

13 **Abstract**

14 The rules governing behavior often vary with behavioral contexts. As a consequence, an action
15 rewarded in one context may be discouraged in another. Animals and humans are capable of
16 switching between behavioral strategies under different contexts and acting adaptively
17 according to the variable rules, a flexibility that is thought to be mediated by the prefrontal
18 cortex (PFC)¹⁻⁴. However, how the PFC orchestrates context-dependent switch of strategies
19 remains unclear. Here we show that pathway-specific projection neurons in the medial PFC
20 (mPFC) differentially contribute to context-instructed strategy selection. In a decision-making
21 task in which mice have been trained to flexibly switch between a previously established rule
22 and a newly learned rule in a context-dependent manner, the activity of mPFC neurons
23 projecting to the dorsomedial striatum encodes the contexts, and further represents decision
24 strategies conforming to the old and new rules. Moreover, the activity of these neuron is
25 required for context-instructed strategy selection. In contrast, the activity of mPFC neurons
26 projecting to the ventral midline thalamus does not discriminate between the contexts, and
27 represents the old rule even if mice have adopted the new one; furthermore, these neurons act
28 to prevent the strategy switch under the new rule. Our results suggest that the mPFC→striatum
29 pathway promotes flexible strategy selection guided by contexts, whereas the mPFC→thalamus
30 pathway favors fixed strategy selection by preserving old rules. Balanced activity between the
31 two pathways may be critical for adaptive behaviors.

32

33 **Main text**

34 The ability to rapidly adjust behaviors according to the context is crucial for everyday life. The
35 prefrontal cortex (PFC), a major hub in the brain supporting cognitive flexibility⁵⁻¹⁰, is thought
36 to have a role in context-dependent behavioral responses. For example, the PFC is involved in
37 regulating contextual fear expression¹¹⁻¹⁴ and encodes contextual cues that inform rules^{2,15,16,6}.
38 In particular, when monkeys engage in a perceptual decision-making task requiring context-
39 guided selection of sensory features (color or motion), PFC neurons represent contextual
40 information in the form of population activity unfolding in a multidimensional space^{2,17}, with
41 individual PFC neurons showing mixed selectivity^{5,7,18-20}. However, it is unclear whether
42 contextual information in the PFC is essential for context-dependent behavioral responses and,
43 if so, how this information is conveyed to influence such behavioral responses.

44 The medial PFC (mPFC) projects extensively to the dorsomedial striatum (DMS)²¹,
45 forming a pathway that has been strongly implicated in decision making²²⁻²⁸ and behavioral
46 flexibility²⁸⁻³⁶. Another major target of the mPFC is the thalamus. More specifically, the ventral

47 midline thalamic nuclei (VMT) has gained attention in flexible behaviors^{37,38}, and the
48 projections from the mPFC to the nucleus reuniens – a part of the VMT – are critical for the
49 formation of contextual fear memories¹². Previous studies also suggest a role for mPFC-VMT-
50 hippocampal interactions in memory processes³⁹⁻⁴¹. Interestingly, recent work indicates that
51 mPFC neurons projecting to the midline thalamus and those projecting to the DMS are both
52 required for flexible behaviors³⁴. These findings point to the possibility that the mPFC exerts
53 its functions in flexible behaviors through, at least in part, projections to the DMS and/or VMT.

54 In this study, we tested the hypothesis that mPFC neurons control context-instructed
55 behavioral flexibility via mPFC→DMS or mPFC→VMT circuit. For this purpose, we first
56 trained mice in an auditory decision-making task based on a two-alternative choice (2AC) task
57 in which animals discriminate “cloud-of-tones” stimuli⁴²⁻⁴⁴. We further trained the mice to use
58 two contextual cues informing different rules. The first rule required mice to make decisions
59 based on only the sensory evidence in the “clouds” – as they had initially been trained. The
60 second rule required mice to adopt a new decision strategy that relied on both the sensory
61 evidence and reward values. With training, mice learned to make decisions based on the old
62 and new rules in a context-dependent manner, and on a trial-by-trial basis. At different training
63 stages, we imaged the activities of mPFC neurons, including those projecting to the DMS or
64 VMT, and further optogenetically manipulated these neurons while mice performed the task.

65 We found that mPFC neurons encode, and are essential for, context-guided strategy
66 selection. Notably, through learning, the DMS-projecting (mPFC^{DMS}) and VMT-projecting
67 (mPFC^{VMT}) neurons acquire opposite functions: whereas mPFC^{DMS} neurons represent
68 contextual changes and are required for switching to the new strategy in a context-dependent
69 manner, mPFC^{VMT} neurons keep a stable representation of the context associated with the initial
70 strategy and impedes the switch. These results uncover distinct roles of mPFC subpopulations
71 in behavioral flexibility and stability.

72

73 **mPFC neurons encode and are required for context-dependent decisions**

74 We first trained mice to perform a 2AC task inspired by a previous study⁴³. In this task (Fig.
75 1a), mice initiated a trial by licking the central spout, which was followed by the presentation
76 of a cloud-of-tones stimulus. The “cloud” contained a mixture of tones in a high-frequency (12-
77 17 kHz) or low-frequency (1-6 kHz) range, which were the target tones predicting the delivery
78 of a water reward (3 μ l) from the left or right spout, respectively. Thus, the rates of high-
79 frequency tones and low-frequency tones in the cloud determined the strength of sensory
80 evidence for decision making, and hence the difficulty of a trial. The easiest trials had clouds

81 composed of purely the high-frequency or low-frequency target tones (which were 1 and -1 in
82 evidence strength, respectively; Methods), while more difficult trials had clouds consisting of
83 mixed target tones (Fig. 1b). Mice improved performance with training in categorizing different
84 clouds, as suggested by the psychometric function (Fig. 1c, d).

85 Next, we further trained the mice to use contextual information to guide the switch
86 between decision strategies. We added two distinct “contexts” comprising multisensory cues to
87 the 2AC task (Fig. 1e, f; Methods). In each trial, one of the contexts was presented immediately
88 before the onset of the cloud to indicate that one of two rules would be applied. Context A
89 (CXA) informed that the original rule (the “old rule”) would be in effect, so that the mice should
90 keep making choices based on the sensory evidence in the subsequent cloud. Context B (CXB)
91 informed that a large reward (10 μ l) would be delivered if mice made a correct leftward choice
92 according to the old rule; however, no rightward choice would lead to any reward. Thus, under
93 this “new rule”, mice still need to use the sensory evidence in the clouds in order to make correct
94 leftward choices, but should ignore the clouds indicating a rightward choice under the old rule.
95 We randomly interleaved CXA trials and CXB trials, but kept CXA trials as the majority (Fig.
96 1e-g; Methods).

97 Through training with the contexts, mice gradually adapted their choice strategies to the
98 different rules, as indicated by the increasing bias towards the left spout in CXB trials (Fig. 1h,
99 i). This led to a shift of the psychometric curve towards the left in CXB trials compared with
100 CXA trials, particularly in trials where sensory evidence in the cloud was weak (Fig. 1j, k;
101 Extended Data Fig. 1a). This leftward bias disappeared when the contextual cues were removed
102 (Fig. 1l-n). It is somewhat surprising that the mice still made rightward choices under CXB,
103 especially in trials where the sensory evidence for a rightward choice was strong under the old
104 rule. This is likely because the mice were over trained with the old rule, and the old rule
105 remained in effect in the majority of trials (Methods). Such situations may cause the old rule to
106 interfere with the new rule, an effect that has been previously reported⁴⁵. Taken together, the
107 CX2AC task revealed that mice adaptively select their decision strategies established on the
108 basis of specific action-outcome contingencies (or rules). Importantly, this selection can be
109 flexibly instructed by contextual cues on a trial-by-trial basis.

110 Previous studies suggest that the dorsolateral PFC in monkeys is involved in context-
111 guided strategy switching^{2,46}, and the mPFC in mice controls cognitive flexibility^{33,47,48} and
112 contextual fear learning¹¹. To understand how mPFC neurons are recruited and contribute to
113 context-guided strategy selection, we imaged the activity of these neurons in mice performing
114 the CX2AC task. To this end, we injected the mPFC in mice with an adeno-associated virus

115 (AAV) expressing the calcium indicator GCaMP6f⁴⁹, and implanted a cannula into the same
116 location (Fig. 2a, b; Extended Data Fig. 1b-d). We trained the mice in the CX2AC task until
117 they reached a stable performance (Fig. 2c; Extended Data Fig. 1e). At different training stages,
118 we used a wide-field microscope to image the GCaMP6 signals in mPFC neurons at cellular
119 resolution through the implanted cannula^{50,51} (Fig. 2a-c).

120 We simultaneously imaged the responses of large populations of neurons, for up to 655
121 neurons per mouse (381 ± 190.69 (mean \pm SD), $n = 6$ mice, for a total of 2286 neurons; Fig.
122 2d; Extended Data Fig. 1b; Extended Data Fig. 2a, b). At the late learning stage, we observed
123 that individual neurons responded to either CXA or CXB (Fig. 2e), with the overall average
124 response to CXB being higher than that to CXA (Extended Data Fig. 2c, d). This difference
125 was not observed at the early training stage. We further analyzed the CXB- or CXA-responsive
126 neurons, defined as the neurons showing significant response (excitatory or inhibitory; $P < 0.05$,
127 permutation test) to presentations of CXB or CXA, respectively. Notably, at the late, but not
128 early stage of training, the CXB-excited neurons displayed stronger excitatory response to CXB
129 than CXA (Fig. 2f, g; Extended Data Fig. 2e-g). In contrast, the CXA-excited neurons displayed
130 similar excitatory response to CXA and CXB at both stages (Fig. 2f, g; Extended Data Fig. 2e-
131 g). It is noteworthy that this increased activity during CXB was consistent across trials with
132 different cloud-of-tones stimuli (Extended Data Fig 2h, i). At both the early and late training
133 stages, there were sizable CXB- or CXA-inhibited populations (Fig. 2f-i; Extended Data Fig.
134 2a, b, e-g) that responded differently to the two contexts (Fig. 2f, g; Extended Data Fig. 2e, f).

135 Since mPFC neurons showed a selective change in their response to CXB only after
136 mice fully learned to adaptively switch between the newer, CXB-guided “go left” rule and the
137 original, CXA-guided “follow the cloud” rule, these neurons may contribute to the context-
138 guided rule selection. Indeed, decoding analysis with linear classifiers (support vector machine,
139 SVM) revealed that mPFC neuron activity during the contextual period discriminated between
140 CXA and CXB with high accuracy at a single trial level, and at the late but not at the early stage
141 of training (Fig. 2j, m), suggesting that mPFC neurons encode the learned behavioral strategies.
142 Similar results were obtained when the analysis was repeated on the trials in which the same
143 cloud was presented and mice made opposite choices under the two contexts (Fig. 2k, l, m;
144 trials marked with red arrows in c). These results indicate that the neuron activity predicts
145 mice’s choices rather than tone-cloud categories. Interestingly, mPFC neurons also encoded the
146 clouds specifically in the late learning phase (Extended Data Fig. 2j, k). Together, these results
147 suggest that mPFC neurons encode the contexts and participate in context-guided decisions.

148 To determine if mPFC neurons are also required for context-guided decisions, we
149 optogenetically inhibited these neurons during the contextual period in the CX2AC task (Fig.
150 2n; Extended Data Fig. 2l, m). We found that this manipulation completely abolished mice's
151 leftward bias under CXB (Fig. 2o-q). In contrast, optogenetic inhibition of mPFC neurons in
152 the absence of the contextual cues had no effect on mice's performance in the 2AC task
153 (Extended Data Fig. 2n-p), suggesting that this manipulation does not alter general cognitive
154 functions such as attention. Our results hence indicate that mPFC neurons not only participate
155 in, but also are essential for context-guided strategy selection.

156

157 **mPFC^{DMS} neurons encode context-dependent decisions**

158 The mPFC sends a vast network of top-down projections to many subcortical areas,
159 among which the DMS has been shown to encode goal-directed behavior²². We reasoned that
160 the DMS-projecting mPFC (mPFC^{DMS}) neurons participate in the context-guided strategy
161 selection. To test this hypothesis, we selectively targeted mPFC^{DMS} neurons for imaging with
162 an intersectional viral strategy, by injecting the DMS with a retrograde AAV expressing Cre,
163 and injecting the ipsilateral mPFC of the same mice with an AAV expressing GCaMP6 in a
164 Cre-dependent manner (Fig. 3a; Extended Data Fig. 3a-c). These mice were implanted with
165 cannulae in the mPFC and, after viral expression, were trained in the CX2AC task as described
166 above (Fig. 3b; Extended Data Fig. 3d, e).

167 We imaged the activity of mPFC^{DMS} neurons during the CX2AC task at different stages
168 of training (early stage, 696 neurons in 5 mice; late stage, 550 neurons in 6 mice; Fig. 3c;
169 Extended Data Fig. 3f; Extended Data Fig. 4a). On average, these neurons showed sustained
170 activation during CXB presentation at the late stage of training, which reached a peak following
171 cloud presentation (Fig. 3c). Notably, the activity during CXB presentation was much higher
172 than during CXA presentation at the late stage of training but not at the early stage (Fig. 3c;
173 Extended Data Fig. 4a).

174 We sorted mPFC^{DMS} neurons into either CXB- or CXA-responsive ($P < 0.05$,
175 permutation test) groups, and analyzed each group's response to both contexts. Training
176 significantly increased the percentage of CXB-excited neurons ($p = 0.02$, χ^2 test,) but did not
177 change the percentage of CXA-excited neurons ($p = 0.74$, χ^2 test) (Fig. 3d). Interestingly,
178 training generally decreased the percentage of both CXB-inhibited neurons ($p = 6.01 \times 10^{-6}$, χ^2
179 test) and CXA-inhibited neurons ($p = 0.006$, χ^2 test) (Fig. 3d; Extended Data Fig. 4c-f).

180 At the late training stage, CXB-excited neurons clearly differentiated CXB from CXA,
181 as they showed sustained and ramping-up activation during CXB presentation, up to much

182 higher levels than their activity during CXA presentation (Fig. 3e, f; Extended Data Fig. 4d, f).
183 These neurons did not discriminate between CXB and CXA at the early stage of training
184 (Extended Data Fig. 4b, c, e). In contrast, CXA-excited neurons did not differentiate CXB from
185 CXA at either late or early stage of training (Fig. 3e, f; Extended Data Fig. 4b-f). These results
186 demonstrate that, through learning, mPFC^{DMS} neurons acquire ramping-up excitatory responses
187 specific to CXB.

