laser when the animal entered the “laser” side of the arena. The ratio of
464 time spent in the “non-laser” side of the arena to the “laser” side of the arena was calculated for
465 both baseline (laser off) and test (laser on) periods.
466
467 *Fiber photometry*
468 Recordings were performed using a commercial fiber photometry system (RZ10x, Tucker Davis
469 Technologies) with two excitation wavelengths, 465 and 405 nm, modulated at 211 and 566 Hz
470 respectively. Light was filtered and combined by a fluorescent mini cube (Doric Lenses). Emission
471 was collected through the mini cube and focused onto a femtowatt photoreceiver (Newport, Model

472 2151). Samples were collected at 1017 Hz and demodulated by the RZ10x processor. Time
473 stamps to synchronize experimental events and recordings were sent via TTLs to the RZ10x
474 system via Arduino, controlled by custom MATLAB (MathWorks) code. For trial outcome specific
475 analyses, trials were binned into groups of three as done earlier, using three subsequent trials of
476 the same outcome to account for the stochastic nature of trial outcomes in PMA and yoked fear
477 conditioning experiments.
478
479 **Histology** Animals were deeply anesthetized with isoflurane and transcardially perfused with
480 phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Brains were removed
481 and post-fixed overnight in 4% PFA before being moved to PBS. Brains were then sectioned via
482 vibratome at 60 microns. To detect VTA axons expressing JAWS-GFP in the mPFC, anti-GFP
483 immunohistochemistry was performed. Sections were incubated with chicken anti-GFP polyclonal
484 antibody (1:2000, Aves Labs) overnight at 4℃. Sections were next rinsed and incubated with
485 donkey anti-chicken AlexaFluor 488 conjugate (1:500; Jackson ImmunoResearch) at room
486 temperature for 2 hr. Sections were washed before being mounted with DAPI. Images of
487 fluorescent expression and implant targeting were taken using a Leica DM6 scanning microscope
488 (Leica Microsystems) using a 10x objective.
489
490 **Data Analysis**
491 *Behavioral Analysis*
492 Overhead videos were collected (Teledyne FLIR, Chameleon 3) and animal positioning extracted
493 via supervised deep learning networks[89]. Animal position information was transformed into
494 freezing and interactions with ROI and bout features using BehaviorDEPOT software[90].
495
496 *Fiber Photometry*

Data were analyzed using a custom-written MATLAB pipeline. Raw recordings were downsampled 10x and the isosbestic signal was fit to the 465nm signal using the polyfit MATLAB function. Signals were transformed into z-scores for tone, freezing, and platform periods using a baseline of -5 to -4 seconds relative to event onset and smoothed with a 25 frame moving average. An animal's individual event responses were averaged to generate one trace per animal. Shock, avoid, and tone onset response z-scores were normalized across animals by using the mean response of the first tone period prior to shock delivery. Tone onset responses were calculated as the average value over the first 2 seconds of the tone. Shock/avoid responses were calculated as the average value over the 2 seconds following end of tone/shock. Platform and freezing responses were aligned to the onset or offset of the behavior. Average values of -2 to -1.5 seconds, -0.5 to 0 seconds, and +1.5 to +2 seconds from the event were used as baseline, event, and post-event responses, respectively. Baseline values were set to 0 to calculate the changes in DA preceding and following the event.

Behavioral Modeling

Time spent on the safety platform during conditioned tones was modeled using a Rescorla-Wagner model. We seeded the model with the amount of baseline time animals spent on the platform prior to shock. We reasoned the value of the platform would change as the animal learns the tone-shock association and as the animal learns the avoid-safety contingency.

We therefore updated the platform value based on failure (shock) trials. The value of the platform was calculated as:

$\Delta v_t = \alpha * (R_t - v_t)$, where:

- Δv_t is the change in associative strength of the platform at trial *t*
- α is the learning rate (free parameter)
- R_t is the outcome at trial *t*

- v_t is associative strength at time t (initially the baseline p(time on platform))

with the update rule:

Δv_t = {

$\alpha * (R_t - v_t)$ if success indicator at time t indicates = 0

0 otherwise

}

This is fit by minimizing a loss function $MSE = (1 / N) * \Sigma_i (v_i - data_i)^2$, where:

- MSE is the mean squared error
- N is the # of data points
- v_i is the model's predicted value at trial i
- $data_i$ is the observed data at trial i

We also considered the alternative that success and failure trials both contribute to updating the platform value. We therefore implemented a model with two complementary learning rates (α): $\alpha_{failure}$, for learning from failure trials, and $\alpha_{success}$, for learning from successful avoid trials. Fit was performed as above. The value of the platform was calculated as:

Δv_t = {

$\alpha_{success} * (R_t - v_t)$ if trial outcome was a success,

$\alpha_{failure} * (R_t - v_t)$ if trial outcome was a failure

}

Dopamine Modeling

Dopamine dynamics were modeled by individually fitting a temporal difference reinforcement learning model (Schultz e al., 1997) to DA signals from each task trial for PMA, FC, and Yoked-trained mice. This model assumes that dopamine signals encode prediction errors, reflecting discrepancies between expected and actual outcomes during fear conditions and avoidance learning. For each trial, a value function of the form:

$$V(t) = \beta(t) + b$$

Where β is a coefficient indexing the influence of *uncertainty* on the value of rewards, and b indexes the *baseline* measure of dopamine on a given trial at timestep t. The value function defines a time-dependent reward prediction based on uncertainty and expected value. The temporal difference (TD) error was computed as:

$$\delta(t) = S(t) + \gamma * V(t+1) - Vt$$

Where $S(t)$ is an external stimulus (shock/tone, modeled as binary),$V(t+1)$ is the predicted value at the subsequent timestep, and γ is the *discount* factor controlling the influence of future rewards on the current prediction. Dopamine signals were then modeled as

$$DA_{predicted}(t + 1) = Vt + \alpha * \delta(t)$$

where α is the learning rate controlling the integration of TD errors into value predictions.

The TD model was fit to signals from each trial for each mouse using maximum likelihood estimation with the SciPy Optimize package in Python. Model error was calculated using a normalized root mean square error.

To facilitate comparison between the dynamics of different parameters across trials, optimized parameters for each subject were normalized between 0 and 1 across trials. To account for differences in numbers of given trial types between subjects, trials were grouped by trial outcome (shock or nonshock) and then averaged into thirds for early, middle, and late learning phases. To

assess change in parameter dynamics across learning, the difference from early learning was calculated.

Statistical analysis

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

Data and code availability

Custom MATLAB and Python code available upon request.

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Author Contributions

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

Declaration of Interests

The authors declare no competing interests.

