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10   **The Infralimbic, but not the Prelimbic Cortex is needed for a Complex Olfactory Memory**

11   **Task.**

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24   **Abstract**

25   The medial prefrontal cortex (mPFC) plays a key role in memory and behavioral flexibility, and

26   a growing body of evidence suggests that the prelimbic (PL) and infralimbic (IL) subregions

27   contribute differently to these processes. Studies of fear conditioning and goal-directed learning

28   suggest that the PL promotes behavioral responses and memory retrieval, while the IL inhibits

29   them. Other studies have shown that the mPFC is engaged under conditions of high interference.

30   This raises the possibility that the PL and IL play differing roles in resolving interference. To

31   examine this, we first used chemogenetics (DREADDs) to suppress mPFC neuronal activity and

32   tested subjects on a conditional discrimination task known to be sensitive to muscimol

33   inactivation. After confirming the effectiveness of the DREADD procedures, we conducted a

34   second experiment to examine the PL and IL roles in a high interference memory task. We

35   trained rats on two consecutive sets of conflicting odor discrimination problems, A and B,

36   followed by test sessions involving a mid-session switch between the problem sets. Controls

37   repeatedly performed worse on Set A, suggesting that learning Set B inhibited the rats' ability to

38   retrieve Set A memories (i.e. retroactive interference). PL inactivation rats performed similarly

39   to controls. However, IL inactivation rats did not show this effect, suggesting that the IL plays a

40   critical role in suppressing the retrieval of previously acquired memories that may interfere with

41   retrieval of more recent memories. These results suggest that the IL plays a critical role in

42   memory control processes needed for resolving interference.

43

44   **Keywords:** *memory retrieval, odor memory, medial prefrontal cortex, prelimbic cortex,*

45   *infralimbic cortex*

1. Introduction:

The medial prefrontal cortex (mPFC) is known to be involved in executive control (Euston et al., 2012; Funahashi, 2001; Rossi et al., 2009) and it plays a critical role in memory retrieval (Bontempi et al., 1999; Miller & Cohen, 2001; Tomita et al., 1999; Yadav et al., 2022). The PFC is critical for a variety of cognitive tasks that require subjects to resolve conflicting rules and responses (Miller, 2000), a function commonly referred to as behavioral flexibility (Ragozzino et al., 1999; Ragozzino, 2007). Consistent with this idea, PFC damage impairs strategy shifting tasks in humans and rodents (Birrell & Brown, 2000; Demakis, 2003; Milner, 1963; Ragozzino et al., 1999; Rich & Shapiro, 2007; Stuss et al., 2000). Our previous studies of olfactory memory in rodents have found that the mPFC is necessary when the memory demands of the task produce high levels of interference, but not when interference is minimal, suggesting that the presence of interference may be the critical factor that drives PFC engagement (Peters et al, 2013; Peters and Smith, 2020).

An extensive literature indicates that the rodent mPFC is not homogenous (Hoover & Vertes, 2007). Instead, the prelimbic (PL) and infralimbic (IL) cortices appear to play distinct roles in memory processes. Studies of fear conditioning (Milad & Quirk, 2002; Quirk et al., 2000) and action-outcome learning (Corbit & Balleine, 2003) have suggested that the PL and IL support opposing processes. Based on these and other similar findings, Gourley & Taylor (2016) proposed a "PL-go/IL-stop" model of the mPFC role in complex behaviors (but see Moorman & Aston Jones, 2015). Specifically, in studies of fear conditioning and extinction, the PL has been shown to promote the retrieval of a fear memory (Courtin et al., 2014; Do-Monte et al., 2015; Laurent & Westbrook, 2009; Sierra-Mercado et al., 2006,) while the IL inhibits retrieval (Laurent & Westbrook, 2009; Morgan et al., 1993; Quirk et al., 2000; Sierra-Mercado et al.,

69   2011). The subregions also appear to differentially modulate stimulus and context associations

70   (George et al., 2023). Similar to findings in fear studies, the PL has been observed to drive

71   cocaine seeking behavior, while the IL suppresses the behavior after extinction (Mesa et al.,

72   2022; Moorman et al., 2015). Furthermore, a study on avoidance and reward seeking

73   demonstrated distinct roles of the subregions such that IL inactivation broadly impaired active

74   and inhibitory avoidance while PL inactivation disrupted only active avoidance (Capuzzo &