188 We reasoned that such CXB-specific response might instruct CXB-dependent decisions.
189 To test this hypothesis and better understand how mPFC^{DMS} neuron response is related to
190 mice's decisions under different contexts, we identified all the neurons activated (z-score > 3)
191 during the cloud period and analyzed the relationship between their activity and the animal's
192 choices under either CXA or CXB. In the early training stage, these neurons (n = 68) responded
193 similarly to the clouds regardless of the choice or context (Fig. 3g, h), with the response not
194 correlated with choice selection under either CXA or CXB (Fig. 3i). Strikingly, in the late
195 training stage, these neurons (n = 88) displayed markedly different response profiles (Fig. 3j-
196 l). Under CXA, their response to the clouds scaled with the evidence strength during either
197 leftward or rightward choices (Fig. 3j, k). As a result, the amplitudes of responses to different
198 clouds correlated with the probabilities of making leftward or rightward choices (Fig. 3l).
199 However, under CXB, such predictive relationships were present only for leftward choices; for
200 rightward choices, the cloud response of these neurons had no apparent relationship with either
201 evidence strength or mice's choices (Fig. 3j-l). These results suggest that the cloud response of
202 PFC^{DMS} neurons in well-trained mice guides decision making in a context-dependent manner.
203 Under CXA (and the "follow the cloud" rule), the response preserves instructive information
204 for making both leftward and rightward choices; however, under CXB (and the "go left" rule),
205 the information is present for leftward choices but absent for rightward choices.

206 To further understand how the contexts influence decision-related cloud response in
207 PFC^{DMS} neurons, we analyzed these neurons' activity during both the context period and the
208 cloud period in different choices (Fig. 3m-p). For leftward choices in the late training stage,
209 PFC^{DMS} neurons showed increased activity following CXB presentations, and further enhanced
210 their activity upon cloud presentations. Consequently, the response to both the context and the
211 clouds (including clouds with varying evidence strength) was markedly higher under CXB than
212 under CXA (Fig. 3m, n; Extended Data Fig. 5c-h). In contrast, for rightward choices, although
213 these neurons had increased activity following CXB presentations, their activity was reduced
214 during the subsequent cloud presentations (Fig. 3o, p; Extended Data Fig. 5c-h). Of note, in the

215 early training stage, the context-induced changes in PFC^{DMS} activity were either small or absent
216 (Extended Data Fig. 5a, b).

217 Together, these results suggest that through learning, mPFC^{DMS} neurons acquire activity
218 representing context-dependent rules, which can modulate decision making and influence
219 choice behavior. In particular, CXB presentation, which instructed the “go left” rule, not only
220 increased the activity of mPFC^{DMS} neurons during the contextual period, but also promoted
221 their response to the clouds specifically during leftward choices (Fig. 3n). The latter effect is
222 likely responsible for the leftward shift of the psychometric curve under CXB (Fig. 3b, late
223 learning).

224

225 **mPFC^{VMT} neurons encode decisions independent of contexts**

226 Since silencing the VMT (including the nucleus reuniens and adjacent nuclei) has been shown
227 to impair contextual fear learning¹² and strategy shifting³⁷, we next tested whether VMT-
228 projecting mPFC (mPFC^{VMT}) neurons could also participate in context-instructed strategy
229 switching. We targeted mPFC^{VMT} neurons using the intersectional viral strategy (Fig. 4a;
230 Extended Data Fig. 6a-c) and imaged the activity of these neurons in mice in the CX2AC task
231 at different stages of training (early stage, 714 neurons; late stage, 572 neurons; Fig. 4b;
232 Extended Data Fig. 6d). On average, there was an increase in mPFC^{VMT} neuron activity in
233 response to both CXA and CXB after mice learned the task, with the activity coupled with each
234 of the task-related events: task initiation, context presentation, and cloud presentation (Fig. 4c;
235 Extended Data Fig. 6e). However, unlike mPFC^{DMS} neurons, mPFC^{VMT} neurons as a whole did
236 not show any significant difference in activity in CXB trials compared with CXA trials, at either
237 early or late stage of training (Fig. 4c; Extended Data Fig. 7a-f). Training did not change the
238 percentage of CXA- or CXB-responsive neurons either (CXA-excited, $p = 0.5222$; CXA-
239 inhibited, $p = 0.6772$; CXB-excited, $p = 0.7043$; CXB-inhibited, $p = 0.6577$; χ^2 test; Fig. 4d).
240 In addition, CXA- or CXB-excited neurons did not show any preference to either context
241 throughout training (Fig. 4e, f; Extended Data Fig. 7a, b).

242 To determine if mPFC^{VMT} neuron response would be related to mice’s decision under
243 different contexts, we identified all the neurons activated (z -score > 3) during the cloud period,
244 and analyzed the relationship between their response and animal’s choice under either CXA or
245 CXB. Like mPFC^{DMS} neurons, in the early training stage, PFC^{VMT} neurons responded similarly
246 to the clouds regardless of the choice or context (Fig. 4g-i), and the response did not correlate
247 with choice selection under either context (88 neurons; Fig. 4j). However, in the late training
248 stage, the cloud response of mPFC^{VMT} neurons ($n = 111$) became predictive of the tones in the

249 clouds (Fig. 4k-m), and of animal's choices (Fig. 4n), under both CXA and CXB. In particular,
250 under CXB, the activity of these neurons correlated with not only leftward choices, but also
251 rightward choices (Fig. 4m, n). In fact, the cloud-response relationship during either the
252 rightward or the leftward choices were highly similar between CXB and CXA conditions (Fig.
253 4o). This observation is markedly different from that of mPFC^{DMS} neurons, which did not show
254 such predictive properties during rightward choices under CXB (Fig. 3l, p).

255 A fraction of mPFC^{VMT} neurons was inhibited by context or cloud presentations across
256 training (Fig. 4d-f; Extended Data Fig. 7; Extended Data Fig. 8a-d). Interestingly, we found
257 that the inhibitory response during leftward choices was more potent under CXB than under
258 CXA, specifically in the late phase of training (Extended Data Fig. 8a-d). Such difference was
259 absent during rightward choices (Extended Data Fig 8b, d), and was also not observed in
260 mPFC^{DMS} neurons throughout training (Extended Data Fig. 8e-h).

261 Together, these results suggest that mPFC^{VMT} neurons have very different encoding
262 properties compared with mPFC^{DMS} neurons. In particular, a subset of PFC^{VMT} neurons show
263 learning-dependent excitatory response that does not represent contextual changes and the
264 associated rule switch. Instead, these neurons represent the old "follow the cloud" rule even
265 though mice make choices according to the new "go left" rule under CXB. In other words, these
266 neurons seem to keep a fixed representation of the original strategy and its associated choice
267 despite the actual changes in strategy and choice. In contrast, another subset of mPFC^{VMT}
268 neurons show learning-dependent increase in inhibition following CXB presentation
269 specifically during left choices, suggesting that inhibition of these neurons is important for the
270 CXB-dependent change in strategy.

271

272 **mPFC^{DMS} and mPFC^{VMT} have opposing roles in context-dependent decisions**

273 Since mPFC^{DMS} neuron activation following CXB presentation predicts the switch of
274 behavioral strategy from "follow the cloud" to "go left", we reasoned that suppressing this
275 neuronal activation would impair the strategy switch. On the other hand, mPFC^{VMT} neuron
276 activation stably represents the original strategy, but inhibition of a subpopulation of mPFC^{VMT}
277 neurons is associated with the strategy switch. Hence, it is possible that inhibition of mPFC^{VMT}
278 neurons might facilitate the switch.

279 To test these predictions, we used optogenetics based on *Guillardia theta* anion-
280 conducting channelrhodopsins (GtACR)^{52,53} to inhibit mPFC^{DMS} or mPFC^{VMT} neurons (Fig. 5a-
281 d; Extended Data Fig. 9a, b). We found that inhibition of mPFC^{DMS} neurons specifically during
282 the contextual period in the CX2AC task caused a reduction in leftward choice under CXB,

283 especially in the trials in which mice were most influenced by the “go left” rule (i.e., the trials
284 with clouds of -0.1 evidence strength; Fig. 5b). The same manipulation did not affect behavior
285 once the contextual cues were omitted (Extended Data Fig. 9c). In sharp contrast, inhibition of
286 mPFC^{VMT} neurons during the contextual period led to an increase in leftward choice under
287 CXB, specifically in the trials with clouds of -0.1 evidence strength (Fig. 5d). This effect
288 disappeared when the contextual cues were absent (Extended Data Fig. 9d). Of note, inhibiting
289 mPFC^{DMS} or mPFC^{VMT} neurons had no effect on CXA trials (Fig. 5b, d). Control experiments
290 showed that light illumination in the mPFC alone had no effect on behavior in the CX2AC task
291 (Fig. 5e-h; Extended Data Fig. 9e, f). Thus, inhibiting mPFC^{DMS} or mPFC^{VMT} neurons during
292 the presentation of CXB prevents or facilitates, respectively, the use of the CXB-guided “go
293 left” rule. These results suggest that mPFC neurons are causally involved in context-dependent
294 selection of strategy, with mPFC^{DMS} neurons being critical for the selection of a newly learned
295 rule whereas mPFC^{VMT} neurons favoring the use of the original rule.

296

297 **Discussion**

298 In the CX2AC task, mice learned that one context (CXA) is associated with a previously learned
299 rule (the “old rule”) that requires decisions be solely based on sensory evidence, while another
300 context (CXB) is associated with a newly learned rule, which requires that decisions be made
301 on the basis of both the sensory evidence and reward values. Thus, this task captured critical
302 features of adaptations that animals and humans make in real life, including the adjustment of
303 decisions according to reward values, the learning of a new rule on top of an already established
304 one, and the use of contexts to guide strategy selection.

305 The CX2AC task combined with *in vivo* imaging and optogenetics allowed us to
306 demonstrate, to our knowledge, for the first time that individual mPFC neurons acquire
307 response through learning that encodes contexts and is necessary for context-dependent
308 selection of decision strategies. The activity of a subset of mPFC^{DMS} neurons discriminated
309 sensory stimuli and predicted choices when mice make decisions based on sensory evidence.
310 Importantly, these neurons showed increased response to CXB compared with that to CXA,
311 and further adapted their response during subsequent sensory discrimination under CXB, with
312 the response increasing to higher levels and predicting choices if mice selected the correct
313 decision strategy. Moreover, optogenetic inhibition of mPFC^{DMS} neurons during CXB
314 presentation prevented the strategy selection. These results suggest that a brief exposure to
315 contextual cues profoundly modulates the subsequent response profile of mPFC^{DMS} neurons
316 during decision making, thereby influencing choices.

317 In contrast, although a subset of mPFC^{VMT} neurons increase context-related activity with
318 learning, these neurons do not discriminate between CXA and CXB. In addition, these neurons
319 have similar activity profiles during sensory discrimination under the old and new rules,
320 responding as if the old rule were in place and the original decision strategy were followed,
321 even when the rule has changed and the mice have adopted the new strategy. Interestingly, the
322 subset of mPFC^{VMT} neurons showing inhibitory response does discriminate between contexts,
323 with the inhibition being more potent during CXB than CXA. Furthermore, optogenetic
324 inhibition of mPFC^{VMT} neurons during CXB facilitated switching to the new strategy. These
325 results suggest that a decrease in activity in mPFC^{VMT} neurons may permit switching to the new
326 rule.

327 Altogether, our results suggest that the two mPFC top-down projections exert distinct
328 and complementary functions in decision making: while mPFC→DMS projections support
329 flexible context-guided strategy selection, mPFC→VMT projections keep the stability of
330 decision strategies. A balance of activity between the two pathways may be critical for adaptive
331 behaviors.

332

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342

343 **Author contributions**

344 O.G. and B.L. conceived and designed the study. O.G. conducted the experiments and analyzed
345 data. D.V.D.L., T.Y., X.Z. and R.S. assisted with experiments and analysis. T.Y. developed the
346 one-photon wide-field imaging system and methods. O.G. and B.L. wrote the paper with inputs
347 from all authors.

348

349 **Competing interests**

350 The authors declare no competing interests.

351

352 **Material and Methods**

353 **Animals**

354 Male and female mice with age of 2-4 months were used in all the experiments. Mice were
355 housed under a 12-h light/dark cycle (7 a.m. to 7 p.m. light) in groups of 2-5 animals, with the
356 room temperature being 22°C and humidity being 50%. Food and water were available *ad*
357 *libitum* before behavioral training. All behavioral experiments were performed during the light
358 cycle. Littermates were randomly assigned to different groups prior to experiments. All mice
359 were C57BL/6J. All experimental procedures were approved by the Institutional Animal Care
360 and Use Committee of Cold Spring Harbor Laboratory (CSHL) and performed in accordance
361 to the US National Institutes of Health guidelines.

362

363 **Stereotaxic surgery**

364 All surgery was performed under aseptic conditions and body temperature was maintained with
365 a heating pad. Standard surgical procedures were used for stereotaxic injection and implantation
366 as previously described^{50,54}. Briefly, mice were anesthetized with isoflurane (2% in a mixture
367 with oxygen, applied at 1.0 L/min), and head-fixed in a stereotaxic injection frame, which was
368 linked to a digital mouse brain atlas to guide the targeting of different brain structures (Angle
369 Two Stereotaxic System, myNeuroLab.com). Lidocaine was injected subcutaneously in the
370 head and neck area as a local anesthetic.

371

372 We first made a small cranial window (1-2 mm²) above the target brain region. For imaging or
373 optogenetic inhibition of mPFC neurons, we lowered a glass micropipette (tip diameter, ~5 µm)
374 containing the AAV1.Syn.GCaMP6f.WPRE.SV40 or rAAV9/CAG-ArchT-GFP viral solution,
375 respectively, to reach the mPFC (coordinates: 1.8 mm anterior to Bregma, 0.3 mm lateral from
376 midline, and 2.3 mm vertical from brain surface). About 0.3-0.4 µl of viral solution was
377 delivered with pressure applications (5-20 psi, 5-20 ms at 1 Hz) controlled by a Picospritzer III
378 (General Valve) and a pulse generator (Agilent). The rate of injection was ~20 nl/min. The
379 pipette was left in place for 10 min following the injection, and then slowly withdrawn.

380

381 To target the PFC^{VMT} pathway for imaging and optogenetics, we injected a retrograde AAV
382 (rAAV2-retro-Syn-Cre (HHMI-Janelia Research Campus) for imaging, or pAAV-Ef1a-
383 mCherry-IRES-Cre (Addgene) for optogenetics; 0.3-0.4 μ l) bilaterally into two different
384 locations of the VMT (coordinates: (1) 1.46 mm posterior to Bregma, 0.0 mm lateral from
385 midline, and 3.85 mm vertical from brain surface; (2) 0.82 mm posterior to Bregma, 0.0 mm
386 lateral from midline, and 3.8 mm vertical from brain surface). To target the PFC^{DMS} pathway
387 for imaging and optogenetics, we injected the same viruses (0.4-0.5 μ l) bilaterally into two
388 different locations of the DMS (coordinates: (1) 0.86 mm anterior to Bregma, 1.3 mm lateral
389 from midline, and 3.8 mm vertical from brain surface; (2) 0.38 mm anterior to Bregma, 1.4 mm
390 lateral from midline, and 3.85 mm vertical from brain surface). We then injected the
391 pAAV.Syn.Flex.GCaMP6f.WPRE.SV40 (Addgene; 0.3-0.4 μ l; unilateral for imaging), or
392 AAV_hSyn1-SIO-stGtACR1-FusionRed (Addgene; 0.3-0.4 μ l; bilateral for inhibition) into the
393 mPFC (coordinates: 1.8 mm anterior to Bregma, 0.3 mm lateral from midline, and 2.3 mm
394 vertical from brain surface).