During the preparation of this work the authors used the generative AI to check writing for grammar and clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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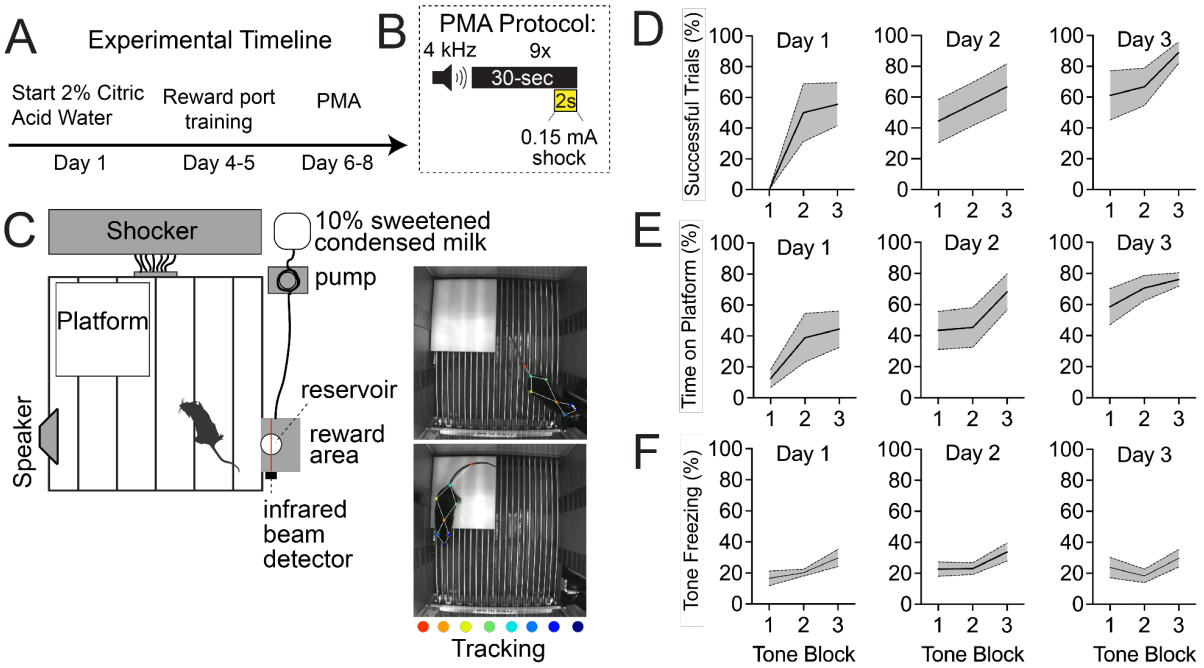

Figure 1. Mice learn avoidance contingencies and tone-shock relationships in platform mediated avoidance (PMA) assay.
A. Experimental timeline.
B. Behavioral training protocol.
C. Schematic of PMA chamber (left) and frames from videos (right).
D-F. Behavior across 3 days of PMA. On each day, data is 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).

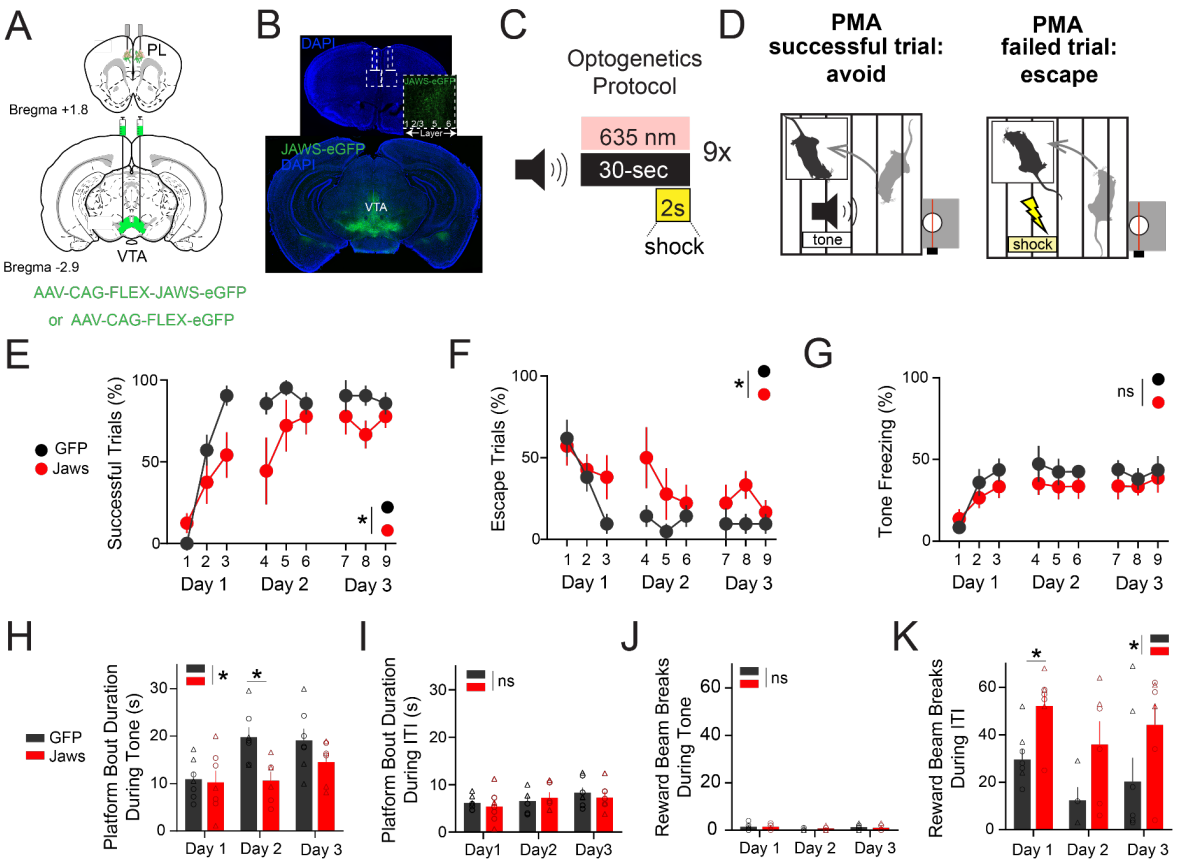

Figure 2. Optogenetic inhibition of DAergic axon terminals in mPFC impairs avoidance contingency learning but not tone-shock learning.

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. Percent 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. Percent 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. Percent 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 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.

I. Platform bout duration during ITI (inter-trial interval) across days in GFP vs. Jaws mice ($F_{time}(1.75, 18.43) = 3.4$, p=0.057; $F_{opsin}(1, 12) = 0.8$, p=0.7; $F_{interaction}(2, 21) = 0.67$, p=0.5).

J. 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.

K. Number of reward beam breaks during ITI across days in GFP and Jaws mice ($F_{time}(1.9, 20.1) = 4.3$, $p=0.02$; $F_{opsin}(1, 12) = 8.2$, $p=0.01$; $F_{interaction}(2, 21) = 0.02$, $p=0.98$; GFP vs Jaws Day 1 $p=0.02$; Day 2 $p=0.2$; Day 3 $p=0.3$).
Triangles represent males and circles represent females.
*P<0.05,. Graphs represent mean ± SEM.