75   Floresco, 2020).

76   In the present study, we examined the role of the mPFC and its PL and IL subregions in two

77   high interference olfactory memory tasks. In the first experiment, we used chemogenetics

78   (DREADDs, Designer Receptors Exclusively Activated by a Designer Drug) to suppress

79   neuronal activity in both the PL and IL, and we tested subjects on a conditional odor

80   discrimination task that had previously been shown to be sensitive to muscimol inactivation of

81   the mPFC (Devito et al, 2010). After replicating the previous study and confirming that our

82   DREADDs procedure was effective, we conducted a second experiment aimed at determining

83   whether the PL and IL play differing roles in high interference odor memory. For this

84   experiment, we trained rats on two conflicting odor discrimination problem sets and used

85   DREADDs inactivation to examine the role of each subregion during high interference test

86   sessions involving a mid-session switch between the two problem sets.

87   **2. Method:**

88   *Experiment 1: Conditional discrimination task*

89   Subjects and Surgery:

90   The subjects were 12 adult Long-Evans rats (6 females, 6 males, Charles River Laboratories,

91   Wilmington, MA). One female was excluded from the analysis due to poor perfusion and tissue

92   quality. The rats were housed singly and maintained on a 12-hr light-dark cycle, food restricted

93   to 80-85% of their ad libitum weight and given free access to water. Prior to training, the rats

94   were anesthetized with isoflurane, placed in a stereotaxic device, the skull was exposed, and

95   craniotomies were drilled for DREADDs virus infusion. An adeno-associated virus, pAAV-

96   hSyn-hM4D(Gi)-mCherry (AAV8), viral titer 2 x 10^{13} vg/mL, was injected bilaterally into the

97   PL cortex (AP 2.9mm, ML ± 0.6, DV -4.2) and the IL cortex (AP 2.9, ML ±0.6, DV -5.4) using a

98   Hamilton syringe and microinjection pump for a total volume of 250 nL per injection site. For

99   the control group, pAAV-hSyn-mCherry (AAV8), viral titer 2.6 x 10^{13} vg/ml, was injected at the

100   same coordinates. The rats were given an antibiotic (5 mg/kg Baytril) and an analgesic (5 mg/kg

101   Ketoprofen) just prior to surgery. Rats were allowed to recover for 7-10 days before beginning

102   behavioral training. Temporary inactivation of the medial prefrontal cortex was induced by i.p.

103   injection of DREADDs agonist clozapine N-oxide (CNO, 5 mg/kg) twenty minutes prior to the

104   relevant training sessions. All experiments were conducted in compliance with guidelines

105   established by the Cornell University Institutional Animal Care and Use Committee.

106   Behavioral Training Procedures:

107   Prior to training, rats were acclimated to the apparatus and then trained to dig in cups of

108   corncob cage bedding material for buried rewards (45 mg purified formula precision pellets,

109   Bioserve, Inc., Frenchtown, NJ). The apparatus was a wooden box (48 cm wide x 81 cm long x

110   51 cm deep) with three compartments, a black side, a white side and a neutral (tan woodgrain)

111   compartment in the middle, which was equipped with dividers that could be removed to allow

112   rats to access the black or white compartments. After acclimation and shaping, the rats were

113   trained to perform a conditional discrimination task. Each trial began with the rat in the neutral

114   center compartment, the divider was removed to allow the rat to access either the black or white

115   compartment, and the rat was presented with two cups containing odorized bedding material

116   (heptanol and ethyl valerate; pure odorants mixed into 10 mL of mineral oil to create a partial

117   vapor pressure of 1 Pa and mixed into 2 liters of bedding material and stored in airtight

118   containers; Cleland et al., 2002). The same two odors were presented in separate cups on every

119   trial, with the conditional rule that one odor predicted a buried reward in the black compartment

120   while the other odor contained the reward in the white compartment (i.e., black X+/Y- and white

121   X-/Y+). The assignment of the odorant valence within each compartment was counterbalanced

122   across rats, and the left and right position of the cups was randomized across trials. A digging

123   response was recorded if the rat displaced any of the bedding, except incidental displacement

124   (e.g., stepping into the cup while walking over it). The rat was allowed to dig until the reward

125   was retrieved, then returned to the center compartment for an intertrial interval (ITI) of

126   approximately 15 seconds while the experimenter prepared the cups for the next trial.