395

396 For optogenetics, we further implanted optic fibers bilaterally 200-300 μ m above the injection
397 locations in the mPFC with a 6° angle using an optic fiber holder (ThorLabs). The optic fibers
398 (core diameter, 200 μ m; length, 3 or 4 mm; NA, 0.22; Inper, Hangzhou, China) used for the
399 photostimulation transmitted light with >90% efficiency when tested before implantation. Optic
400 fibers were attached to the skull using a UV light-sensitive dental cement (3M RelyX Unicem).
401 A home-made stainless-steel head-bar was also mounted next to the posterior part of the optic
402 fiber for head restraint. Additional dental cement was added to seal the preparation.

403

404 To prepare mice for imaging, 3-5 days following the virus injection, the mice underwent
405 another surgery as described above, during which we implanted a cannula with a glass coverslip
406 at its bottom (two types of cannulae were used: (1) outer diameter, 1.8 mm; length, 4 mm; (2)
407 outer diameter, 1 mm; length, 3 mm; Inscopix) into the mPFC. Before implanting the cannula,
408 drops of diluted anti-inflammatory solution (dexamethasone, Metacan) were added to the
409 cranial window and washed 30-60 s later with a saline solution. The cannula was slowly (~20
410 μ m/min) lowered to the mPFC with a cannula holder, to depths that were above the viral
411 injection location (coordinates: 1.8 mm anterior to Bregma, 0.3 mm lateral from midline, and
412 2.3-2.4 mm vertical from brain surface). The cannula was attached to the skull using UV light-
413 sensitive dental cement. A head-bar (for head restraint) was subsequently mounted to the skull
414 as described above. The skin of mice was sutured using medical glue (3M Vetbond Tissue

415 Adhesive). We waited for at least 4 weeks before starting the imaging experiments in these
416 mice.

417

418 **Behavioral apparatus**

419 Mice were head-restrained with the head-bar on a home-made head-fixation system. Three
420 metal spouts were placed approximately 5 mm below mouse's mouth. The distance between
421 the adjacent spouts was ~4 mm. The spouts were arranged such that the mice could reach each
422 spout with their tongue. The spouts were made of needles (CML supply, industrial dispensing
423 tips, 16 gauge, 1-1/2" long) connected to silicon tubes, which were further connected to 50-ml
424 syringes containing water. Gravity flow of water through the tubes was controlled by electronic
425 valves (Lee Company, LHD series solenoid valve). The spouts were held together using a 3D
426 printed plastic holder⁴³, which was attached to a 3-axis manual micromanipulator (Thorlabs,
427 DT12XYZ). The placement of the spouts was adjusted with the micromanipulator, and was
428 monitored with a webcam placed under the spouts. Each spout also served as part of a custom
429 "lickometer" circuit, which registered a lick event each time a mouse completed the circuit by
430 licking the spout.

431

432 Stimulus playback and trial control were performed via a Bpod/PulsePal open-source Arduino-
433 based system (Sanworks, Stony Brook, NY, USA). Custom scripts written in MATLAB based
434 on Bpod commands were used to control the delivery of different stimuli and record licking
435 events. Auditory stimuli were uploaded to the audio adaptor board using the Bpod control
436 system.

437

438 **Behavioral training**

439 Mice were kept on a water-restriction schedule (1 ml of water per day for each mouse), starting
440 48 h before the onset of training in the 2AC task. The training protocol for the 2AC task was
441 derived from previous studies^{42,43,55}. A white light signaled the start of a trial. Mice were taught
442 to initiate the trial sequence by licking the central spout, which was rewarded with 0.5 μ l of
443 water. Five seconds after the trial initiation, a 1-s "cloud-of-tones" stimulus was presented.
444 Mice were trained to lick the left spout when hearing a cloud containing high-frequency tones
445 (12-17kHz) and the right spout when hearing a cloud containing low-frequency tones (1-6kHz).
446 A correct choice was rewarded by a 3- μ l drop of water, whereas an error choice led to nothing
447 in that trial. A choice was defined as correct if mice committed to licking the same spout during
448 the second half of the cloud period. The white light was switched off when mice responded to

449 the cloud, irrespective of the choice being correct or incorrect. The inter-trial interval (ITI)
450 lasted for 10-15 seconds.

451 The tones in the cloud were drawn according to a protocol modified based on previously
452 described ones^{42,43,55}. The cloud consisted of a stream of 30-ms pure tones presented at a rate
453 of 100 tones per second. We used eighteen possible tones with frequencies logarithmically
454 spaced between 1 kHz and 18 kHz. On easy trials, tones were drawn exclusively from those
455 with the target frequencies: low (1-6 kHz) for rightward choice, and high (12-18 kHz) for
456 leftward choice. On intermediate trials, the clouds contained a mixture of tones with different
457 frequency ranges. The difference in the rates between high-frequency tones and low-frequency
458 tones in a cloud determined the strength of sensory evidence:

459 Strength of evidence for leftward choice = $[\text{Tone}_{\text{High}} - \text{Tone}_{\text{Low}}]$ (tones per s) / 100
460 (total tones per s)

461 For example, a cloud with an evidence strength of 0.6 meant that for each time slot in
462 the cloud, the chance for the tone to be picked from the high-frequency range (12-18 kHz) was
463 60% higher than the chance for it to be picked from the low-frequency range (1-6 kHz). We
464 used clouds with evidence-strength values of -1, -0.6, -0.1, 0.1, 0.6, and 1 for all the imaging
465 and pathway-specific optogenetics experiments. The negative values corresponding to the
466 strengths favoring rightward choice.

467 We started by training mice with easy trials. The different types (leftward-choice and
468 rightward-choice) of these trials were either randomly interleaved, or alternated between two
469 different blocks, with each block containing 5 trials of the same type. Once mice reached a
470 reasonable performance (70-80% correct trials, 5-20 days of training), intermediate trials with
471 clouds of fixed evidence strength were gradually introduced. For imaging experiments, all mice
472 were first trained in alternating blocks, with each block containing 5 trials of the same type
473 (leftward-choice or rightward-choice). Mice were able to categorize the clouds at 3 weeks after
474 training started and increased the performances over a 7-10-week training period.

475 After mice were able to discriminate the intermediate clouds, we started to train them
476 in the CX2AC task by adding two distinct contextual cues to the 2AC task, context A (CXA)
477 and context B (CXB). CXA consisted of a 3-s UV light and a 1-s 4.5-kHz pure tone. CXB
478 consisted of a 3-s green light and a 1-s 12kHz pure tone. In each trial, one of the two contextual
479 cues were presented at 2 s following the onset of trial initiation to indicate that one of two rules
480 would be applied. CXA informed that the original rule (the “old rule”) would be in effect, so
481 that the mice should keep making choices based on the sensory evidence in the subsequent
482 cloud. CXB informed that a large reward (10 μ l) would be delivered if mice made a correct

483 leftward choice according to the old rule; however, no rightward choice would lead to any
484 reward. Thus, under this “new rule”, mice still need to use the sensory evidence in the clouds
485 in order to make correct leftward choices, but should ignore the clouds indicating a rightward
486 choice under the old rule. We randomly interleaved CXA trials and CXB trials, but kept the
487 former as the majority of trials (70% for all the mice in Fig. 1, 2 & 5, and 4 mice in Fig. 3 & 4;
488 60% for 4 mice in Fig. 3 & 4). After 3-6 weeks of training with the two contextual cues, ~80%
489 of mice showed a substantial leftward bias in CXB trials but not in CXA trials in psychometric
490 function. The bias was assessed by averaging the behavioral performance in 6 sessions for each
491 mouse.

492

493 **Behavior analysis**

494 The psychometric curve was generated by fitting a sigmoidal function using a built-in Matlab
495 routine. The context-dependent bias index was computed as the strength of sensory evidence in
496 the cloud (between -1 and 1) where the psychometric curve crossed the chance level, that is,
497 where the fraction of leftward choice was equal to 50%. The change in bias index (Δ bias) was
498 calculated as the bias index in CXB trials minus that in CXA trials. The fraction of leftward
499 choice was calculated as the fraction in the psychometric curve corresponding to a strength of
500 sensory evidence of 0.

501

502 **Optogenetic experiment**

503 We used a green laser (532 nm, OEM Laser Systems Inc., Bluffdale, Utah, USA) for
504 photoinhibition of mPFC neurons. We delivered the light bilaterally into the mPFC during the
505 contextual period in all trials (power, 10-15 mW measured at the tip of optic fibers; 3-s constant
506 light illumination between contextual cue onset and cloud onset). Data from six sessions were
507 used for analysis.

508 For the pathway-specific photoinhibition, we delivered the light bilaterally into the
509 mPFC during the contextual period in 30% of trials. We used data from three sessions that met
510 the following criteria for analysis: (1) the bias index (see above) in CXA trials was not below -
511 0.3, indicating that mice did not have a substantial baseline bias towards the left side; and (2)
512 the difference in the bias index between CXA trials and CXB trials was larger than 0.25,
513 indicating that the mice used the contextual cues to guide decisions. Data were averaged across
514 the three sessions for each mouse.

515

516 **Calcium imaging acquisition and analysis**

517 All imaging experiments were conducted on behaving head-restrained mice in a dark, sound
518 attenuated box. A custom-built wide-field imaging system was used to image GCaMP6
519 fluorescence signals. The system consisted of four major components: excitation light source,
520 imaging optics, CCD camera and acquisition software, and mechanical parts. An LED (470 nm;
521 PE-100, CoolLED) was used as the excitation light source. During imaging, the light power
522 was adjusted to 0.1–0.4 mW, and was set to be constant for the same animal across imaging
523 sessions. A filter cube (U-MF2, Olympus), which contained the appropriate optical filters, was
524 used to ensure that only fluorescence signals with the desired wavelengths are transmitted. The
525 filters were: excitation (FF02-482/18-25, Semrock), dichroic (FF409/493/573/652-Di01,
526 Semrock) and emission (FF01-520/35-25, Semrock). An objective lens (10x, NA 0.3, WD 11
527 mm; MPLFLN10X, Olympus) was used to focus the excitation light, and collect fluorescence
528 signals through the implanted cannulae. A tube lens (180 mm; TTL180-A, Thorlabs) was paired
529 with the objective for magnification and forming images onto a monochrome CCD camera (pco,
530 digital 14 bit CCD camera, image sensor ICX285AL, pco.pixelfly), which was used to collect
531 fluorescence signals. A custom Imaging Acquisition software written in LabVIEW (National
532 Instruments) was used to interface the camera with a dedicated desktop computer and record
533 the GCaMP6 signals at a frame rate of 10 frames/s. To synchronize imaging with behavioral
534 events, Imaging Acquisition was triggered with a TTL (transistor-transistor logic) signal from
535 the Bpod State Machine (Sanworks) used for behavioral control. During imaging, the
536 timestamps of different events, including the trigger signals sent to Imaging Acquisition, the
537 onset of different stimuli, and licking events were all recorded with Bpod.

538
539 For imaging data processing and analysis, we first used Inscopix Data Processing software
540 (v.1.2.0., Inscopix) to spatially down-sample all the raw images by a factor of 4 to reduce file
541 size, and to correct the image stack for motion artifacts. The motion-corrected images were
542 cropped to remove post-registration borders and margin areas. The pre-processed image stack
543 was exported as a .tif file. Next, we used the extended constrained non-negative matrix
544 factorization optimized for one-photon imaging (CNMF-E)^{50,51,54,56,57} to demix neural signals
545 and get their denoised and deconvolved temporal activity, termed $\Delta F^{56,57}$, for further analysis.

546
547 To determine whether a neuron was significantly ($P < 0.05$) excited or suppressed by a stimulus,
548 and thus can be classified as being “responsive” to the stimulus, we used a permutation test to
549 compare the mean ΔF values during baseline (the 3-s period immediately before trial initiation)
550 with those during the contextual period (between 2 s and 5 s after trial initiation), or with those

551 during both the contextual period and the cloud period (between 2 s and 6 s after trial initiation),
552 taking all the trials into consideration. We used the Chi-square test to compare the percentages
553 of responsive neurons before and after learning. For further analyses, we used z-scores to
554 represent the dynamic activities in each neuron. To obtain the temporal z-scores for a neuron,
555 we first obtained the mean activity trace for the neuron by averaging the fluorescence signals
556 (ΔF) at each time point across all trials, and then computed the z-scores as $(F(t) - F_{\text{mean}})/F_{\text{SD}}$,
557 where $F(t)$ is the ΔF value at time t , F_{mean} , and F_{SD} are the mean and standard deviation,
558 respectively, of the ΔF values over the baseline period. To analyze the responses of neuronal
559 populations during leftward or rightward choices in trials with different cloud-of-tones stimuli,
560 we selected individual neurons with z-scores higher than 3 during CXA or CXB period. We
561 further averaged the responses of the selected neurons for each condition.

562

563 **Decoding analysis**

564 We performed population decoding analysis using the linear support vector machine (SVM) in
565 MATLAB (fitsvm) to determine whether the types of trials could be predicted on the basis of
566 the trial-by-trial population activities. We used the activities of all the simultaneously imaged
567 neurons in each session in each mouse to perform the population decoding analysis. We used a
568 subset of the low dimensional trial-by-trial neuronal activity data as the training dataset to train
569 a classifier with linear kernel function ('linear') for two-class decoding (e.g., classifying CXA
570 trials and CXB trials). Finally, we validated the classifier by using the 'predict' function to
571 classify the trial-by-trial neuronal activities in the test dataset. Activities from randomly
572 selected 80% of trials of each type were used to train the classifier, and activities from the
573 remaining 20% of trials of each type were used to test decoding accuracy. To generate the
574 shuffled data, we randomly reassigned a trial type to each of the trial-by-trial neuronal activities.
575 We then followed the same procedure as that used for classifying the actual data to decode the
576 shuffled data. We repeated this classification process 50 times for both the actual test dataset
577 and the shuffled data, and calculated the average accuracy as the decoding accuracy.