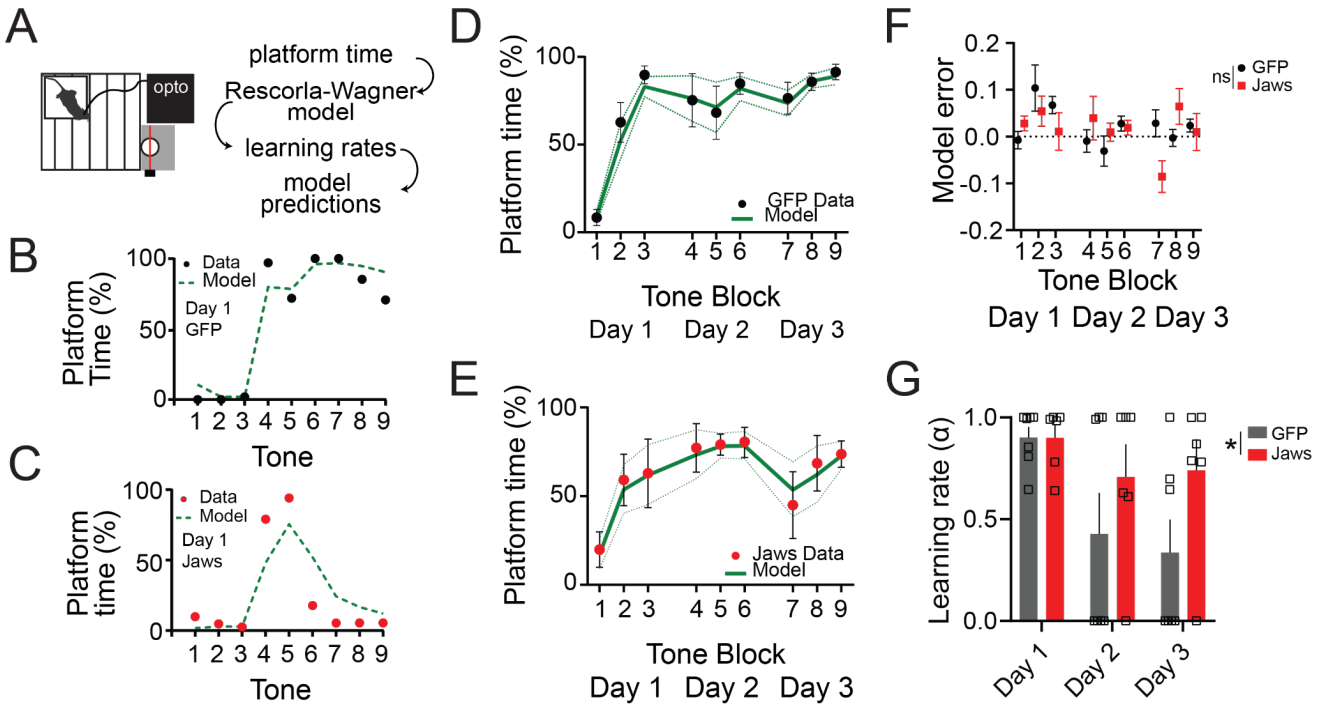

Figure 3. Inhibition of mPFC DA release decreases learning rates during PMA.
A. Schematic of optogenetic experiment and modeling approach.
B. Example model performance for a GFP-expressing animal.
C. Same as B for a Jaws-expressing animal.
D. Percent time on platform during the tone for GFP-expressing animals, both observed and model predictions.
E. Same as D for Jaws-expressing animals.
F. Quantification of modeling prediction error reveals no difference in the model's performance between GFP and Jaws groups. Mixed effects model ($F_{time}(3.32, 39.87)=2.1$, $p=0.1$; $F_{opsin}(1, 96)=0.69$, $p=0.6$; $F_{interaction}(8, 96)=2$, $p=0.052$).
G. Model-derived learning rates for GFP vs. Jaws animals. Two-way ANOVA ($F_{time}(1.4, 15.8)=3.2$, $p=0.07$; $F_{opsin}(1, 11)=5.4$, $p=0.04$; $F_{interaction}(2, 22)=0.8$, $p=0.4$).