127   Rats were trained on the conditional discrimination rule using a sequence of training steps.

128   First, the rats were given blocks of 10 trials in the black compartment with the relevant

129   discrimination rule (e.g., heptanol is rewarded but ethyl valerate is not), followed by 10 trials in

130   the white compartment with the reversed discrimination rule (e.g., ethyl valerate is rewarded but

131   heptanol is not). When errors were made, corrections to dig in both cups were allowed for the

132   first 3 sessions but were not allowed for subsequent sessions. The ten trial block sessions in each

133   compartment continued until the rat achieved a behavioral criterion of 85% correct choices on

134   two consecutive sessions. The rats were then trained on alternating blocks of 5 trials until they

135   achieved 85% correct. Finally, the rats were given training sessions consisting of 64 trials with

136   the black and white compartments presented in a random sequence. After rats achieved 85%

137   correct on the final stage of training, they were given test sessions with CNO injections or saline

138   control injections. The CNO test sessions took place at least 5 weeks after surgery to allow time

139   for expression of the DREADDs receptors.

140   *Experiment 2: Odor set shifting task*

141   Subjects and Surgery:

142   The subjects were 30 adult Long-Evans rats (16 females, 14 males). Six rats were excluded

143   from the analysis due to inaccurate placement or overexpression of DREADDs receptors outside

144   the target subregions. Surgery took place prior to training and was similar to experiment 1,

145   except that the virus was selectively injected bilaterally into either the PL cortex (AP 2.8mm,

146   ML $\pm$ 0.6, DV 4.6) or the IL cortex (AP 2.8, ML $\pm$3.3, DV 6.6) using a microinjector (Nanoject

147   III, Drummond Scientific, Broomall, PA). Injections targeting the IL cortex were performed at a

148   15-degree angle from the midline in order to avoid inadvertent spread of the virus along the

149   injector track into the overlying PL cortex. For the control group, pAAV-hSyn-mCherry

150   (AAV8), viral titer 2.6x10^{13} vg/ml, was injected bilaterally into the PL or IL cortex. We injected

151   300 nL per site (20 nL pulse every 20 seconds with 10 seconds between pulses). CNO test

152   sessions took place at least 5 weeks after surgery to allow time for expression of the DREADDs

153   receptors.

154   Behavioral Training Procedures:

155   We adapted procedures previously used in our laboratory to test olfactory memory under

156   high interference conditions (Butterly et al., 2012; Peters & Smith, 2020). All training was done

157   in a white Plexiglass chamber (45 cm wide x 60 cm long x 40 cm deep). Other materials as well

158   as the procedures for acclimation and dig training were the same as experiment 1. After the rats

159   learned to reliably retrieve the buried rewards, they began training on the first of two odor

160   discrimination problem sets, each of which contained eight odor pairs (16 individual odors).

161   Twenty-four pure odorants served as cues, prepared as in experiment 1: Propyl butyrate, Ethyl

162   acetate, Anisole, Ethyl isovalerate, Furfuryl propionate, n-Butyl glycidyl ether, 1-Butanol, n-

163   Amyl acetate, Ethyl butyrate, Propionic acid, Benzaldehyde, 1-Octanol, Methyl 2-furoate, Butyl

164   butyrate, Cis-3-Hexenyl acetate, Heptanol, Ethyl valerate, 5-Methylfurfural, D-Limonene,

165   Methyl Butyrate, 2-Phenylethanol, 2-Furyl methyl ketone, 1-Nonanol, and Butyl Pentanoate.

166   For each trial, the rat was presented with the two odors comprising one of the eight

167   discrimination problems, with one of the odors always rewarded and the other not rewarded. The

168   predictive value of the odors (rewarded or non-rewarded) was counterbalanced across subjects

169   and their locations (left or right side of the chamber) were randomized across trials. The daily

170   training sessions consisted of 64 trials (eight trials with each odor pair, presented in an

171   unpredictable sequence). After reaching a criterion of 90% correct choices on two consecutive

172   sessions on the first problem set, the rats were trained on a second problem set, Set B. Each odor

173   pair in Set B consisted of a novel odor and an odor which had previously been presented in Set A

174   (Fig. 2). This ensured that the rats could not adopt a strategy of simply approaching the novel

175   odor (or avoiding the familiar odor) for each new odor pair. The rats were given daily training

176   sessions on Set B until they reached a behavioral criterion of 90% correct choices on two

177   consecutive sessions. While learning Set B, all rats were given an i.p. injection of saline to

178   acclimate them to the injection procedures to be used during the subsequent test sessions.