578

579 **Statistical analysis**

580 Statistical analyses were conducted using MATLAB statistical toolbox (MathWorks). The
581 statistical test used for each comparison is indicated when used. Parametric tests were used
582 whenever possible to test differences between two or more means. Non-parametric tests were
583 used when data distributions were non-normal. Analysis of variance (ANOVA) was used to
584 check for main effects and interactions in experiments with repeated measures, and for one or

585 more factors. All comparisons were two tailed. Statistical hypothesis testing was conducted at
586 a significance level of 0.05, with Bonferroni corrections when multiple tests were performed

587

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1 **Prefrontal top-down projections control context-dependent strategy selection**

2

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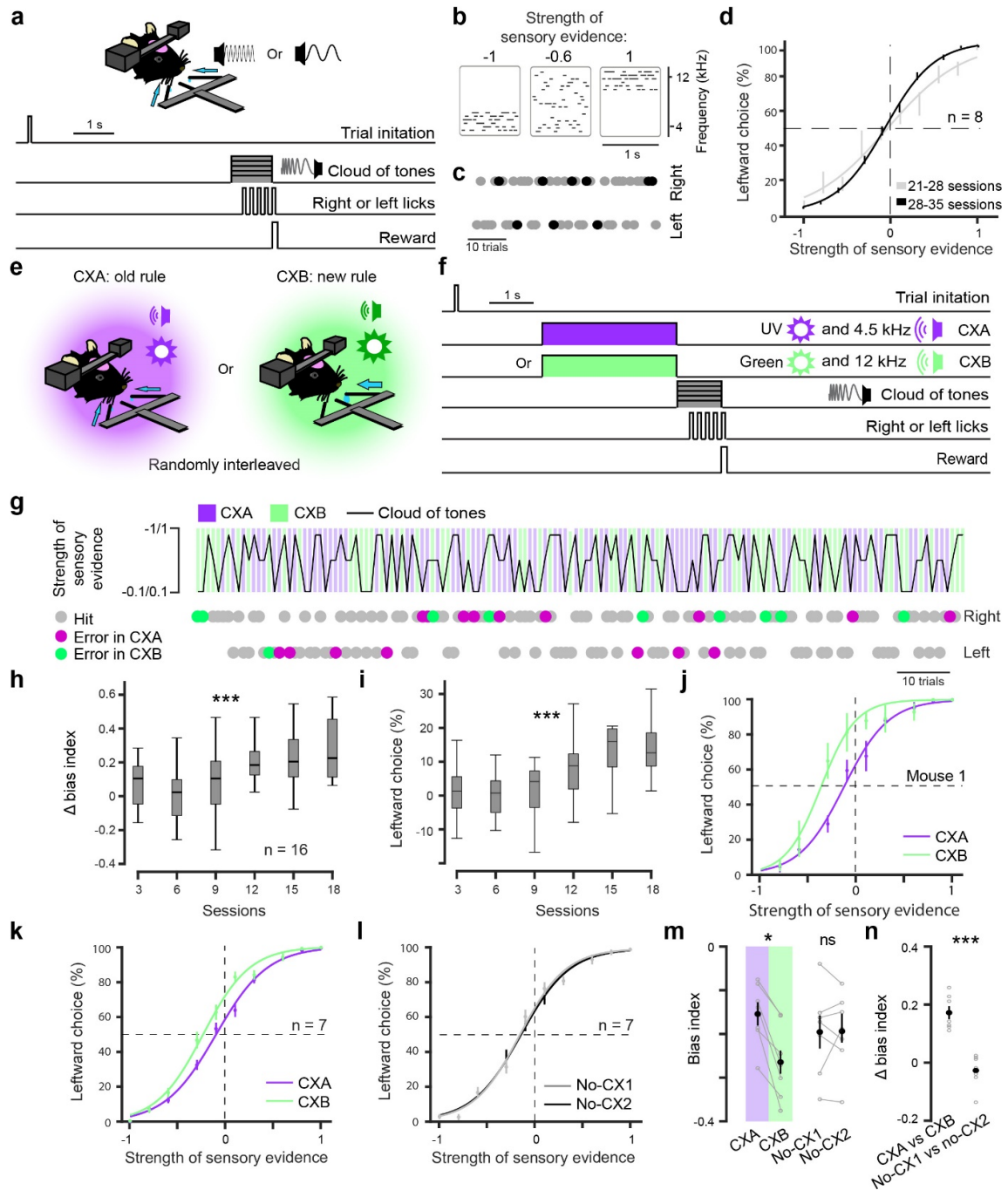
5

6

7 **FIGURES, EXTENDED DATA FIGURES, and LEGENDS**

8

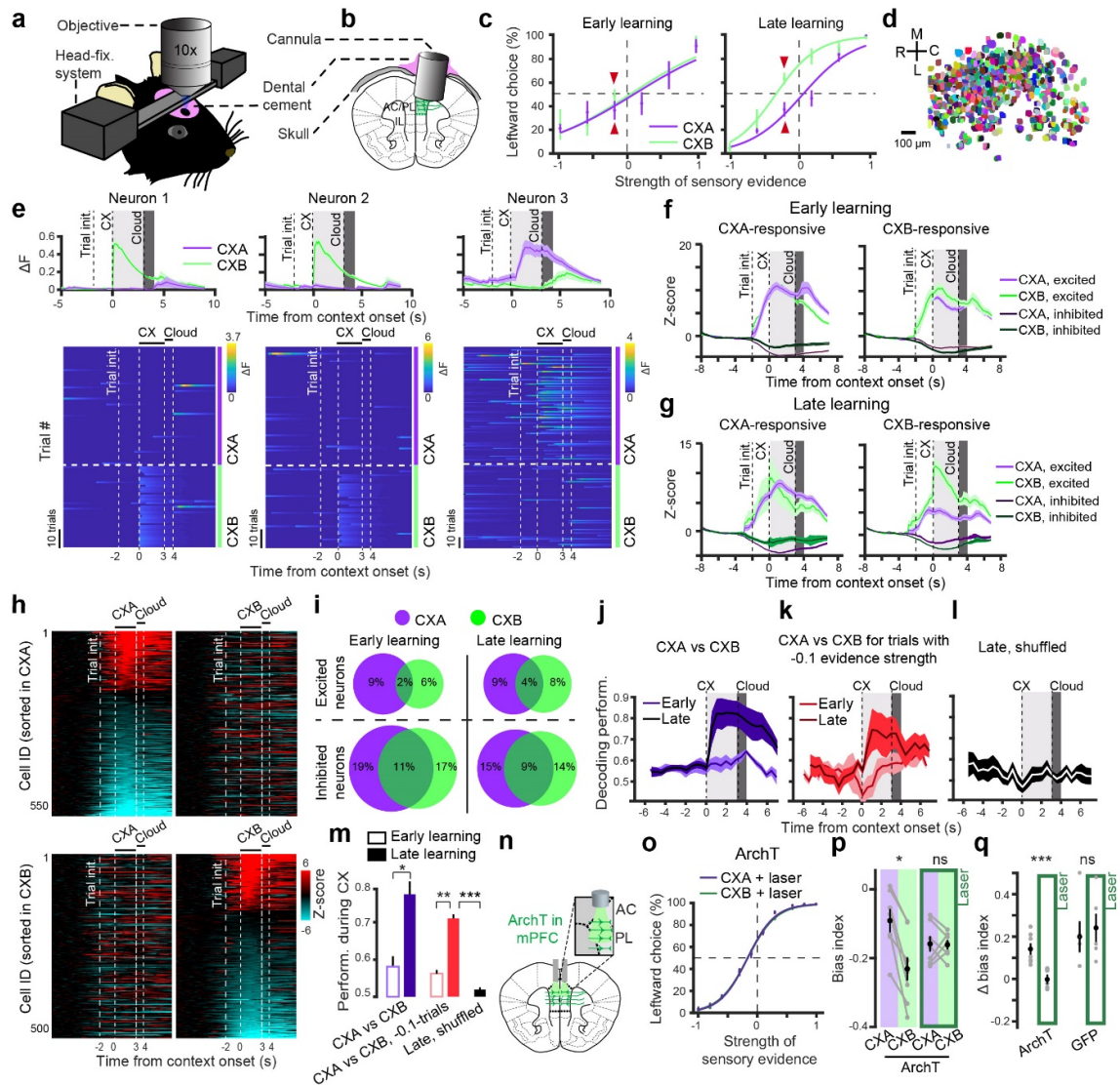
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11 **Fig. 1. Context-guided two-alternative choice (CX2AC) task.** **a**, Schematics of the setup
 12 (upper) and structure (lower) of the 2AC task. **b**, Example cloud-of-tones stimuli, in which the
 13 strength of sensory evidence for leftward choice is -1 (right), -0.6 (middle), and 1 (left). **c**,
 14 Choices in an example session. Gray and black dots represent correct and error trials,
 15 respectively. **d**, Psychometric curve of mice ($n = 8$) averaged across sessions. **e**, Schematics of
 16 the contexts and the associated rules in the CX2AC task. **f**, Schematics of the CX2AC task.
 17 Note that the CXA and CXB trials were randomly interleaved. **g**, A portion of an example

18 session of the CX2AC task, with upper panel showing the trial-by-trial arrangement of the
19 contexts and the cloud-of-tones stimuli (which are randomly interleaved across trials), and
20 lower panel showing animal choices (left or right) in the corresponding trials. **h**, Changes in
21 bias index across sessions (see Methods; $n = 16$ mice, $F(1,15) = 7.21$, $***P = 1.06e-5$, one-way
22 ANOVA). **i**, Leftward choice percentage when sensory evidence strength was 0 ($F(1,15) = 7.51$,
23 $***P = 6.2e-6$, one-way ANOVA). (**j**, **k**) Psychometric curves of an example mouse (**j**) and 7
24 mice (**k**). **l**, Psychometric curves of the same mice as those in (**k**) in the absence of contextual
25 cues, plotted for two different sets of sessions (no-CX1 & no-CX2). **m**, Bias index calculated
26 from the psychometric curves in (**k**) and (**l**) (* $P = 0.0378$, ns (nonsignificant), $P = 0.98$, paired
27 Student t-test). **n**, Changes in bias index calculated from the curve in (**k**) and (**l**) ($***P = 0.00058$,
28 paired Student t-test). Psychometric curves are averaged across six sessions. Data are presented
29 as mean \pm s.e.m.
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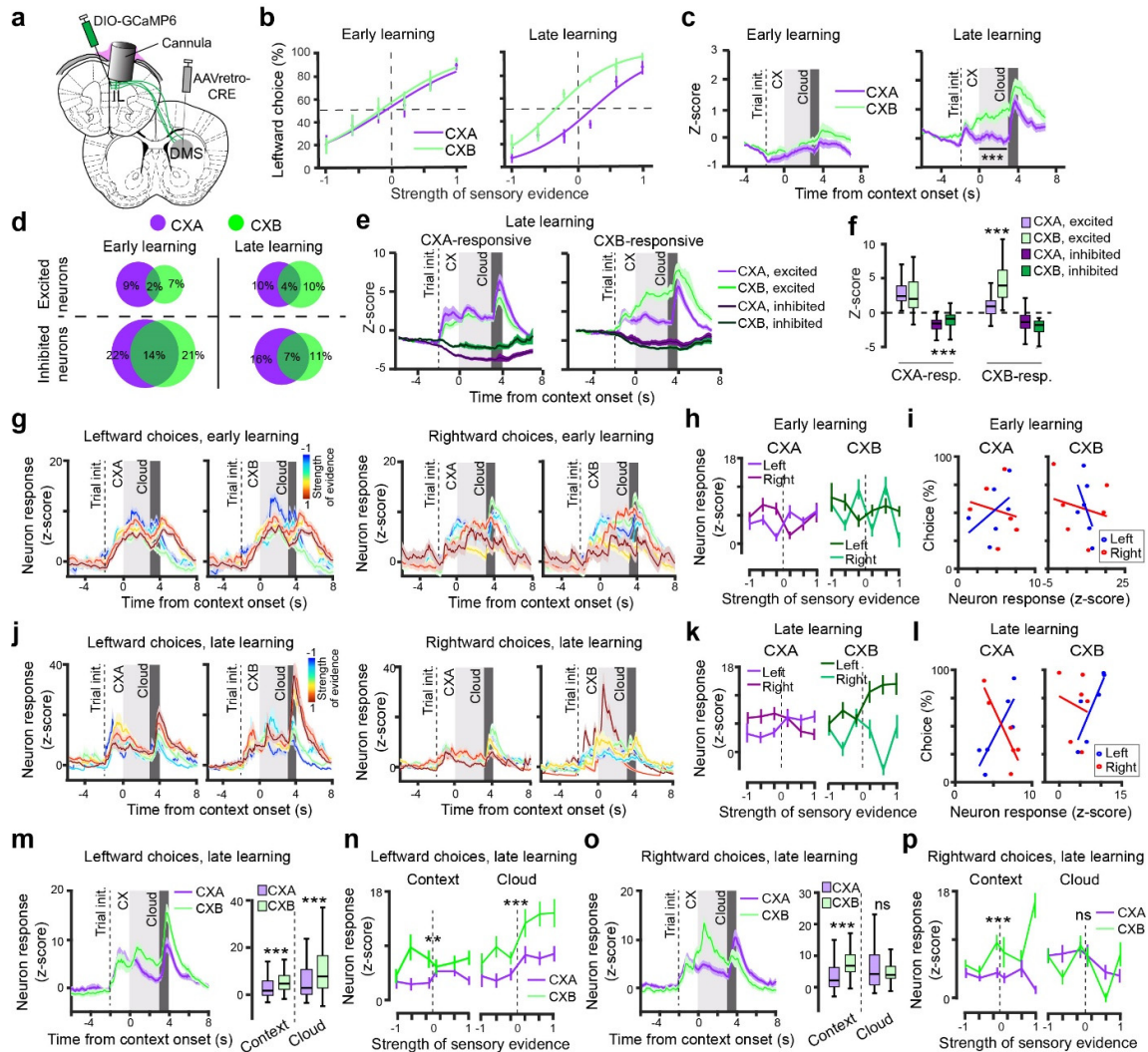


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32 **Fig. 2. Learning-induced response in mPFC neurons essential for context-guided decisions.**

33 (a, b) Schematics of the imaging setup (a) and cannula implantation (b). c, Average
 34 psychometric curves for mice at the early (left, n = 4) and late (right, n = 6) training stages in
 35 the CX2AC task. d, Neurons detected and isolated from an example mouse. e, Example neurons
 36 responding to CXB (neuron 1 and 2) or CXA (neuron 3) at the late learning stage. Top panel,
 37 average activity over all trials in each context. Bottom panel, heatmaps of trial-by-trial activity
 38 in each context. f, Average activity of CXA-responsive (left) or CXB-responsive (right)
 39 neurons in different trial types. Activity was acquired during the early learning stage. g, Same
 40 as (f) but for activity acquired during the late learning stage. h, Heatmaps of the activity (z-
 41 scored) of the individual neurons in (g). Each row represents a neuron. Top: neurons sorted
 42 according to their activity during CXA. Bottom: neurons sorted according to their activity
 43 during CXB. i, Venn diagrams showing the percent distributions of neurons significantly

