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1 **Noradrenergic signaling in the rodent orbitofrontal cortex is required to update goal-**
2 **directed actions**

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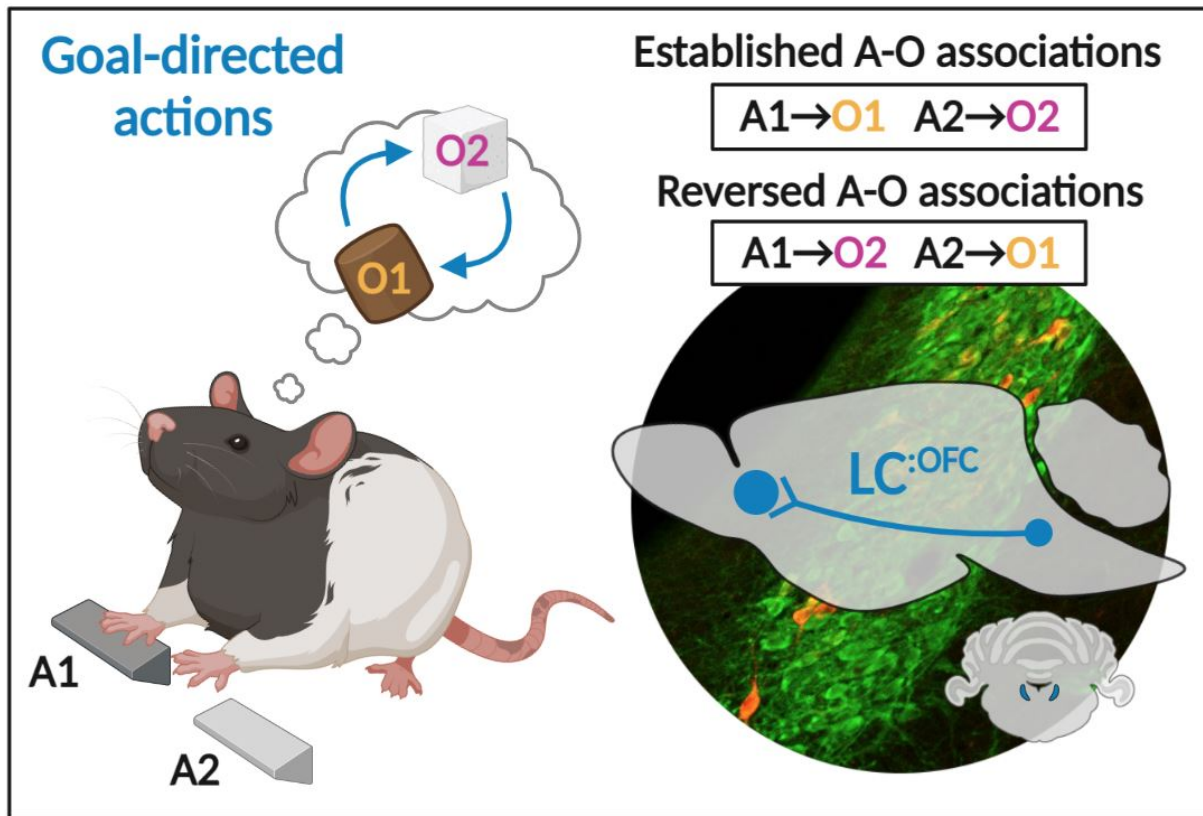
27
28 **Running title:** NA in OFC and goal-directed action

29 **SUMMARY**

30 In a constantly changing environment, organisms must track the current relationship between
31 actions and their specific consequences and use this information to guide decision-making.
32 Such goal-directed behavior relies on circuits involving cortical and subcortical structures.
33 Notably, a functional heterogeneity exists within the medial prefrontal, insular, and
34 orbitofrontal cortices (OFC) in rodents. The role of the latter in goal-directed behavior has
35 been debated, but recent data indicate that the ventral and lateral subregions of the OFC are
36 needed to integrate changes in the relationships between actions and their outcomes.
37 Neuromodulatory agents are also crucial components of prefrontal functions and behavioral
38 flexibility might depend upon the noradrenergic modulation of prefrontal cortex. Therefore,
39 we assessed whether noradrenergic innervation of the OFC plays a role in updating action-
40 outcome relationships. We used an identity-based reversal task and found that depletion or
41 chemogenetic silencing of noradrenergic inputs within the OFC rendered rats unable to
42 associate new outcomes with previously acquired actions. Silencing of noradrenergic inputs
43 in the medial prefrontal cortex or depletion of dopaminergic inputs in the OFC did not
44 reproduce this deficit. Together, our results indicate that noradrenergic projections to the OFC
45 are required to update goal-directed actions.