179   After achieving the criterion for Set B, the rats were given three consecutive days of test

180   sessions involving a mid-session switch between the two sets. The first half of each session (32

181   trials) was always the same as the problem set from the last half of the previous day's session

182   (Fig. 3A). For example, the first manipulation session consisted of 32 trials of Set B immediately

183   followed by 32 trials of Set A. The mid-session switch from one problem set to the other was not

184   cued. The second session started with 32 trials of Set A immediately followed by 32 trials of Set

185   B, and so on for the last session.

186   Perfusion and Tissue Processing:

187   After the completion of the experiment, rats were deeply anesthetized with isoflurane and

188   transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4%

189   paraformaldehyde dissolved in 0.1 M PBS. Brains were extracted, post-fixed overnight in 4%

190   paraformaldehyde dissolved in 0.1 M PBS before cryoprotection in 30% sucrose dissolved in

191   PBS for 48 h before slicing. The brains were sectioned into 40-μm coronal slices, mounted on

192   slides and stained with DAPI (ProLong Gold with DAPI). The brains of 2 control and 2

193   DREADDs rats were processed for cFos to visualize the effects of the inactivation procedures.

194   Sections were washed 3 times in PBS for 5 minutes each, then incubated in 2% normal goat

195   serum blocking solution with 0.1% Triton-X 100 for 1 hour at room temperature. The sections

196   were then incubated in primary antibody (anti-c-Fos, #2250S, 1:2000, Cell Signaling, Danvers,

197   USA) diluted in blocking solution overnight at room temperature. The following day, the

198   sections were washed 3 times in PBS for 10 minutes each, then incubated in secondary antibody

199   (Alexa Fluor 488 goat anti-Rabbit, 1:500, ThermoFisher, A-11008) at room temperature for 2

200   hours. After 3 more washes in PBS for 5 minutes each, the sections were mounted onto slides

201   and cover-slipped with ProLong Gold Antifade Mountant with DNA Stains DAPI.

202   Data Analysis:

203   Statistical analyses were performed using R. Two-way ANOVAs and Mixed-Factor ANOVA

204   were computed as needed with factors of group, treatment, session, set, and set order. Tukey

205   HSD *post hoc* tests were used to assess significance of differences between groups with alpha set

206   to 0.05.

207

208   **3. Results:**

209   *3.1 Experiment 1: The mPFC Role in Conditional Discrimination.*

210   We assessed the effects of mPFC inactivation on the conditional odor discrimination task

211   using a linear mixed effect model. Our control condition was comprised of hM4Di rats given

212   saline injections (n = 7) as well as mCherry rats given CNO injections (n = 4). Therefore, we

213   included in our model the fixed effects of group (hM4Di and mCherry) and treatment (saline and

214   CNO) and random effect of rat ID. The two control conditions are shown separately in Figure 1.

215   We found a significant interaction of the group and treatment conditions ($F(1,9)=12.82$, p =

216   0.006, Fig. 1). Post hoc (Tukey) comparisons confirmed that the two control groups were not

217   different (p = 0.99), so we combined them into a single control group and found that the hM4Di

218   CNO group performed significantly worse than controls ($t(14.6) = 4.99$, p = 0.0002, custom

219   contrast used with Kenward-Roger approximation). Interestingly, two of the DREADDs subjects

220   performed similarly to controls (Fig. 1). The reasons for this are unclear, as the hM4Di

221   expression was not noticeably different for these subjects. Overall, the behavioral impairment is

222   similar to a previous experiment using muscimol, suggesting that our chemogenetic approach

223   was effective (also see Fig. 3E). We also examined differences in males and females and found

224   no main effect of sex, and interactions of sex, group, and treatment were also not significant.

225   *3.2 Experiment 2: The Role of PL and IL in a Proactive Interference Task.*

226   For this experiment, we trained rats on two conflicting odor discrimination problem sets (Fig.

227   2 and Method) and then gave them a series of three CNO test sessions involving a mid-session

228   switch between the problem sets (Fig. 3A). Since the first CNO test session began with 32 trials

229   of problem Set B (Fig. 3A, red arrow), the same problem set the rats had been performing for

230   several days, our design offered the opportunity to determine whether PL or IL inactivation

231   impaired ongoing performance on problem set B separately from the switch manipulation. We

232   found that inactivation of either subregion significantly impaired performance (Fig. 3B). A two-

233   way ANOVA on the data of problem set B on test day one with treatment group and sex as

234   between-subject factors revealed a main effect of group ($F(2,18) = 10.2$, $p = 0.001$). Post hoc

235   comparisons revealed that both PL ($p = 0.002$) and IL ($p = 0.002$) inactivated groups

236   significantly differed from the control group but did not differ from each other ($p = 0.99$).