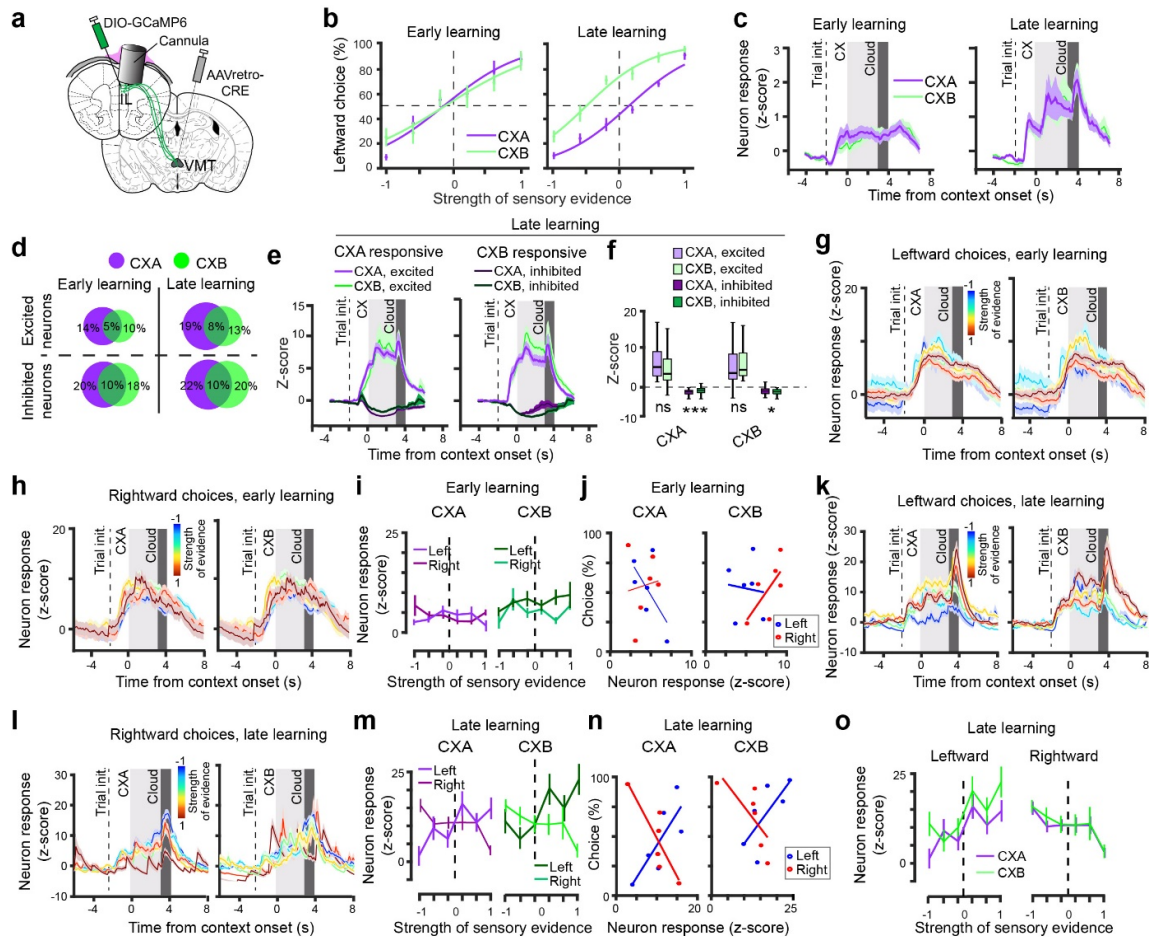
44 (permutation test, $P < 0.05$) excited (top) or inhibited (bottom) by different contexts during
45 early (left) and late (right) learning stages. Numbers indicate the overall percentages of neurons.
46 **j**, SVM classifier performance for classifying CXA trials vs CXB trials during the early ($n = 4$
47 mice) and late ($n = 6$ mice) stages of learning. **k**, SVM classifier performance for classifying
48 CXA trials versus CXB trials where the sensory evidence strength was the same (-0.1 ; indicated
49 with red arrowheads in d) but mice made opposite choices, during the early ($n = 4$ mice) and
50 late ($n = 6$ mice) stages of learning. **l**, Classification as in (k) but using trials shuffled between
51 the two conditions. **m**, Quantification of performance during the contextual period for the
52 results in (j-l) (* $P = 0.0142$, ** $P = 0.0069$, *** $P = 6.69e^{-6}$; paired t-test). **n**, Schematics of the
53 approach for optogenetic inhibition of mPFC neurons. **o**, Psychometric curves in different
54 contexts for mice ($n = 6$) in which the mPFC neurons were photo-inhibited by laser during
55 context presentation. **p**, Quantification of bias indices in CXA and CXB for the ArchT mice in
56 (o), at baseline (left) and when the mPFC neurons were photo-inhibited during the contextual
57 period (right) ($n = 7$, * $P = 0.0378$), ns (nonsignificant), $P = 0.901$, paired t-test). **q**,
58 Quantification of change in bias index caused by the contextual change. Photo-inhibition of
59 mPFC neurons reduced the change (ArchT mice, $n = 7$, *** $P = 0.00058$; GFP mice, $n = 4$, ns,
60 $P = 0.7099$; paired t-test). Psychometric curves are averaged over six sessions. Data are
61 presented as mean \pm s.e.m.
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63

64 **Fig. 3. mPFC^{DMS} neuron activity represents context-dependent decisions.** **a**, Schematics of
65 the experimental design. **b**, Average psychometric curves for mice at the early (left, $n = 5$) and
66 late (right, $n = 6$) training stages in the CX2AC task. **c**, Average activity of all neurons in CXA
67 and CXB trials in the early (left) and late (right) learning stages (late, $F_{(1,30)} = 68.677$, $***P =$
68 $4.354e-15$, two-way ANOVA with repeated measures). **d**, Venn diagrams showing the percent
69 distributions of neurons significantly (permutation test, $P < 0.05$) excited (top) or inhibited
70 (bottom) by different contexts during early (left) and late (right) learning stages. Numbers
71 indicate the overall percentages of neurons. **e**, Average activity of CXA-responsive (left) or
72 CXB-responsive (right) neurons in different trial types. Activity was acquired during the late
73 learning stage. **f**, Quantification of the average activity during context presentation in (e) (CXA-
74 responsive neurons: excitation, $P = 0.62$, inhibition, $***P = 5.9e-6$, paired t-test; CXB-
75 responsive neurons: excitation, $***P = 0.246e-6$, inhibition, $P = 0.08$, Wilcoxon rank sum test).
76 **(g-i)** Data acquired during the early learning stage. **g**, Activity of CXB-excited neurons in trials

77 in which mice made leftward choices (left panel) and rightward choices (right panel). Each
78 trace represents the averaged activity in trials with the same strength of sensory evidence. **h**,
79 Responses of neurons in (g) to the cloud-of-tones stimuli as a function of evidence strength,
80 separately plotted for different choices under either CXA (left) or CXB (right). **i**, Relationship
81 between neuron responses to the cloud-of-tones stimuli (derived from h) and choices under
82 either CXA (left) or CXB (right) (CXA: leftward, $r = 0.468$, $P = 0.34$, rightward, $r = -0.19$, $P =$
83 0.717 ; CXB: leftward, $r = -0.434$, $P = 0.389$, rightward, $r = -0.254$, $P = 0.626$; Pearson
84 correlation analysis). **(j-p)** Data acquired during the late learning stage. **j, k & l**, same as g, h &
85 i, respectively, except that data were acquired during the late learning stage. **l**, Relationship
86 between neuron responses to the cloud-of-tones stimuli (derived from k) and choices under
87 either CXA (left) or CXB (right) (CXA: leftward, $r = 0.843$, $P = 0.034$, rightward, $r = -0.882$,
88 $P = 0.0199$; CXB: leftward, $r = 0.936$, $P = 0.006$, rightward, $r = -0.266$, $P = 0.609$; Pearson
89 correlation analysis). **m**, Left panel: average activity of CXB-excited neurons in trials in which
90 mice made leftward choices under CXA or CXB. Right panel: quantification of the activity
91 during the context and cloud period (context, $***P = 3.07e-5$, cloud, $***P = 2.6e-4$, Wilcoxon
92 rank sum test). **n**, Quantification of neuron activity during context and cloud periods as a
93 function of evidence strength (context, $F(1,88) = 8.5$, $**P = 0.004$, cloud, $F(1,88) = 12.3$, $***P$
94 $= 0.0005$, Friedman test). **o & p**, Same as m & n, respectively, except that trials in which mice
95 made rightward choices were analyzed. **o**, Context, $***P = 2.2e-8$, cloud, $P = 0.83$, Wilcoxon
96 rank sum test. **p**, Context, $F(1,88) = 28.12$, $***P = 2.8e-4$, cloud, $F(1,88) = 0.55$, ns, $P = 0.45$,
97 Friedman test. Data are presented as mean \pm s.e.m. or box-and-whisker plots.
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Fig. 4. PFC^{VMT} neuron activity in the CX2AC task. **a**, Schematics of the experimental design.

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b, Average psychometric curves for mice at the early (left, $n = 6$) and late (right, $n = 6$) training

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stages in the CX2AC task. **c**, Average activity of all neurons in CXA and CXB trials in the early

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(left) and late (right) learning stages. **d**, Venn diagrams showing the percent distributions of

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neurons significantly (permutation test, $P < 0.05$) excited (top) or inhibited (bottom) by different

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contexts during early (left) and late (right) learning stages. Numbers indicate the overall

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percentages of neurons. **e**, Average activity of CXA-responsive (left) or CXB-responsive (right)

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neurons in different trial types. Activity was acquired during the late learning stage. **f**,

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Quantification of the average activity during context presentation in (e) (CXA-responsive

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neurons: excitation, $P = 0.099$, inhibition, $***P = 1.7e-3$, paired t-test; CXB-responsive neurons:

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excitation, $P = 0.167$, inhibition, $*P = 0.044$, Wilcoxon rank sum test). **(g-j)** Data acquired

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during the early learning stage. **(g, h)** Activity of CXB-excited neurons in trials in which mice

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made leftward choices (g) and rightward choices (h). Each trace represents average activity in

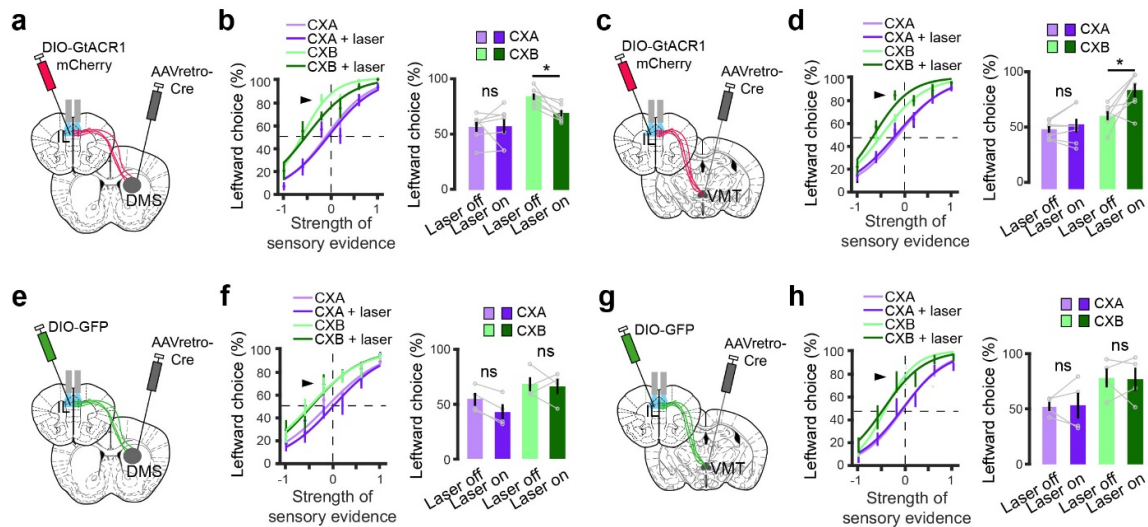
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trials with the same strength of sensory evidence. **i**, Responses of neurons in (g, h) to the cloud-

114

of-tones stimuli as a function of evidence strength, separately plotted for different choices under

115 either CXA (left) or CXB (right). **j**, Relationship between neuron responses to the cloud-of-
116 tones stimuli (derived from i) and choices under either CXA (left) or CXB (right) (CXA:
117 leftward, $r = -0.621$, $P = 0.188$, rightward, $r = 0.836$, $P = 0.109$; CXB: leftward, $r = 0.01$, $P =$
118 0.97 , rightward, $r = 0.766$, $P = 0.075$; Pearson correlation analysis). **(k-o)** Data acquired during
119 the late learning stage. **k-n**, same as g-j, respectively, except that data were acquired during the
120 late learning stage. **n**, Relationship between neuron responses to the cloud-of-tones stimuli
121 (derived from m) and choices under either CXA (left) or CXB (right) (CXA: leftward, $r = 0.796$,
122 $P = 0.058$, rightward, $r = -0.85$, $P = 0.031$; CXB: leftward, $r = 0.819$, $P = 0.045$, rightward, $r =$
123 -0.856 , $P = 0.029$; Pearson correlation analysis.) **o**, Quantification of neuron activity during
124 cloud periods as a function of evidence strength, for both rightward choices and leftward
125 choices (leftward, $F(1,68) = 0.94$, $P = 0.31$, rightward, $F(1,68) = 1.77$, $P = 0.18$, Friedman test).
126 Data are presented as mean \pm s.e.m. or box-and-whisker plots.
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129 **Fig. 5. Inhibiting mPFC^{DMS} or mPFC^{VMT} oppositely influences context-dependent**

130 **decisions. a,** Schematic of the experimental design. mPFC^{DMS} neurons were optogenetically

131 inhibited during context presentation in the CX2AC task. **b,** Left panel: psychometric curves of the

132 GtACR1 mice for different trial types. Data represent the average of 3 sessions from 7 mice.

133 Right panel: quantification of leftward choices in trials where the sensory evidence strength

134 was -0.1 (indicated by the arrowhead on the psychometric curves) ($F(1,6) = 18.54, P = 0.001$;

135 ns (nonsignificant), $P > 0.05$; * $P < 0.05$; two-way ANOVA with repeated measures, followed

136 by Student paired t-tests with Bonferroni corrections). **c,** Schematic of the experimental design.

137 mPFC^{VMT} neurons were optogenetically inhibited during context presentation in the CX2AC

138 task. **d,** Left panel: psychometric curves of the GtACR1 mice for different trial types. Data

139 represent the average of 3 sessions from 7 mice. Right panel: quantification of leftward choices

140 in trials where the sensory evidence strength was -0.1 (indicated by the arrowhead on the

141 psychometric curves) ($F(1,6) = 16.01, P = 0.0018$; ns, $P > 0.05$; * $P < 0.05$; two-way ANOVA

142 with repeated measures, followed by Student paired t-tests with Bonferroni corrections). **(e, f)**

143 Same as (a, b), except that GFP was expressed in mPFC^{DMS} neurons. **f,** Left panel: psychometric

144 curves of the GFP mice for different trial types. Data represent the average of 3 sessions from

145 4 mice. Right panel: quantification of leftward choices in trials where the sensory evidence

146 strength was -0.1 (indicated by the arrowhead on the psychometric curves) ($F(1,3) = 10.7, P =$

147 0.017 ; ns, $P > 0.05$; two-way ANOVA with repeated measures, followed by Student paired t-

148 tests with Bonferroni corrections). **(g, h)** Same as (c, d), except that GFP was expressed in

149 mPFC^{VMT} neurons. **h,** Left panel: psychometric curves of the GFP mice for different trial types.