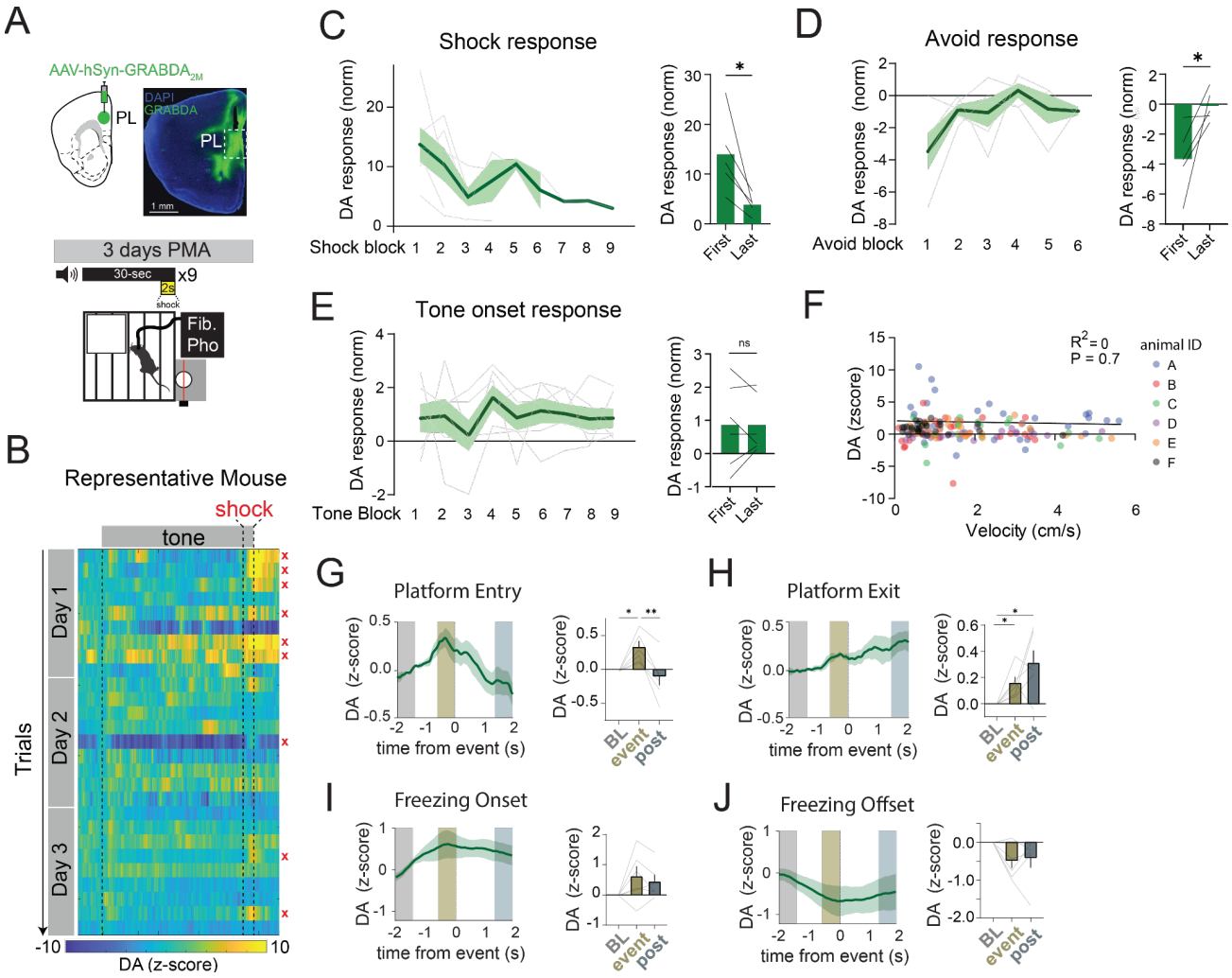

Figure 4. mPFC DA dynamics during PMA

A. Representative coronal section showing AAV-GRAB_DA$_{2m}$ expression and fiber placement. **B.** Heatmap showing z-scored DA fluorescence in a representative mouse. Red x marks shock trials.

C. DA signal decreases following repeated shocks. First block vs last block paired t-test p=0.03.

D. DA signal increases following repeated avoids. First block vs last block paired t-test p=0.04.

E. DA dynamics in response to tone onset do not show overt dynamics. First block vs last block paired t-test p=0.9.

F. Velocity does not correlate with DA fluorescence. Data points are color coded by animal, with the average DA response and velocity at the start (first 3 seconds) of each tone. Pearson's R^2 = 0, p=0.7.

G-J. DA dynamics surrounding avoidance and freezing behavior at baseline (BL), immediately preceding the behavior (event), or after the behavior (post).

G. DA ramps up prior to platform entry then back down following platform entry. Repeated measures one-way ANOVA F(10), p = 0.01. BL vs. event p = 0.02. Event vs. post p=0.007. BL vs. post p= 0.7.

H. DA dynamics ramp up during platform exit. Repeated measures one-way ANOVA F=6, p=0.04. BL vs. event p=0.03. Event vs. post p=0.4. BL vs. post p = 0.04.

I. DA dynamics during freezing onset. Repeated measures one-way ANOVA F=2.8, p=0.1.

J. DA dynamics during freezing offset. Repeated measures one-way ANOVA F=3.1, p=0.1.

N=6 animals. Posthoc testing performed with Tukey's test.

*P<0.05, ** P<0.01. Graphs represent mean ± SEM.

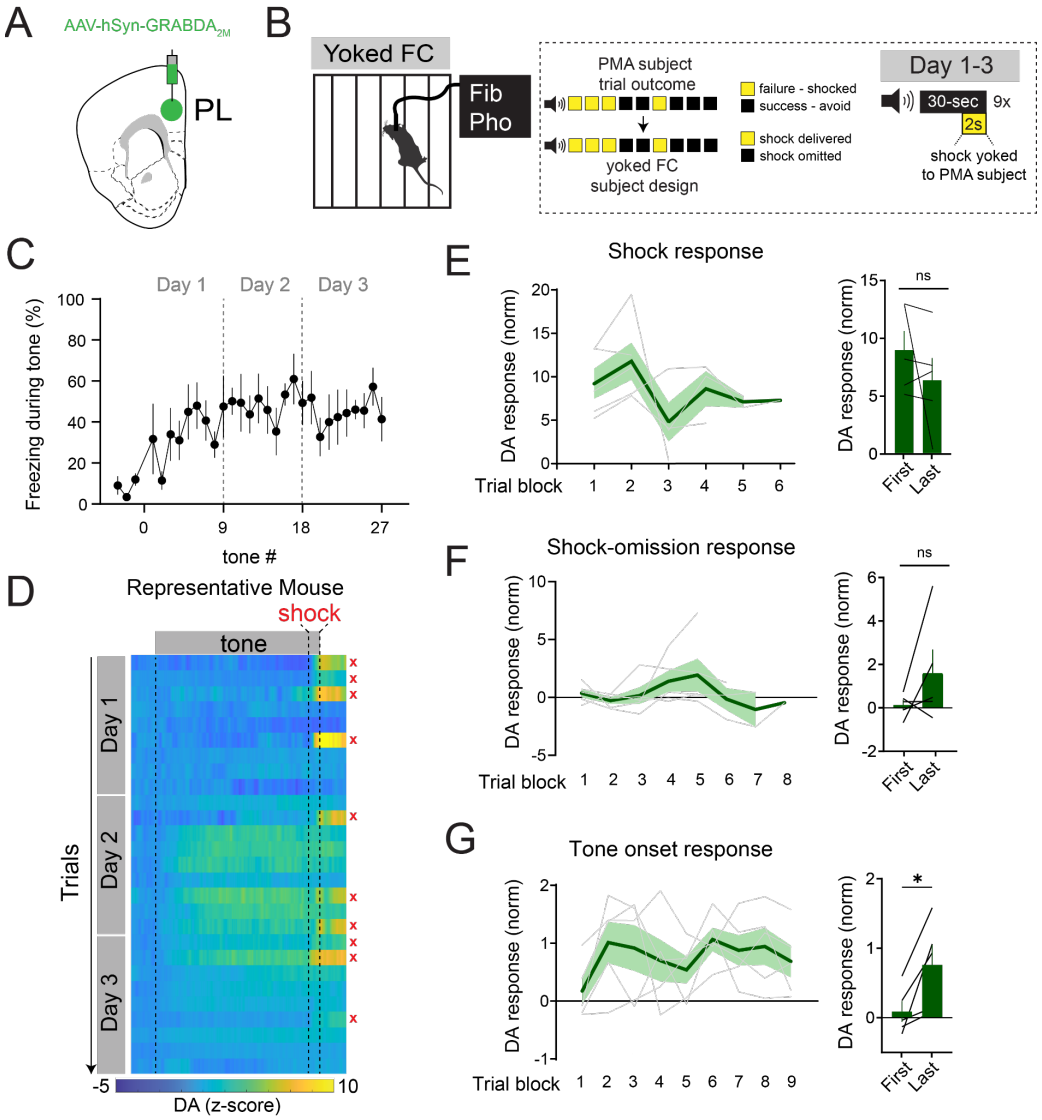

Figure 5. Removing the avoidance contingency from PMA alters mPFC DA representations of trial outcomes and cues.

A. Schematic showing GRABDA recording in prelimbic (PL) cortex.
B. Experimental design: animals were placed in a fear conditioning (FC) arena and exposed to tones. Presence of a co-terminal shock was dependent on the matched trial outcome of a subject from PMA.
C. Heatmap of GRABDA fluoresence (zscored) from representative mouse.
D. Freezing during the tone across days.
E. Left: DA levels to shock across time. Right: Comparison of first to last shock response block. Two-tailed paired t-test p=0.3.
F. Left: DA levels to shock-omission across time. Right: Comparison of first to last shock omission block. Paired t-test p=0.2.