237   We then assessed the rats' performance over the full three-day test sequence. Average

238   performance on each test session and each half-session problem set is illustrated in figure 3C. In

239   order to simplify the analysis and focus on the change in performance across the mid-session

240   switch from one problem set to another, we computed a difference score between the first

241   problem set of each day and the second (Fig. 3D), and we submitted these values to a to a mixed-

242   factor ANOVA, with treatment group as a between-subjects factor (3 levels - Control, PL and

243   IL) and session (3 levels - days 1-3) as a within-subjects factor. This analysis revealed a main

244   effect of session ($F(2,63) = 31.06$; $p < 0.0001$) and a significant interaction of group and session

245   variables ($F(4,63) = 2.79$; $p = 0.03$). We also examined differences in males and females and

246   found no main effect of sex nor significant interactions of sex, group, and session; thus, we do

247   not further discuss effect of sex. Post hoc comparison revealed a difference in the pattern of

248   performance across sessions for the three groups. For both control subjects and subjects with PL

249   inactivation, we observed a pattern in which the rats tended to perform better on set B than set A.

250   Because the order of presentation of the odor sets varied across sessions, B-A, followed by A-B

251   and then B-A (see Fig. 3A), this resulted in difference scores that were high for the first session,

252   low for the second session and high again on the third session. In contrast, subjects with IL

253   inactivation showed no such pattern. Instead, the rats tended to perform better on the first

254   problem set of the day, regardless of whether that was Set A or Set B, resulting in positive

255   difference scores which did not change significantly across the three sessions.

256   **4. Discussion:**

257   In two experiments, we found that the mPFC plays an important role in olfactory memory

258   processes. In our first experiment, we found that combined inactivation of the PL and IL

259   impaired performance on a contextually-cued conditional discrimination task, consistent with

260   previous studies (DeVito et al, 2010). Because the predictive value of the odor cues is reversed in

261   the black and white contexts, subjects had to learn a complex conditional rule, manage

262   conflicting response tendencies, and generally exhibit the kind of behavioral flexibility that is a

263   hallmark of PFC functions (Euston et al., 2012; Navawongse & Eichenbaum, 2013; Ragozzino et

264   al., 2003). However, there are also conflicting memory demands since subjects must remember

265   which odor is associated with reward in the two contexts, so our result is also consistent with

266   theoretical accounts suggesting that the PFC mediates cognitive control over memory retrieval

267   processes (Bontempi et al., 1999; Corcoran & Quirk, 2007; Frankland et al., 2004; Takashima et

268   al., 2006). One observation from our second experiment particularly supports this idea.

269   Previously, we found that mPFC inactivation impaired performance on a single odor

270   discrimination problem set like those employed here (Peters et al., 2013). This impairment was

271   likely attributed to the requirement that subjects simultaneously manage many odor memories,

272   since there was no impairment when they were allowed to learn one discrimination problem at a

273   time. In the present study, we found that inactivation of each individual subregion produced a

274   modest, but statistically reliable impairment in ongoing performance on problem set B (Fig. 3B).

275   This occurred before subjects were exposed to the mid-session switch manipulation, so the

276   impairment could not be due to changing rules or response requirements (also see Peters and

277   Smith, 2020).

278   In our second experiment, we tested subjects' ability to perform a mid-session switch

279   between two conflicting odor discrimination problem sets (Fig. 2). Unlike the conditional

280   discrimination task, where rats could use the background context to determine which odor was

281   rewarded on any given trial, there was no explicit cue to inform the rats about the current

282   problem set. Instead, the rats had to deduce which set of rules was in effect and respond

283   accordingly. Control subjects were readily able to do this, performing well above chance levels

284   throughout the test sessions. This is consistent with numerous studies showing that intact rats are

285   capable of set-shifting and rule-switch tasks (Birrell & Brown, 2000; Dias & Aggleton, 2000;

286   Ragozzino et al., 1999, 2003, 2007; Rich & Shapiro, 2007). However, their performance was

287   notably better for problem set B, the most recently learned of the two problem sets. This finding