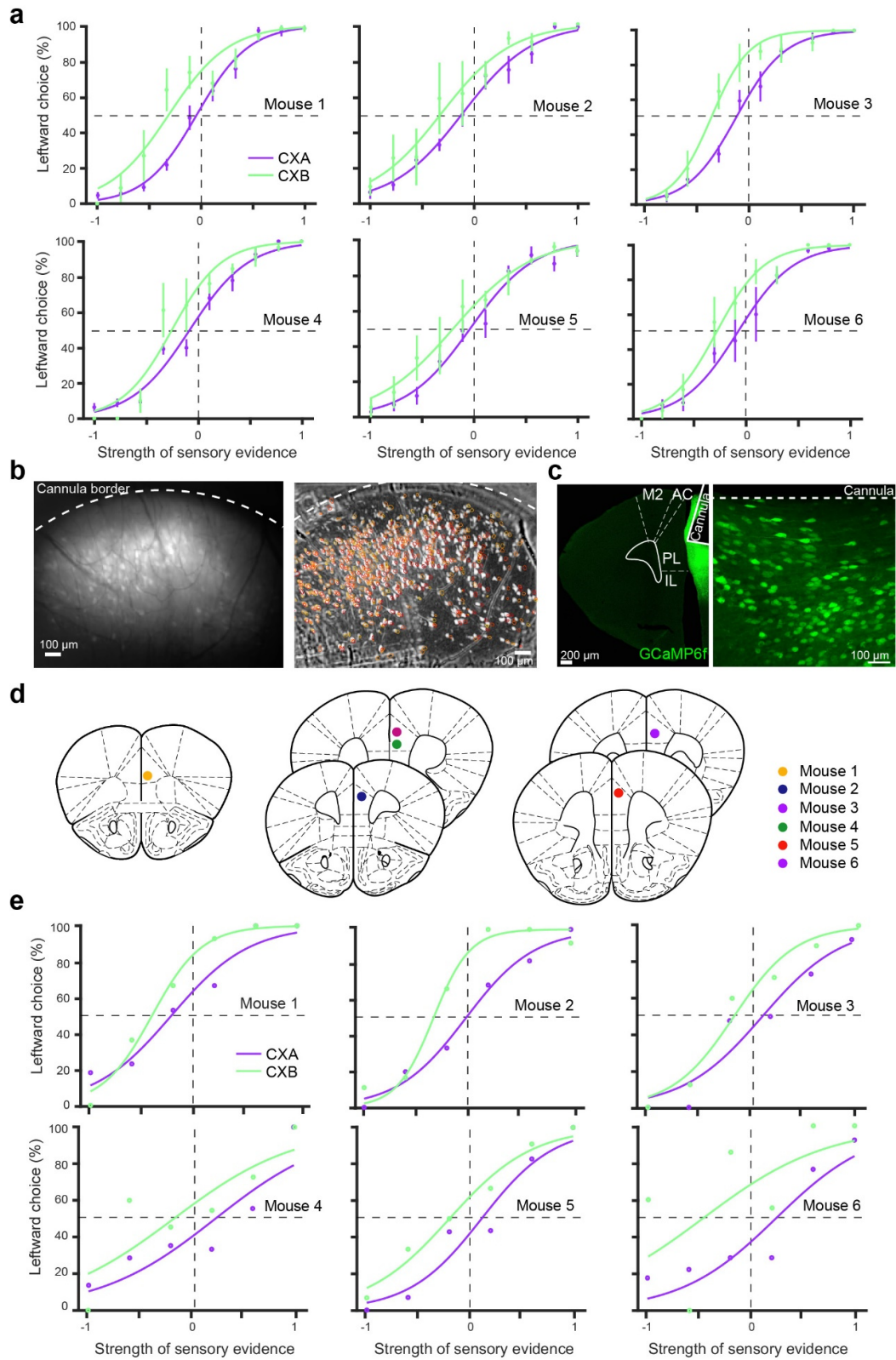
150 Data represent the average of 3 sessions from 4 mice. Right panel: quantification of leftward

151 choices in trials where the sensory evidence strength was -0.1 (indicated by the arrowhead on

152 the psychometric curves) ($F(1,3) = 8.7, P = 0.0256$; ns, $P > 0.05$; two-way ANOVA with
153 repeated measures, followed by Student paired t-tests with Bonferroni corrections). Data are
154 presented as mean \pm s.e.m.

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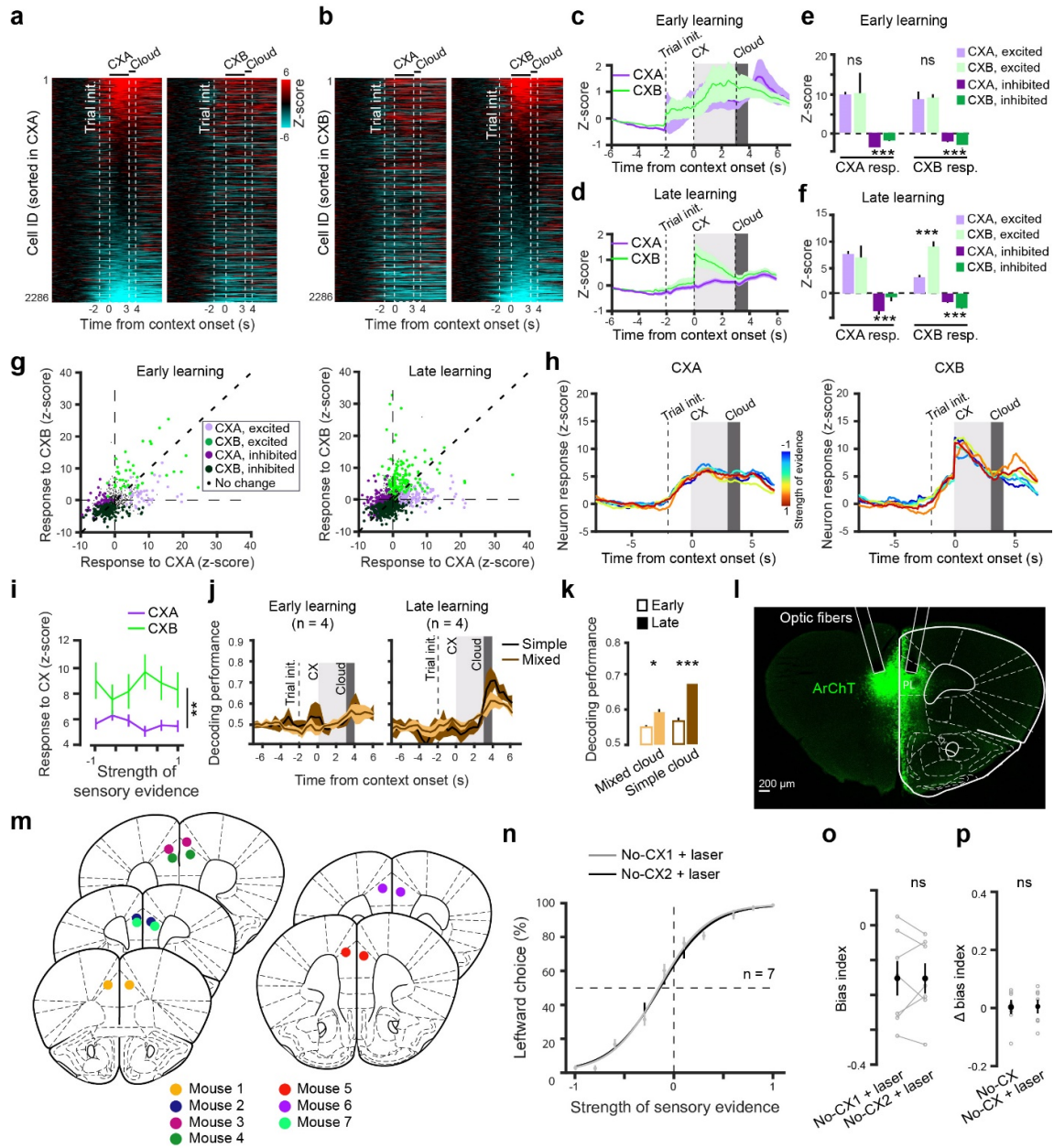


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158 **Extended Data Fig. 1. Behavioral performance and histology of the mice used for imaging**

159 **mPFC neurons. a, Psychometric curves of individual mice. Performance of each mouse under**

160 different contexts was averaged over six sessions for each of the eight cloud-of-tones stimuli,
161 which had evidence strength between -1 and 1 for the leftward choice. **b**, Images of the mPFC
162 of an example mouse. Left: maximum intensity projection of raw images recorded during an
163 imaging session. Dashed line represents the border of the cannula. Right: same field of view as
164 that in the left, with isolated individual neurons marked with circles. **c**, Confocal images
165 showing mPFC neurons expressing GCaMP6f and cannula placement. On the right is a higher
166 magnification image of the mPFC area on the left. **d**, Diagrams showing cannula placement in
167 the mPFC of different mice. **e**, Psychometric curves of individual mice during imaging.
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171 **Extended Data Fig. 2. mPFC neurons encode contextual information in a learning-**

172 **dependent manner. (a, b)** Heatmaps of the activity (z-scored) of all individual neurons under

173 different contexts ($n = 2286$ neurons, 6 mice). Each row represents a neuron. **a**, Neurons are

174 sorted according to their activity during CXA. **b**, Same data as that in (a), but neurons are sorted

175 according to their activity during CXB. **(c, d)** Average activity of all neurons in CXA and CXB

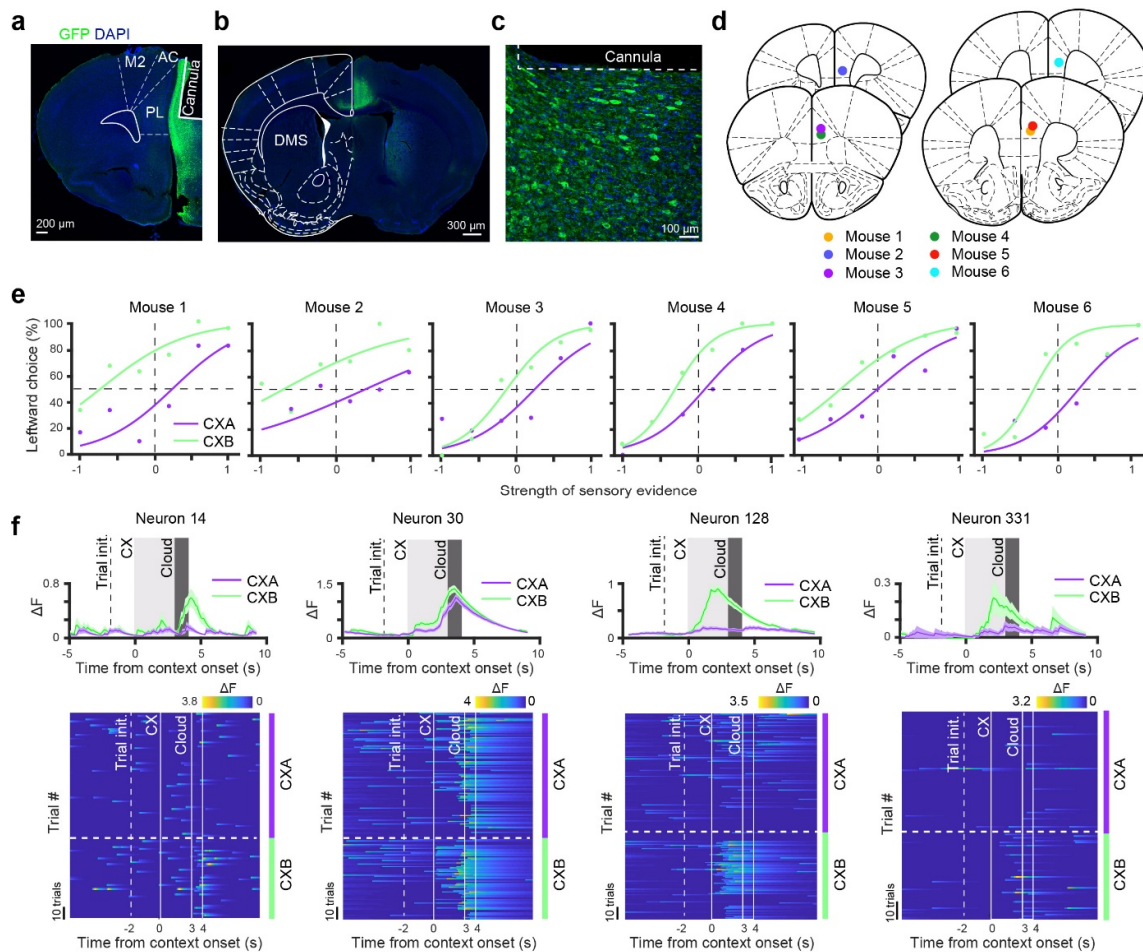
176 trials in the early (c) and late (d) learning stages. **e**, Quantification of the average activity during

177 context presentation in (c) (CXA-responsive neurons: excitation, ns (nonsignificant), $P = 0.623$,

178 inhibition, $***P = 5.751e-10$; CXB-responsive neurons: excitation, ns, $P = 0.31$, inhibition,

179 $***P = 3.535e-6$; Student paired t test). **f**, Quantification of the average activity during context

180 presentation in (d) (CXA-responsive neurons: excitation, ns, $P = 0.055$, inhibition, $***P =$
181 $5.75e-11$; CXB-responsive neurons: excitation, $***P = 1.0189e-7$, inhibition, $***P = 8.6e-12$;
182 Student paired t test). **g**, Relationship between CXA-response and CXB-response for individual
183 neurons displaying a significant activity change or no change ($P < 0.05$ or $P > 0.05$, respectively,
184 permutation test) during the contextual period, in the early (left) and late (right) stages of
185 training. **h**, Activity of CXA-excited neurons (left) and CXB-excited neurons (right) at the late
186 stage of training, with each trace representing the average activity in trials with the same
187 strength of sensory evidence. **i**, Quantification of neuron activity during the context period as a
188 function of evidence strength, for both CXA trials and CXB trials ($F(1,313) = 8.1$, $**P = 0.02$,
189 Friedman test). **j**, SVM classification performance for simple cloud-of-tones (evidence strength,
190 -1 vs 1) and mixed cloud-of-tones (evidence strength, -0.6 or -0.1 vs 0.1 or 0.6). **k**,
191 Quantification of SVM classification performance in (j), averaged over the cloud period (mixed,
192 $*P = 0.0178$; simple, $***P = 1.91e-4$, Student paired t test). **l**, Confocal images showing mPFC
193 neurons expressing ArchT and optic fiber placement. **m**, Diagrams showing optic fiber
194 placement in the mPFC of different mice. **n**, Psychometric curves for mice ($n = 7$ mice) in
195 which the mPFC neurons were photo-inhibited (with laser) during the contextual period in two
196 different sets of sessions in the absence of contextual cues (no-CX1 & no-CX2). **o**,
197 Quantification of bias indices for the mice in **n** ($n = 7$, ns, $P = 0.1527$, Student paired t-test). **p**,
198 Quantification of the change in bias index in the two different sets of sessions ($n = 7$, ns, $P =$
199 0.984 ; Student paired t-test). Psychometric curves are averaged over six sessions. Data are
200 presented as mean \pm s.e.m.
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203 **Extended Data Fig. 3. Imaging mPFC^{DMS} neuron activity during the CX2AC task. (a-c)**

204 Confocal images showing the expression of GCaMP6f and cannula placement in the mPFC (a),

205 the GCaMP6f-labeled axon fibers in the DMS originating from the mPFC (b), and a higher