G. Left: DA levels to tone onset across time. Right: Comparison of first to last tone block onset. Paired t-test p=0.03. *p<0.05.

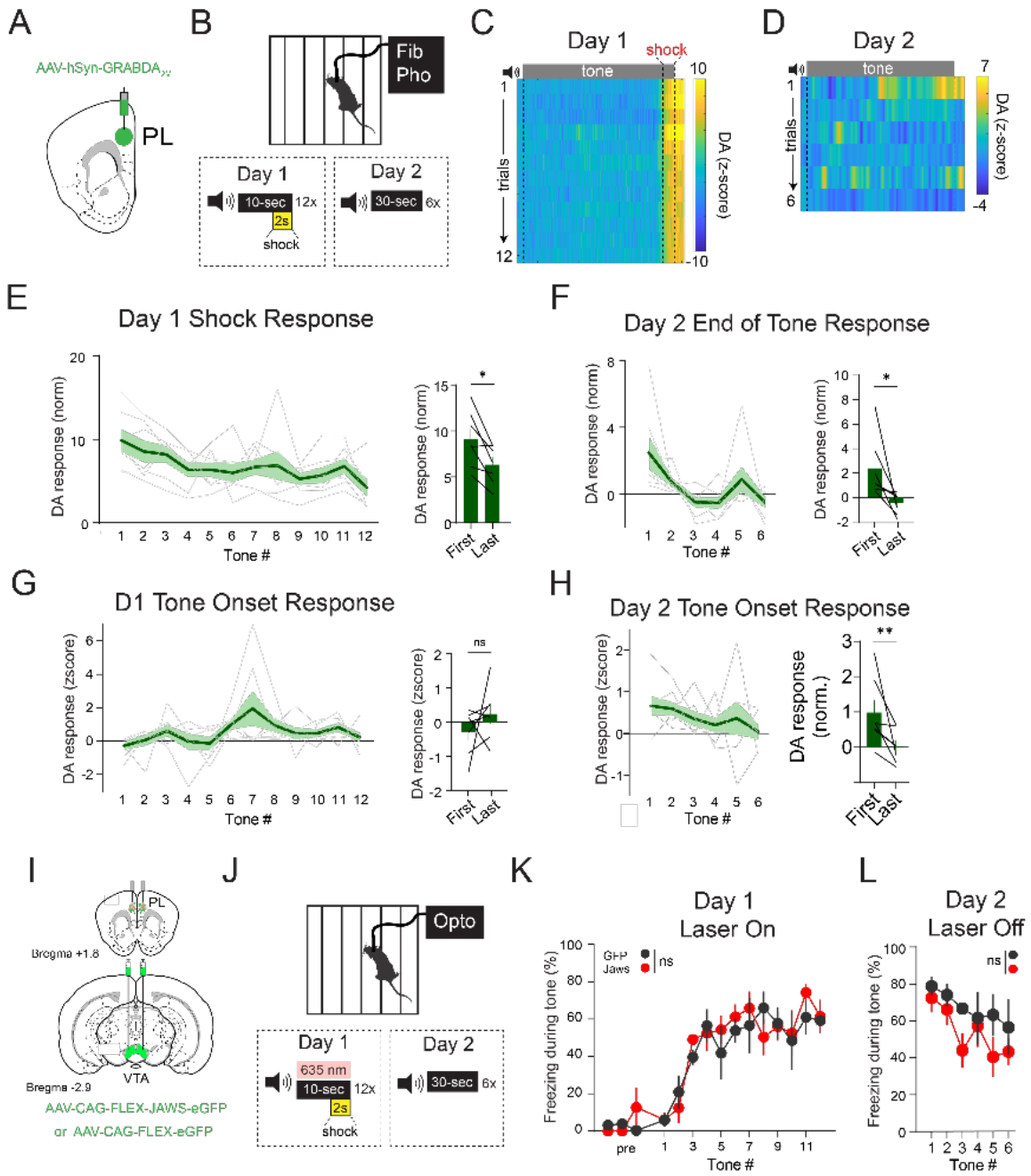

Figure 6. mPFC DA reflects but is not required for cued fear conditioning.
A. AAV and fiber placement GRABDA fiber photometry.
B. Experimental design.
C. Heatmap from representative mouse showing GRABDA fluorescence during fear conditioning. and
D. retrieval.
E. DA levels to shock during fear conditioning decrease across time. (right) Comparison of first to last shock response. Two-tailed paired t-test p=0.01.
F. DA levels during end of tone during the expected footshock on retrieval day. (right) Comparison of first and last tone response. Two-tailed paired t-test p=0.03.
G. DA levels during tone onset during fear conditioning do not linearly change across time. (right) Comparison of first and last tone response. Two-tailed paired t-test p=0.3.

H. DA levels during tone onset during fear memory retrieval decrease across time. (right) Comparison of first and last tone response. Two-tailed paired t-test p=0.008. * P<0.05, ** P<0.01, ns P>0.05 **.**
I. AAV injection and fiber placement for optogenetic inhibition of VTA-mPFC axon terminals.
J. Experimental protocol.
K. 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).
L. 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).

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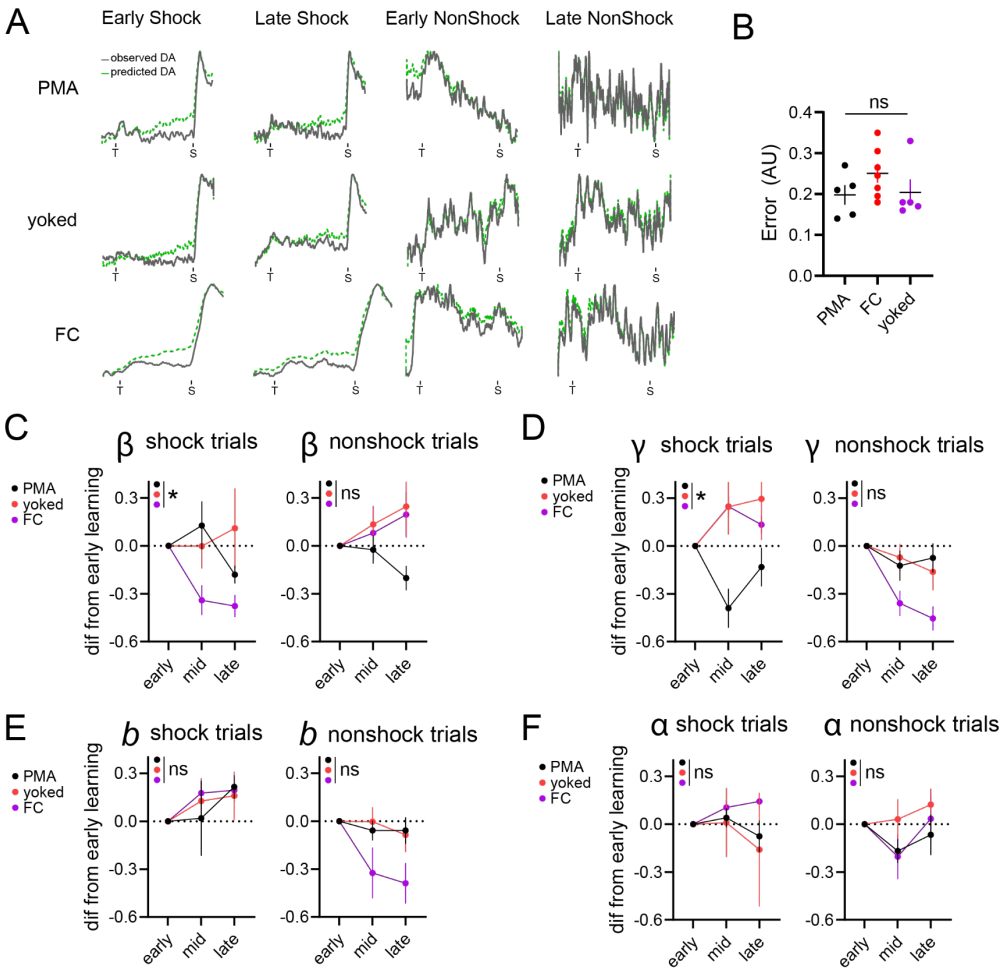