288   suggests that learning problem set B impaired memory for the previously learned problem set A

289   (i.e. retroactive interference, Underwood, 1957). This effect was quite striking. Of the 21 test

290   sessions conducted in seven rats over three days, performance was better on set B more than

291   90% of the time. This occurred despite strong initial learning of problem set A, with all the rats

292   achieving greater than 90% correct, and this effect persisted throughout the three testing sessions

293   even though the rats received 36 trials with problem set A each day. Rodents typically exhibit

294   very strong and persistent odor memory (Tong et al., 2014; Wang et al., 2020), suggesting that

295   the present results are not likely due to passive forgetting of problem set A. Instead, we suggest

296   that this effect is the result of active suppression of problem set A memories that is caused by

297   learning the conflicting memories of problem set B. An extensive body of work has shown that

298   memory control processes mediated by the PFC can involve suppression of conflicting memories

299   (Anderson et al., 1994; Anderson & Neely, 1996; Bekinschtein et al., 2018; Wimber et al., 2015;

300   Wu et al., 2014;). In the case of our control subjects, the poorer performance on problem set A

301   may have been mediated by the functioning of the intact PFC, particularly the IL cortex as we

302   discuss below.

303   In contrast to control subjects, rats with IL inactivation did not show reliably better memory

304   for problem set B. Instead, they tended to perform better on whichever problem set was

305   presented first on each of the test days, regardless of whether it was problem set A or set B. This

306   became apparent during test session two when, unlike controls, IL-inactivation rats performed

307   better on problem set A (see light grey bar for Day 2 in Fig. 3C and positive values in Fig. 3D for

308   IL rats, compared to Control and PL rats). In our experimental design, the first problem set for

309   each test day was the same as the end of the previous day (Fig. 3A). Thus, IL inactivation

310   resulted in better memory for the most recently experienced problem set on test day 2, without

311   the apparent retrieval advantage of set B memories seen in controls. To the extent that intact

312   controls experienced suppression of problem set A memories, as described above, IL inactivation

313   appears to have blocked this memory suppression effect, suggesting that the IL plays an

314   important role in suppressing conflicting memories.

315   Some models of PFC function suggest that the PL and IL play opposing roles in modulating

316   memory retrieval processes. As discussed above, studies of fear conditioning and extinction

317   demonstrate the differential roles of the PL and IL in promoting and inhibiting memory retrieval

318   (Do-Monte et al., 2015; Otis et al., 2017; Quirk et al., 2000). Specifically, stimulation of PL

319   neuronal activity increases fear retrieval (Vidal-Gonzalez et al., 2006) and PL inactivation

320   reduces fear retrieval (Corcoran & Quirk, 2007; Laurent & Westbrook, 2009; Sierra-Mercado et

321   al., 2011). Manipulation of IL neuronal activity has the opposite effect: inactivation increases

322   retrieval (Morgan et al., 1993; Sierra-Mercado et al., 2006, 2011) while stimulation reduced

323   retrieval (Burgos-Robles et al., 2007; Do-Monte et al., 2015; Vidal-Gonzalez et al., 2006).

324   Although this PL-go/IL-stop dichotomy is commonly cited in fear conditioning studies (see

325   Gourley & Taylor, 2016), a growing literature in goal directed learning also supports this idea

326   (Bari et al., 2011; Cholvin et al., 2016; Gutman et al., 2017; Ostlund & Balleine, 2005; Pfarr et

327   al., 2015; Tran-Tu-Yen et al., 2009; Van Holstein & Floresco, 2020). In our study, the results of

328   IL inactivation were consistent with this idea insofar as the loss of IL activity resulted in reduced

329   inhibition of the set A memories.

330   According to this theoretical framework, PL inactivation might have been expected to reduce

331   retrieval of either set A or set B memories, but we found no changes in performance during the

332   mid-session switch sessions. The reasons for this are not clear. However, it is possible that our

333   task prioritizes cognitive control processes that inhibit retrieval over those that promote retrieval.

334   Because the rats received extensive training prior to the test sessions, the odor-reward

335   associations of both problem sets were presumably very strong, and there may have been little

336   need to promote the retrieval of these already-strong memories. Instead, the requirement for

337   subjects to rapidly switch between the two strong, but conflicting sets of memories may have

338   preferentially engaged retrieval inhibition processes, rendering PL neuronal activity irrelevant to

339   performance.