206 magnification image of mPFC^{DMS} neurons expressing GCaMP6f (c). **d**, Diagrams showing

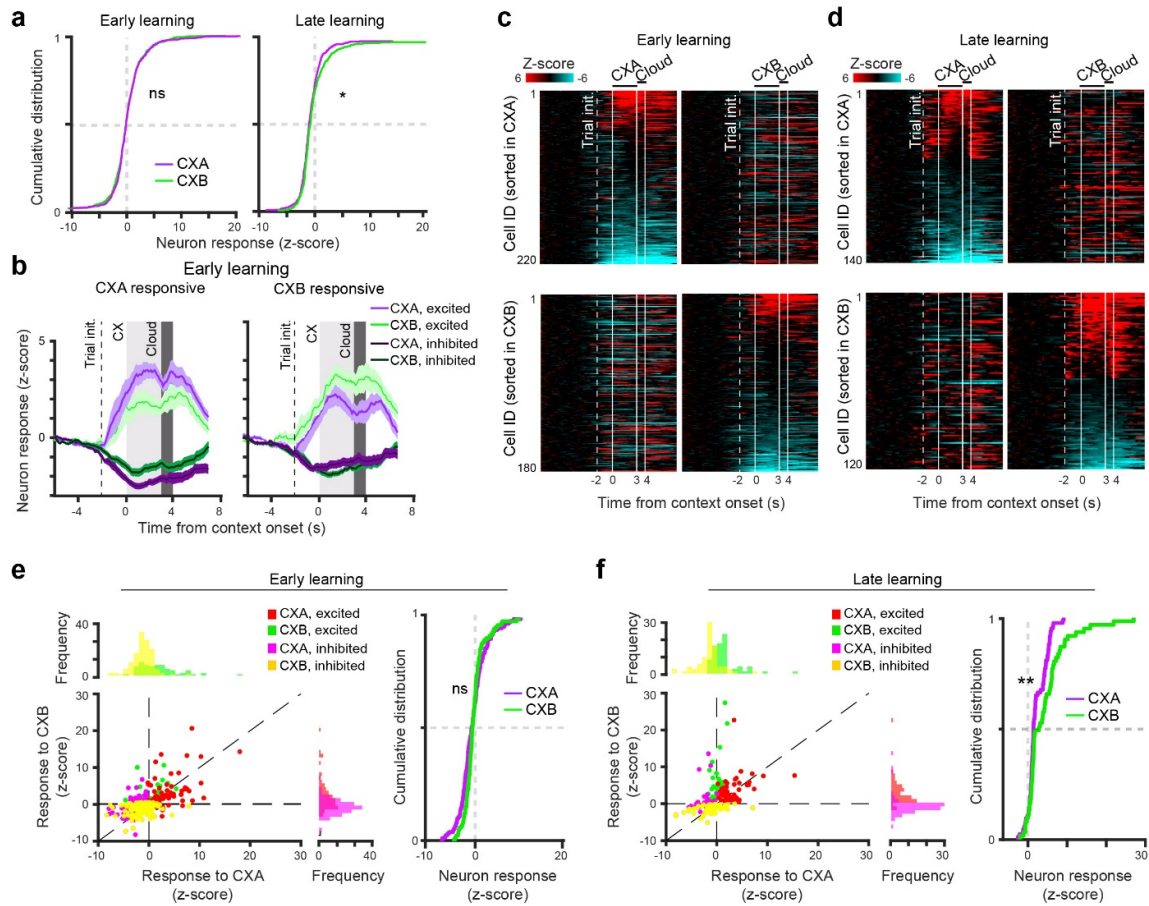
207 cannula placement in the mPFC of different mice. **e**, Psychometric curves of individual mice

208 during imaging. **f**, Responses of example mPFC^{DMS} neurons at the late learning stage. Top panel:

209 average activity over all trials in each context. Bottom panel: heatmaps of trial-by-trial activity

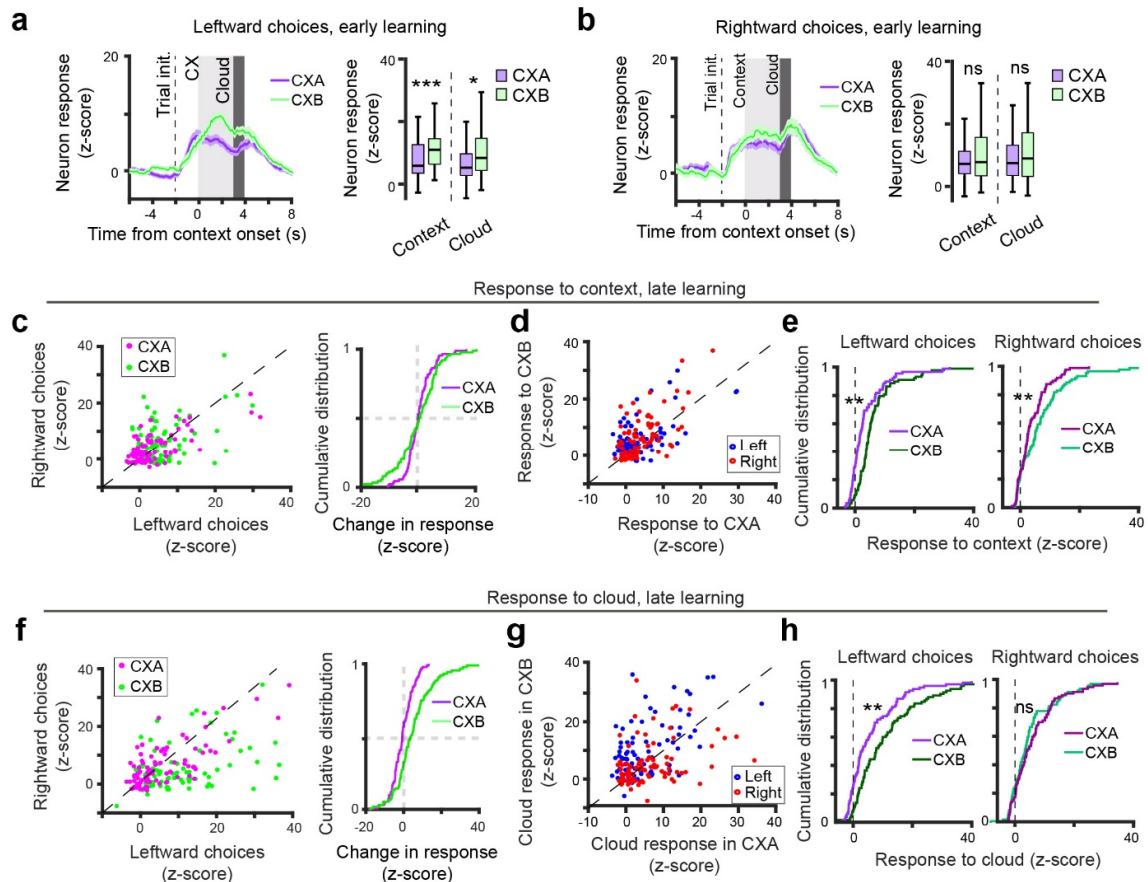
210 in each context. Data are presented as mean \pm s.e.m.

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213 **Extended Data Fig. 4. Characterization of mPFC^{DMS} neuron activity under different**
 214 **contexts in the CX2AC task. a**, Cumulative distribution of the responses of all neurons to
 215 CXA and CXB presentations in the early (left) and late (right) learning stages (early, ns
 216 (nonsignificant), $P = 0.55$, late, $*P = 0.013$, Wilcoxon rank-sum test). **b**, Left: average activity
 217 of CXA-responsive (excited or inhibited) neurons in CXA trials and CXB trials. Right: average
 218 activity of CXB-responsive (excited or inhibited) neurons in CXA trials and CXB trials.
 219 Activity was acquired during the early learning stage. **c**, Heatmaps of the activity (z-scored) of
 220 individual neurons significantly (permutation test, $P < 0.05$) responding to CXA (top) and CXB
 221 (bottom) at the early stage of learning. Each row represents a neuron. Neurons are sorted
 222 according to their activity during CXA (top) or CXB (bottom). **d**, Same as (c), except that data
 223 were acquired during the late learning stage. **e**, Left panel: CXA-responses and CXB-responses
 224 of individual neurons shown in (c). Right panel: cumulative distribution of CXA-responses and
 225 CXB-responses of individual neurons shown in (c) (ns, $P = 0.813$, Wilcoxon rank-sum test). **f**,
 226 Left panel: CXA-responses and CXB-responses of individual neurons shown in (d). Right panel:
 227 cumulative distribution of CXA-responses and CXB-responses of individual neurons shown in
 228 (d) ($**P = 0.0033$, Wilcoxon rank-sum test).



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230 **Extended Data Fig. 5. Characterization of mPFC^{DMS} neuron activity during leftward and**

231 **rightward choices in the CX2AC task. a,** Left panel: average activity of CXB-excited neurons

232 in trials in which mice made leftward choices under CXA or CXB at the early stage of learning.

233 Right panel: quantification of the activity during the context and cloud period (context, ***P =

234 0.001, cloud, *P = 0.022, Wilcoxon rank sum test). **b,** Same as (a), except that data were from

235 trials in which mice made rightward choices (context, ns (nonsignificant), P = 0.357, cloud, ns,

236 P = 0.562, Wilcoxon rank sum test). **(c-e)** Responses of individual neurons to contexts during

237 the late stage of learning. **c,** Left panel: scatter plot of the responses of individual neurons during

238 leftward and rightward choices in CXA or CXB trials (CXA, P = 0.24, CXB, P = 0.002, sign

239 rank test). Right panel: cumulative distribution of response difference between leftward and

240 rightward choices for individual neurons in each context. **d,** Scatter plot of CXA-response and

241 CXB-response of each neuron during leftward or rightward choices. **e,** Cumulative distribution

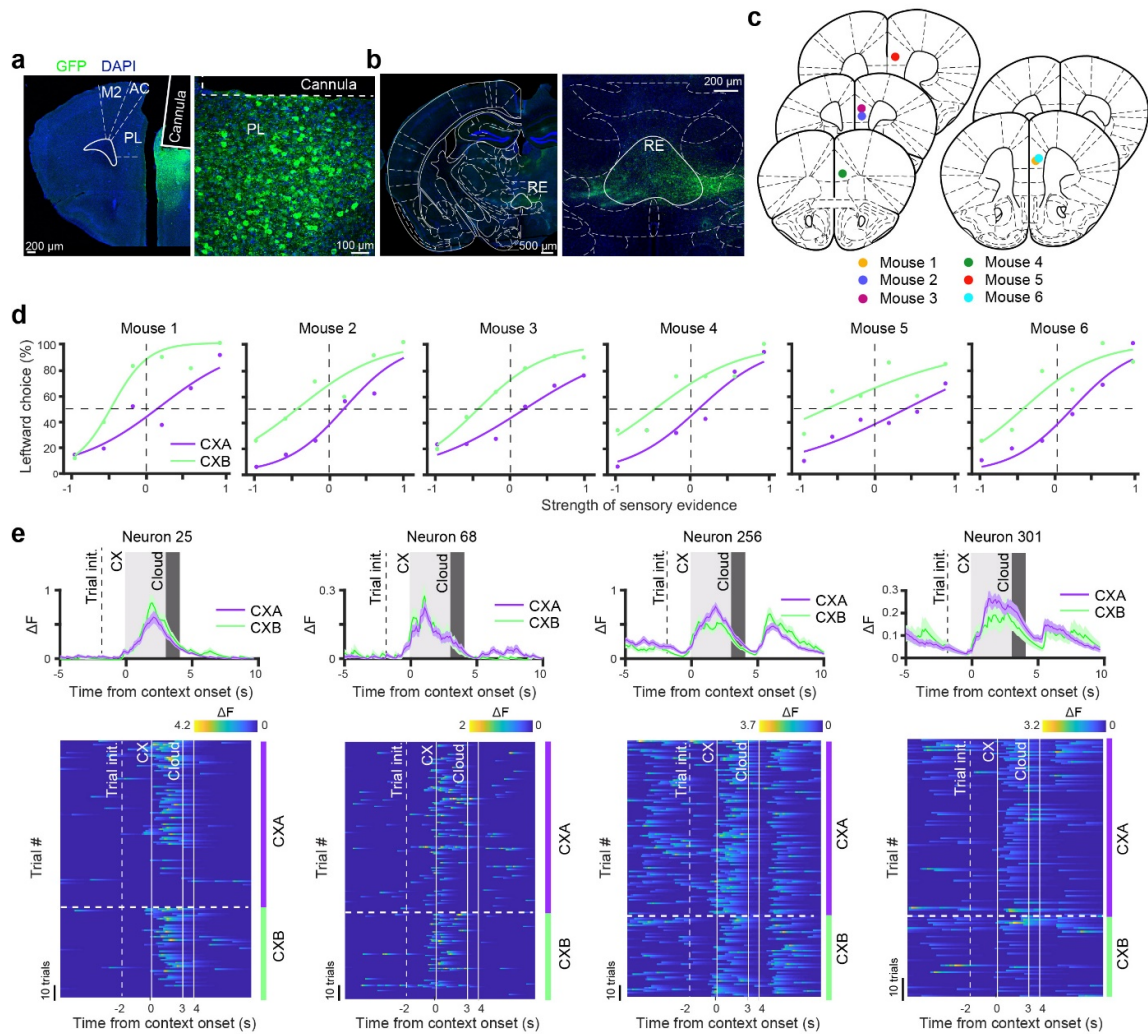
242 of CXA-responses or CXB-responses of individual neurons during leftward choices (left panel;

243 **P = 0.0094, Wilcoxon rank sum test) and rightward choices (right panel; **P = 0.0082,

244 Wilcoxon rank sum test). **(f-h)** Responses of individual neurons to cloud-of-tones stimuli during

245 late stage of learning. **f,** Left panel: scatter plot of the responses during the leftward choices and

246 rightward choices for individual neurons in CXA or CXB trials (CXA, $P = 0.149$, CXB, $P =$
247 $2.01e-5$, sign rank test). Right panel: cumulative distribution of response difference between
248 leftward and rightward choices for individual neurons in each context. **g**, Scatter plot of the
249 responses of each neuron in CXA trials and CXB trials during leftward or rightward choices. **h**,
250 Cumulative distribution of the responses of individual neurons in different contexts during
251 leftward choices (left panel; $**P = 0.0015$, Wilcoxon rank sum test) and rightward choices
252 (right panel; ns, $P = 0.3708$, Wilcoxon rank sum test).
253



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255 **Extended Data Fig. 6. Imaging mPFC^{VMT} neuron activity during the CX2AC task.**

256 Confocal images showing the expression of GCaMP6f and cannula placement in the mPFC. On

257 the right is a higher magnification image of the mPFC area in the image on the left, showing

258 mPFC^{DMS} neurons expressing GCaMP6f. **b**, Confocal images showing the GCaMP6f-labeled

259 axon fibers in the VMT area originating from the mPFC. On the right is a higher magnification

260 image of the VMT area in the image on the left, showing the GCaMP6f-labeled axon fibers in