Figure 7. Temporal difference model captures assay-specific dynamics governing mPFC DA.
A. Representative observed versus model predicted DA responses across assay and learning. Y-axis scaled to min and max values for each trace. X axis tick marks indicate tone onset (T) and shock/shock omission onset (S).
B. The model has a similar error rate across assays. One-way ANOVA F=1.3, p = 0.29.
C. Temporal scaling factor (β) in (left) shock and (right) nonshock trials, averaged into values during early, middle, and late trials. (left) Two-way ANOVA $F_{time}(2,30)=1.9$, p=0.1, $F_{assay}(2,15)=4.1$, p=0.03, $F_{time\ x\ assay}(4,30)=3.1$, p=0.02. Mid PMA vs FC p=0.006, Late yoked vs FC p=0.006. (right) Two-way ANOVA $F_{time}(2,28)=1.4$, p=0.5, $F_{assay}(2,14)=1.5$, p=0.2, $F_{time\ x\ assay}(4,28)=5.5$, p=0.2.
D. Same as C for future discounting factor (γ). Two-way ANOVA $F_{time}(2,30)=1.1$, p=0.3, $F_{assay}(2,15)=5$, p=0.02, $F_{time\ x\ assay}(4,30)=5.2$, p=0.002. Mid PMA vs FC p=0.0002, Mid PMA vs yoked p=0.0007, Late PMA vs yoked p=0.02. (right) Two-way ANOVA $F_{time}(2,28)=9$, p=0.0009, $F_{assay}(2,14)=7.1$, p=0.007, $F_{time\ x\ assay}(4,28)=2.5$, p=0.059.
E. Same as C for base parameter (b). Two-way ANOVA $F_{time}(2,30)=3.1$, p=0.056, $F_{assay}(2,15)=0.1$, p=0.9, $F_{time\ x\ assay}(4,30)=0.3$, p=0.8. (right) Two-way ANOVA $F_{time}(2,28)=3.8$, p=0.03, $F_{assay}(2,14)=2.7$, p=0.1, $F_{time\ x\ assay}(4,28)=1.8$, p=0.15.
F. Same as C for learning rate parameter (α). Two-way ANOVA $F_{time}(2,30)=0.5$, p=0.6, $F_{assay}(2,15)=0.5$, p=0.5, $F_{time\ x\ assay}(4,30)=0.6$, p=0.6. (right) Two-way ANOVA $F_{time}(2,28)=2$, p=0.1, $F_{assay}(2,14)=0.7$, p=0.4, $F_{time\ x\ assay}(4,28)=0.6$, p=0.6.

Supplemental Figures

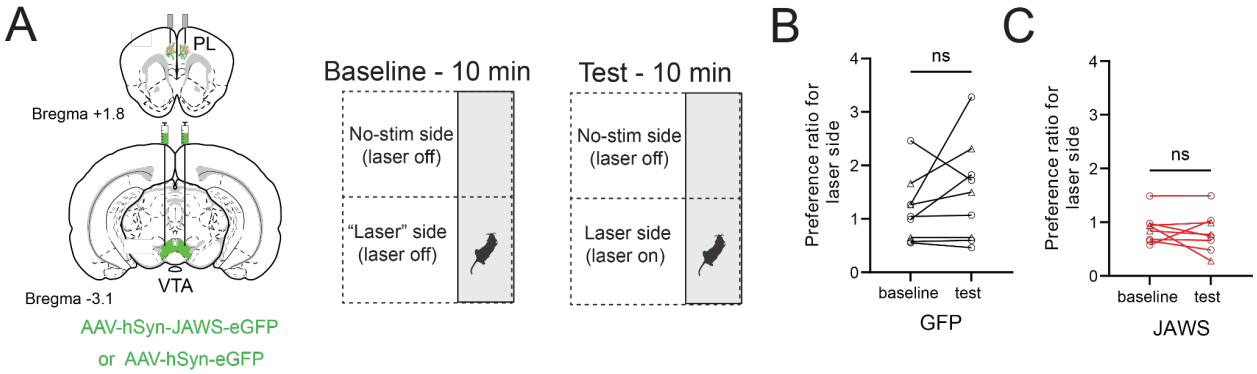

Figure S1. Inhibition of VTA DA terminals in mPFC does not cause real-time place avoidance.
A. Surgical strategy and schematic of real-time place preference assay.
B. Animal preference for "laser" side of the arena during baseline (laser off) period and during testing (laser on) period in GFP-expressing animals. Paired t-test, p=0.2, n=9.
C. Same as B. for Jaws-expressing animals. Paired t-test, p=0.4, n=8.