340   This account positions the mPFC as a modulator of memory retrieval rather than a storage

341   site for the odor memories themselves, and previous studies have shown that the mPFC is not

342   needed for basic memory tasks that do not involve interference or complex rule switching

343   (Birrell & Brown, 2000; Peters et al., 2013; Seamans et al., 1995). However, when interference

344   does present a problem for subjects, the PL and IL are well-positioned to influence the olfactory

345   regions of the brain where odor memories may be stored. In particular, these mPFC subregions

346   have extensive anatomical projections to the anterior olfactory nucleus (Vertes, 2004).

347   Consistent with this idea, inactivation of the anterior olfactory nucleus impairs performance on

348   the conditional discrimination task used in our first experiment (Levinson et al, 2020) and

349   preliminary data from our laboratory show that neurons in this region respond to the odor cues

350   and their valence in the odor set shifting task used for experiment two (Wu et al, 2023). Thus,

351   complex olfactory memory tasks, such as those employed here, may be a particularly useful

352   approach for examining the memory functions of the mPFC and its subregions.

353

360   **Declaration of interest:** none

361   **Authors' contribution:**

362   *D.J. Jun*: conceptualization, methodology, investigation, validation, formal analysis,

363   writing – original draft, writing – review & editing, visualization

364   *R. Shannon*: investigation

365   *K. Tschida*: supervision

366   *D.M. Smith*: conceptualization, methodology, writing – review & editing, resources,

367   supervision, funding acquisition

368

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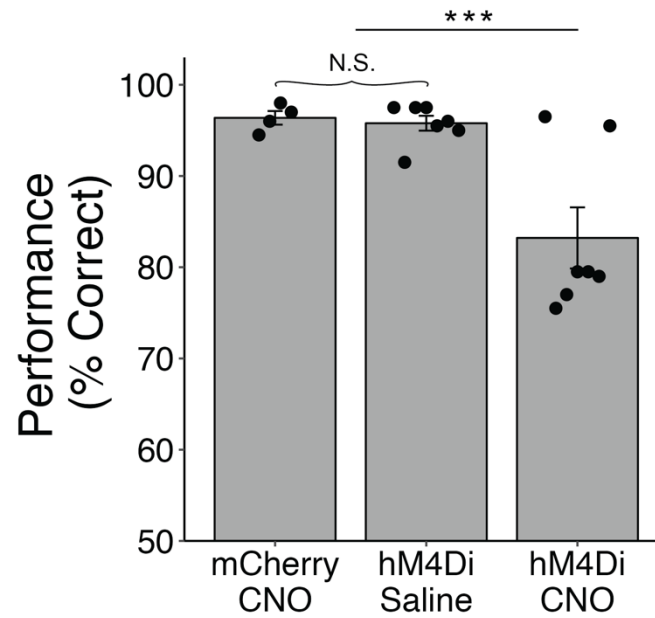
Fig 1. Performance of control subjects (mCherry CNO and hM4Di Saline) and DREADD inactivation subjects (hM4Di CNO) on the conditional discrimination task (*** indicates p < .001) with performance of individual subjects indicated by the dots.