261 the nucleus reuniens (RE) of the VMT. **c**, Diagrams showing the cannula placement in the

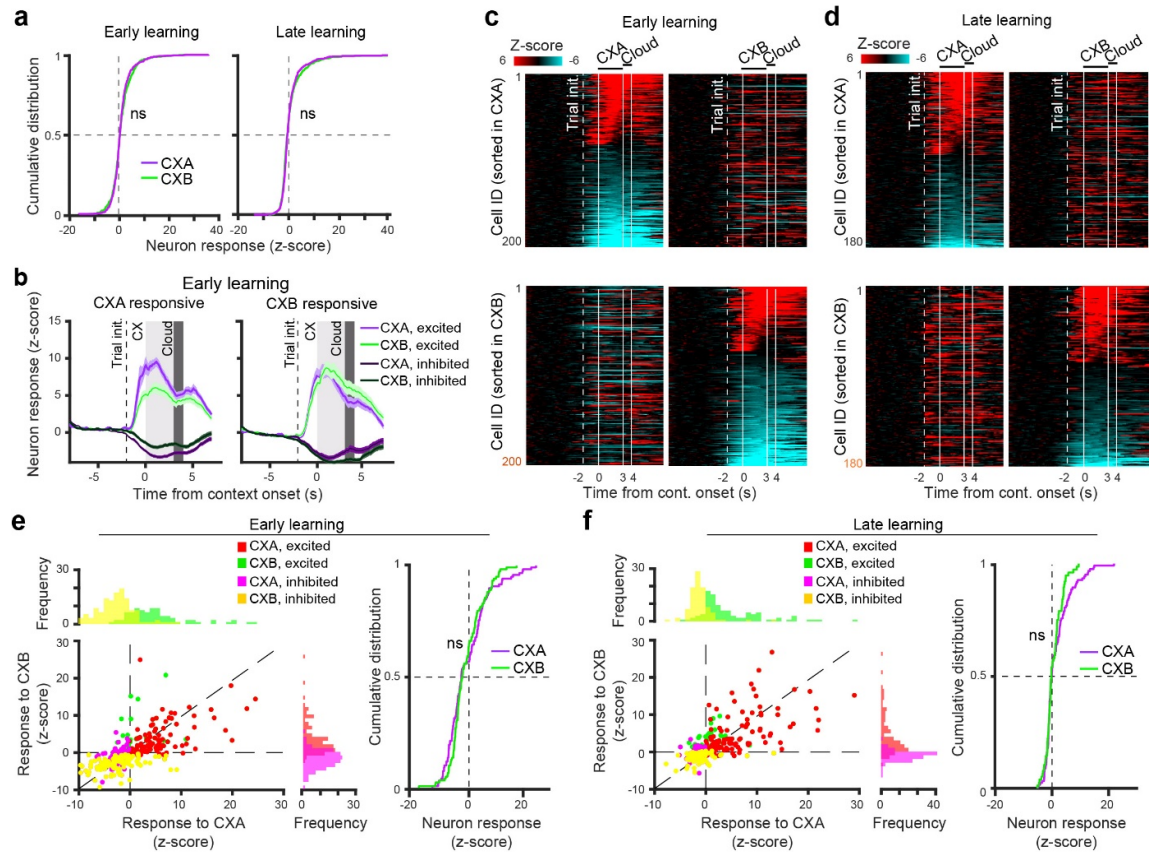
262 mPFC of different mice. **d**, Psychometric curves of individual mice during imaging. **e**,

263 Responses of example mPFC^{VMT} neurons at the late learning stage. Top panel, average activity

264 over all trials in each context. Bottom panel, heat-maps of trial-by-trial activity in each context.

265 Data are presented as mean \pm s.e.m.

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267

268 **Extended Data Fig. 7. Characterization of mPFC^{VMT} neuron activity under different**

269 **contexts in the CX2AC task. a,** Cumulative distribution of the responses of all neurons to

270 CXA and CXB presentations in the early (left) and late (right) learning stages (early, ns

271 (nonsignificant), $P = 0.41$, late, ns, $P = 0.97$, Wilcoxon rank-sum test). **b,** Left:

272 average activity of CXA-responsive (excited or inhibited) neurons in CXA trials and CXB trials.

273 Right: average activity of CXB-responsive (excited or inhibited) neurons in CXA trials and CXB trials.

274 Activity was acquired during the early learning stage. **c,** Heatmaps of the activity (z-scored) of

275 individual neurons significantly (permutation test, $P < 0.05$) responding to CXA (top) and CXB

276 (bottom) at the early stage of learning. Each row represents a neuron. Neurons are sorted

277 according to their activity during CXA (top) or CXB (bottom). **d,** Same as (c), except that data

278 were acquired during the late learning stage. **e,** Left panel: CXA-responses and CXB-responses

279 of individual neurons shown in (c). Right panel: cumulative distribution of CXA-responses and

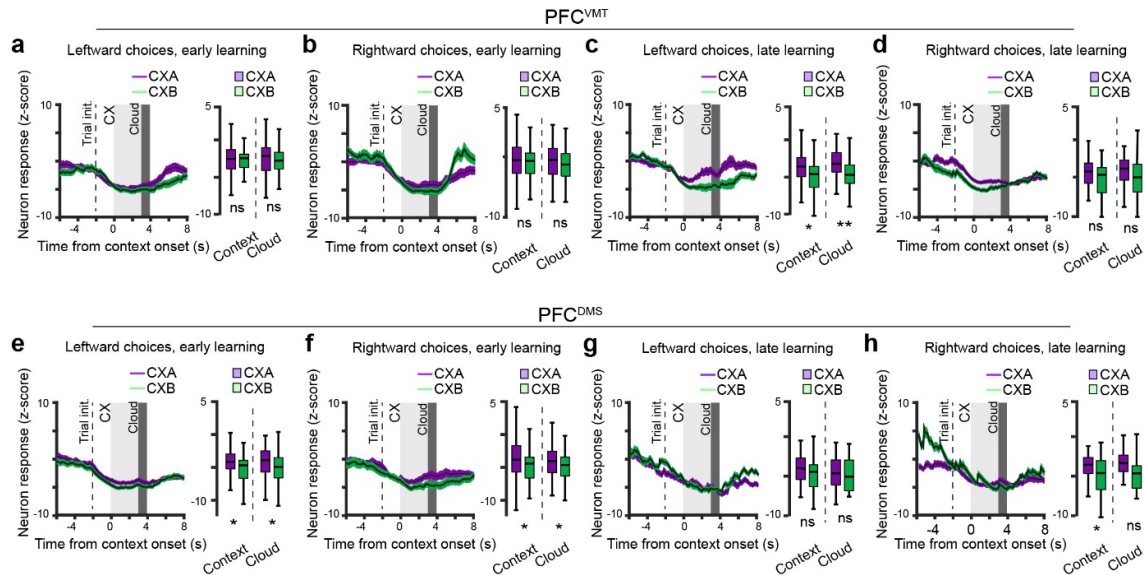
280 CXB-responses of individual neurons shown in (c) (ns, $P = 0.718$, Wilcoxon rank-sum test). **f,**

281 Left panel: CXA-responses and CXB-responses of individual neurons shown in (d). Right panel:

282 cumulative distribution of CXA-responses and CXB-responses of individual neurons shown in

283 (d) (ns, $P = 0.424$, Wilcoxon rank-sum test). Data are presented as mean \pm s.e.m.

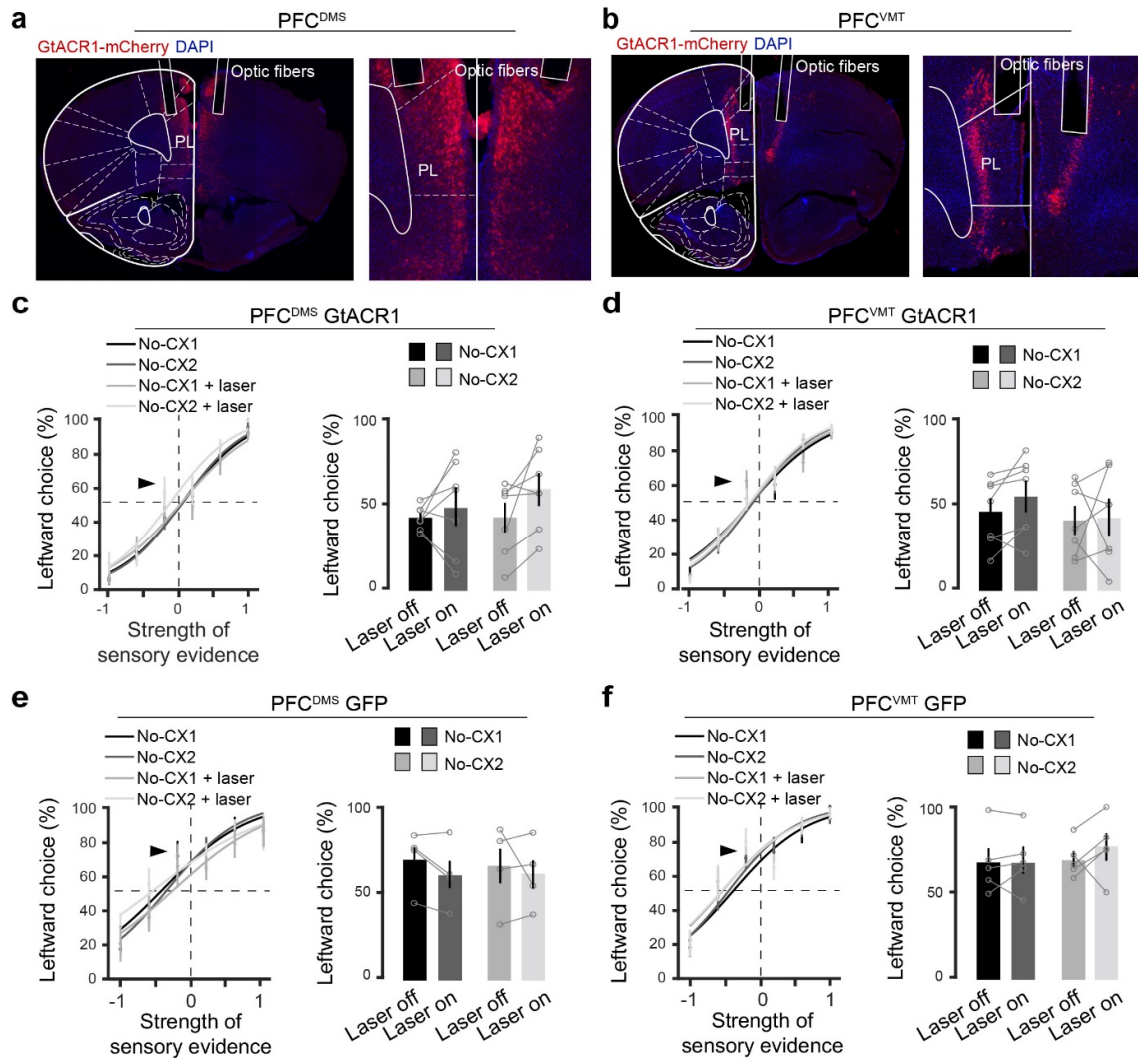
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286 **Extended Data Fig. 8. mPFC^{VMT} neurons, but not mPFC^{DMS} neurons, show decreased**
 287 **activity during context-dependent decisions. (a-d)** Activity of mPFC^{VMT} neurons showing
 288 significant reductions (z-score < -3) in activity during the contextual period. **a**, Left panel:
 289 average activity in trials in which mice made leftward choices under CXA or CXB at the early
 290 stage of learning. Right panel: quantification of the activity during the context and cloud period
 291 (context, ns (nonsignificant), $P = 0.469$, cloud, ns, $P = 0.142$, Wilcoxon rank sum test). **b**, Same
 292 as (a), except that the analysis was for trials in which mice made rightward choices (context,
 293 ns, $P = 0.246$, cloud, ns, $P = 0.178$, Wilcoxon rank sum test). **(c, d)** Same as (a, b), respectively,
 294 except that data were acquired during the late stage of learning. **c**, Context, $*P = 0.0143$, cloud,
 295 $**P = 0.002$, Wilcoxon rank sum test. **d**, Context, ns, $P = 0.069$, cloud, ns, $P = 0.189$, Wilcoxon
 296 rank sum test. **(e-h)** Activity of mPFC^{DMS} neurons showing significant reductions (z-score < -
 297 3) in activity during the contextual period. **e**, Left panel: average activity in trials in which mice
 298 made leftward choices under CXA or CXB at the early stage of learning. Right panel:
 299 quantification of the activity during the context and cloud period (context, $*P = 0.031$, cloud,
 300 $*P = 0.02$, Wilcoxon rank sum test). **f**, Same as (e), except that the analysis was for trials in
 301 which mice made rightward choices (context, $*P = 0.015$, cloud, $*P = 0.026$, Wilcoxon rank
 302 sum test). **(g, h)** Same as (e, f), respectively, except that data were acquired during the late stage
 303 of learning. **g**, Context, ns, $P = 0.438$, cloud, ns, $P = 0.714$, Wilcoxon rank sum test. **h**, Context,
 304 $*P = 0.171$, cloud, ns, $P = 0.068$, Wilcoxon rank sum test.

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Extended Data Fig. 9. Histological verification and control experiments for the optogenetic inhibition of mPFC^{DMS} neurons and mPFC^{VMT} neurons. **a**, Confocal images showing the expression of GtACR1 in mPFC^{DMS} neurons and optic fiber placement in the mPFC. On the right is a higher magnification image of the mPFC area in the image on the left. Note that mPFC^{DMS} neurons are located in layer 2/3. **b**, Confocal images showing the expression of GtACR1 in mPFC^{VMT} neurons and optic fiber placement in the mPFC. On the right is a higher magnification image of the mPFC area in the image on the left. Note that mPFC^{VMT} neurons are located in layer 5/6. **c**, Left panel: psychometric curves of mice in the CX2AC task, in which mPFC^{DMS} neurons expressed GtACR1. In randomly interleaved trials, the mPFC^{DMS} neurons were photo-stimulated during the period between trial initiation (licking at the center spout) and the onset of the cloud-of-tones, but in the absence of any contextual cues. This procedure was repeated in two different sets of sessions without contextual cues (no-CX1 & no-CX2). Right panel: quantification of leftward choices in the trials with cloud-of-

320 tones of “-0.1” evidence strength (indicated by the arrowhead on the psychometric curves)
321 ($F(1,7) = 0.7$, $P = 0.44$, two-way ANOVA with repeated measures). **d**, Same as (c), except that
322 data were from mice in which mPFC^{VMT} neurons expressed GtACR1 ($F(1,7) = 0.565$, $P = 0.44$,
323 two-way ANOVA with repeated measures). **e**, Same as (c), except that data were from mice in
324 which mPFC^{DMS} neurons expressed GFP ($F(1,3) = 0.872$, $P = 0.91$, two-way ANOVA with
325 repeated measures). **f**, Same as (c), except that data were from mice in which mPFC^{VMT} neurons
326 expressed GFP ($F(1,3) = 3.88$, $P = 0.08$, two-way ANOVA with repeated measures).