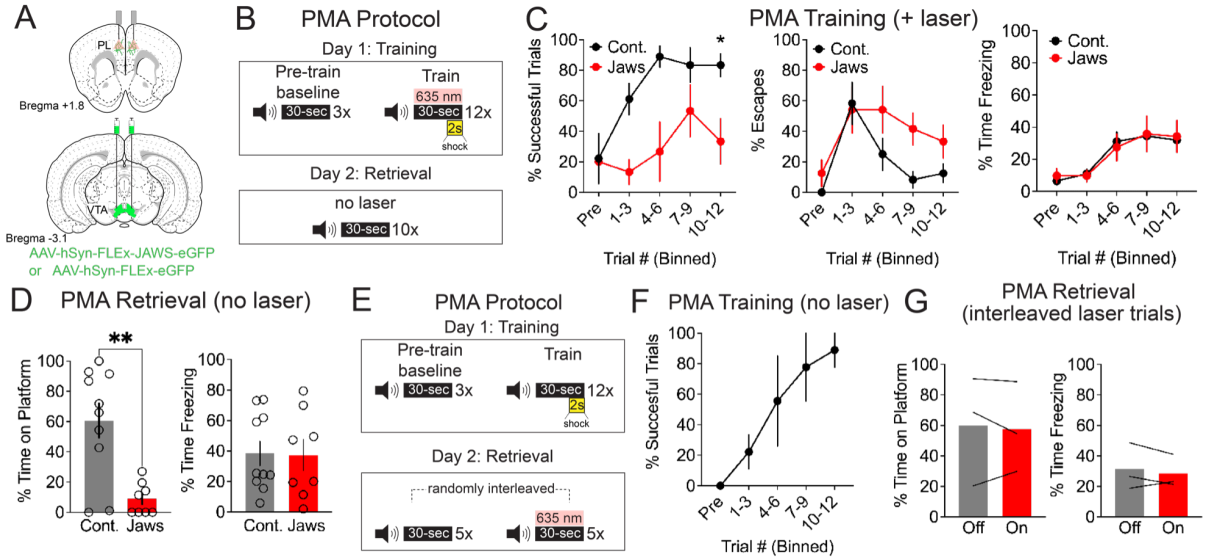

Figure S2. Optogenetic inhibition of VTA-mPFC DA terminals during PMA without motivational conflict.

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.

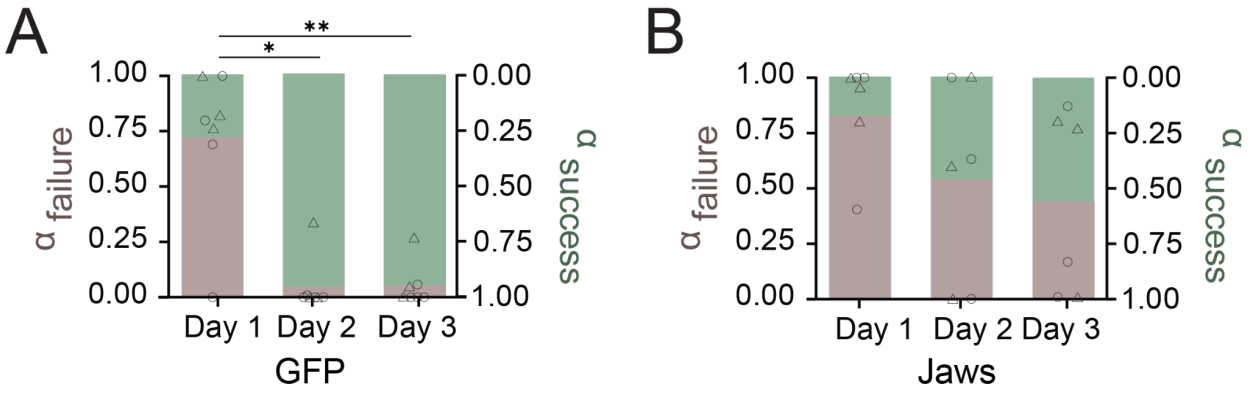

Figure S3. Inhibition of mPFC DA release influences learning rates based on failures and successes.

A. Learning rates per day as calculated by a Rescorla-Wagner model (Figure 3) factoring in trial types (success or failure) for GFP-expressing animals. Model-derived learning rates for GFP animals show a transition from failure-dominant learning to success-dominant learning. Mixed-effects model $F(1.3, 7.4)=20.8$, $p=0.001$. Points represent failure learning rate. *$P<0.05$, ** $P<0.01$.

B. Same as A for Jaws-expressing animals. Model-derived learning rates for Jaws animals do not show a change in learning strategy across time. Mixed-effects model $F(1.3, 6.7)=3.1$, $p=0.1$. Points represent failure learning rate.

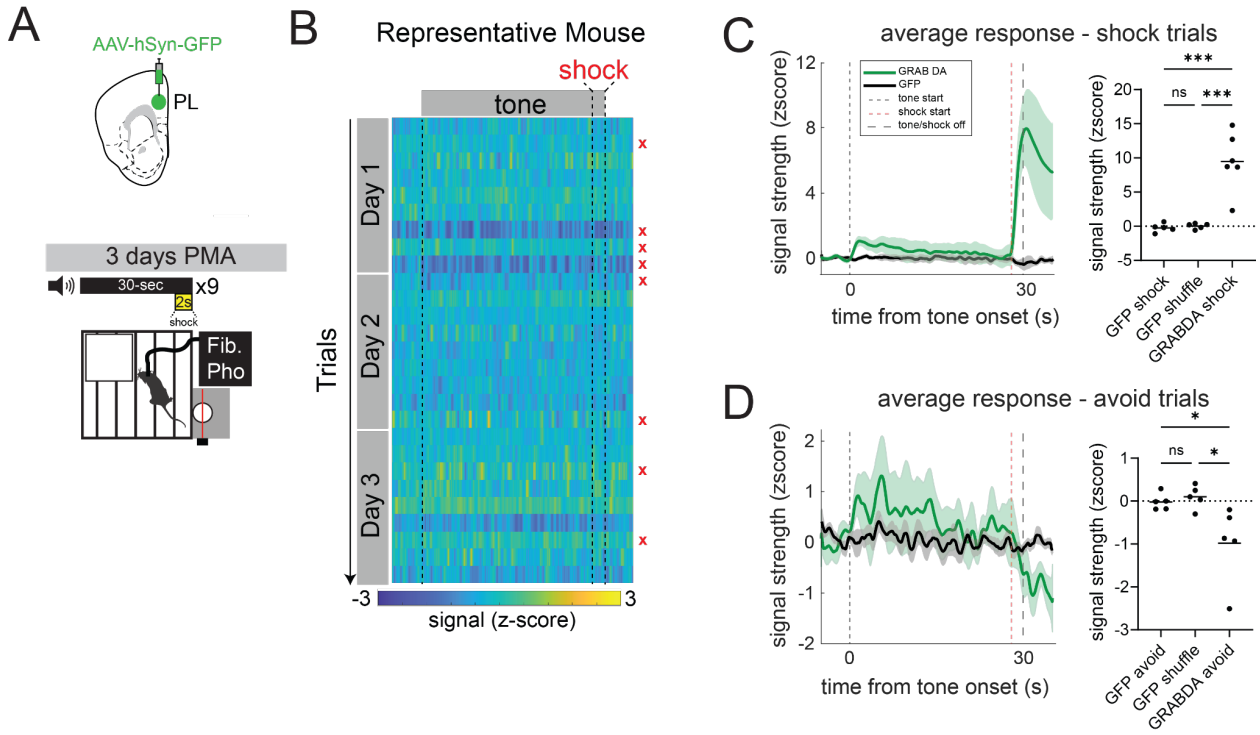

Figure S4. Dopamine responses are not caused by movement artifacts.
A. Surgical strategy to record fluorescent control (GFP) signal in PL (top); schematic of PMA (bottom).
B. Representative heatmap of GFP signal recorded during PMA.
C. GRAB DA animals had increased signal during shock compared to GFP recordings. Averaged signal during shock trials for GFP animals (black) and GRAB DA animals (green) (left). Quantification of shock response in GFP animals, shuffled GFP signal, and GRAB DA animals (right). ANOVA F=22.6, p<0.0001. GFP vs GRABDA p=0.0002, GFP shuffle vs GRABDA p=0.0002, GFP vs GFP shuffle p=0.9. *** p<0.001.
D. GRAB DA animals had decreased signal during shock compared to GFP recordings. Averaged signal during avoid trials for GFP animals (black) and GRAB DA animals (green) (left). Quantification of avoid response in GFP animals, shuffled GFP signal, and GRAB DA animals (right). ANOVA F=6.1, p=0.01, GFP vs GRABDA p=0.02, GFP shuffle vs GRABDA p=0.04, GFP vs GFP shuffle p=0.9. * p<0.05.