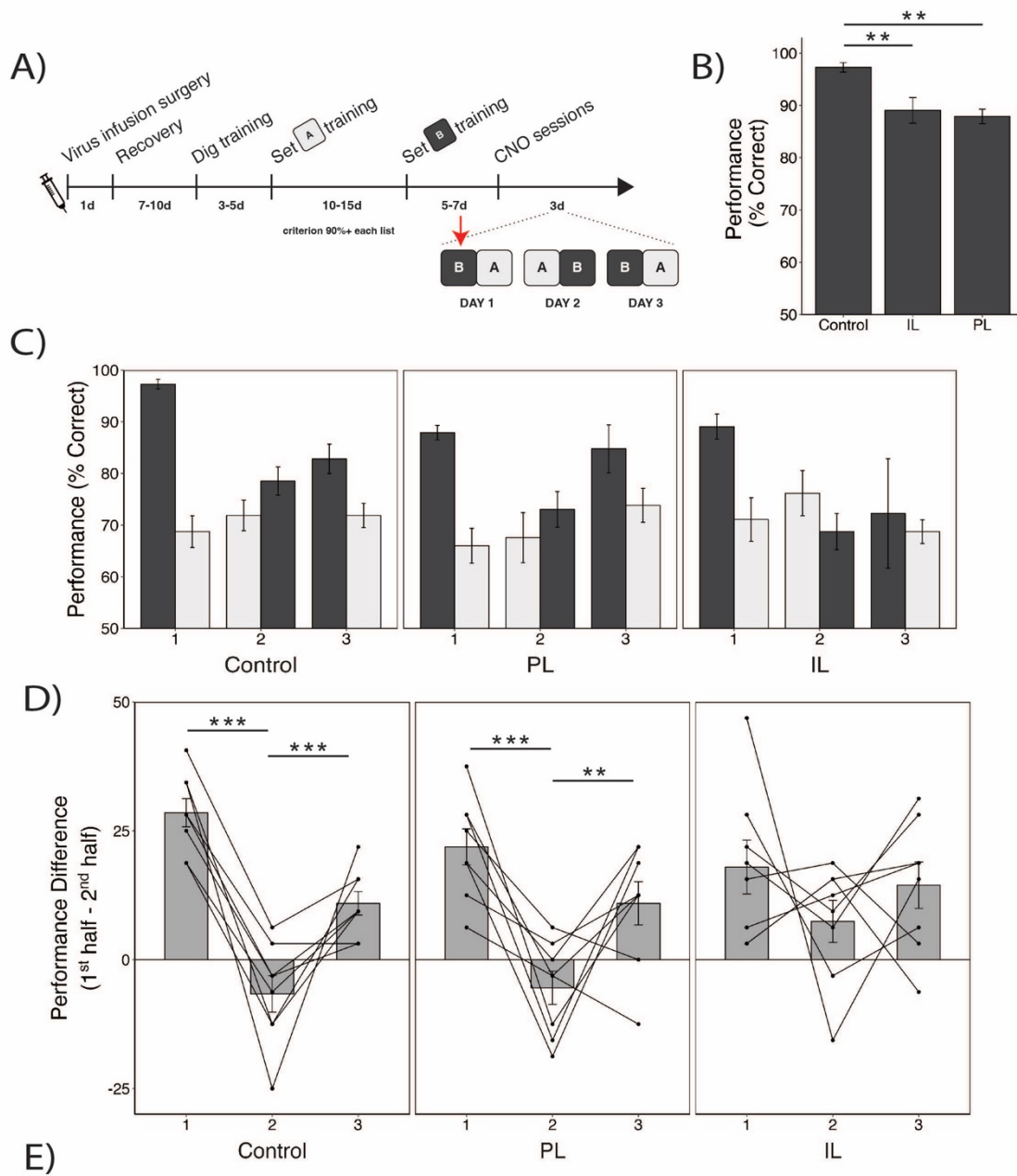| Set A		Set B	
1+	2-	17+	1-
3+	4-	4+	18-
5+	6-	19+	5-
7+	8-	8+	20-
9+	10-	21+	9-
11+	12-	12+	22-
13+	14-	23+	13-
15+	16-	16+	24-

Fig 2. The odor discrimination problem sets presented in the odor set shifting task. Each number represents a distinct odor cue, and each problem set contains 16 odors (8 pairs). Set B consists of 8 novel odors and 8 familiar odors from Set A with their reward contingencies reversed.

589

590 **Fig 3.** A) Experimental timeline for the odor set shifting task. B) Effects of CNO on ongoing
591 performance on problem set B prior to any mid-session switch manipulations (red arrow).
592 Inactivation of each subregion significantly impaired performance (*** indicates $p < .001$, ** $p <$
593 0.01). C) Performance of the three experimental groups during each of the three mid-session switch
594 test sessions. Rats were given CNO injections prior to each of the test sessions. Performance is
595 shown separately for the first and second halves of each test day, with problem set A shown in
596 light grey and problem set B shown in black. Note that each test session began with the problem
597 set from the previous day, as shown in A. D) Difference scores reflecting the change in
598 performance (% correct) from the first half of each test session to the second half. The means ($\pm$
599 SEM) are indicated by the bars, with the performance of individual subjects indicated by the dots
600 and lines. E) Confocal image of a sagittal section from an example rat is shown, with hM4Di-
601 mCherry expression targeted to the IL (red), DAPI (blue) and *c-Fos* (green). The rat was given
602 CNO and 32 trials in the odor set shifting task. Note that *c-Fos* expression is apparent in the PL,
603 where hM4Di was not expressed, but is largely absent in the IL. Scale bars = 50 um.