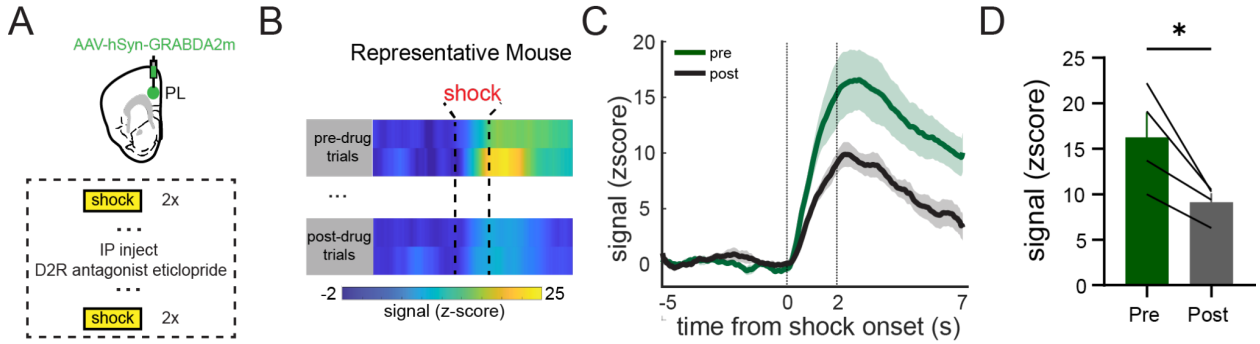

Figure S5. GRABDA response is dependent on D2 receptor function.
A. Experimental schematic: GRABDA signal recorded in PL during shocks prior to then following systemic administration of eticlopride, a D2 receptor antagonist.
B. Representative heatmap of GRABDA signal peri-shock prior to and following eticlopride.
C. Average GRABDA signal peri-shock prior to and following eticlopride.
D. GRABDA shock signal decreases following eticlopride. Paired t-test p=0.03.

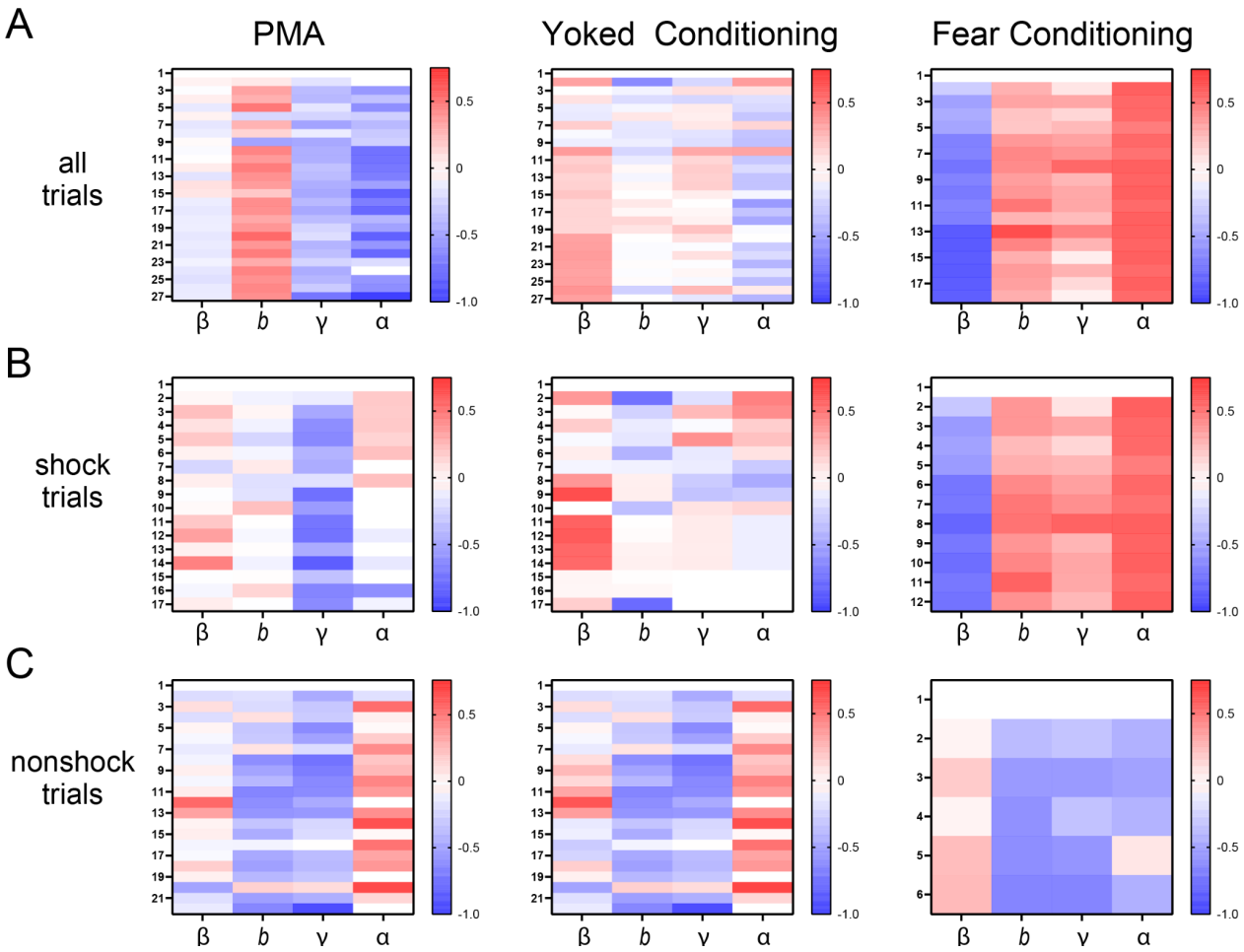

Figure S6. Temporal Difference model parameters across trials.
A. Average parameters displayed across all trials for PMA (left), yoked conditioning (middle), and fear conditioning (right). Values shown as normalized differences from the first trial.
B. Shock outcome only trials. Values presented as normalized differences from the first shock trial.
C. Nonshock outcome only trials. Values presented as normalized differences from the first nonshock trial. Number of shock and avoid trials in PMA and yoked conditioning differed for each animal but total 27 trials per animal.