46

47 **Keywords:** neuromodulation, noradrenaline, prefrontal cortex, locus coeruleus, reversal, rat



49

50 **HIGHLIGHTS**

- 51 • Rats learn initial action-outcome associations in an instrumental task
- 52 • Noradrenergic depletion in the OFC prevents the encoding and expression of these
- 53 associations following reversal learning
- 54 • Dopaminergic depletion in the OFC does not result in behavioral deficits
- 55 • LC:^{OFC} noradrenergic projections are required to update action-outcome associations

56 **IN BRIEF**

57 Cerpa et al. investigate whether noradrenergic projections from the locus coeruleus (LC) to
 58 the orbitofrontal cortex are involved in updating previously established goal-directed actions
 59 following environmental change. They find that these LC projections are required to both
 60 encode and express reversed action-outcome associations in rats.

61 **INTRODUCTION**

62 Animals use their knowledge of an environment to engage in behaviors that meet their basic
63 needs and desires. In a dynamic environment, an animal must also be able to update its
64 understanding of the setting, particularly when the outcomes or consequences of its actions
65 change. Numerous studies indicate that goal-directed behaviors are supported by the
66 prefrontal cortex (PFC), and current research suggests a parcellation of functions within
67 prefrontal regions in rodents (for recent reviews see Balleine, 2019, O'Doherty et al 2017,
68 Coutureau & Parkes 2018). Specifically, the prelimbic region of the medial PFC (mPFC) is
69 needed to initially acquire goal-directed actions and learn the relationship between distinct
70 actions and their outcomes (Corbit & Balleine 2003, Killcross & Coutureau 2003, Tran-Tu-Yen
71 et al 2009), whereas the gustatory region of the insular cortex is required to recall the current
72 value of these outcomes to guide choice between competing actions (Balleine & Dickinson
73 2000, Parkes & Balleine 2013, Parkes et al 2015). In addition, the ventral (VO) and lateral (LO)
74 subregions of the orbitofrontal cortex (OFC) are required to update previously established
75 goal-directed actions (Parkes et al 2018). These data suggest that the VO and LO play critical
76 roles in tracking the relationships between actions and their consequences.

77 Behavioral flexibility also requires activity in noradrenergic (NA) neurons of the locus
78 coeruleus (LC), which are thought to track uncertainty in the current situation (Bouret & Sara
79 2004, Cope et al 2019, Jahn et al 2018, McGaughy et al 2008, Tait et al 2007, Tervo et al 2014,
80 Uematsu et al 2017). Most notably, compelling theoretical models hypothesize that the LC
81 interacts with the PFC to support behavioral flexibility (Sara & Bouret 2012). Taken together,
82 these data raise the intriguing possibility that LC NA innervation of the OFC might be needed
83 to update previously established goal-directed actions (Chandler et al 2013, Agster et al 2013,
84 Sadacca et al 2016, Cerpa et al 2019, Cerpa et al 2021). To investigate this possibility, we

85 examined whether LC NA inputs to the OFC are required to learn that a specific outcome
86 associated with a given action has changed, and to recall this information to guide choice.
87 Specifically, after learning initial action-outcome (A-O) associations, rats were required to
88 flexibly encode and use new associations during an instrumental reversal task. First, we
89 depleted NA fibers in the OFC using anti-D β H-saporin and observed a profound deficit in the
90 ability to use the reversed A-O associations to guide choice. We then found that this deficit is
91 not present when we depleted dopaminergic signaling in the OFC. Finally, we investigated the
92 temporal and anatomical specificity of this effect using viral vector-mediated expression of an
93 inhibitory DREADD. We found that silencing LC:^{OFC}, but not LC:^{mPFC}, projections impaired the
94 ability to acquire and express the reversed instrumental contingencies. Collectively, these data
95 demonstrate that LC NA projections to the OFC are required for both encoding and recalling
96 the identity of an expected instrumental outcome, specifically when that identity has changed.

97 **RESULTS**

98 **Initial goal-directed learning does not require NA signaling in the OFC**

99 We first assessed if the initial acquisition and expression of goal-directed actions requires NA
100 signaling in the OFC using the behavioural design (**Figure 1A**). Rats in the Pre group were
101 injected with the anti-D β H saporin toxin (SAP) before the initial instrumental training, during
102 which responding on one action (A1) earned O1 (sucrose or grain pellets, counterbalanced),
103 and responding on the other (A2) earned O2 (grain or sucrose pellets, counterbalanced). Rats
104 in group Post were similarly trained, but were injected with SAP following this initial stage. To
105 deplete NA projections, rats were given bilateral injections of SAP or inactive anti-IgG saporin
106 (CTL) targeting the VO and LO. SAP infusion resulted in extensive NA fiber loss in the VO and
107 LO, as revealed by a main effect of group ($F_{(1,53)} = 276.95$, $p < 0.001$) and region ($F_{(1,53)} = 12.31$,
108 $p < 0.01$), and a significant group (CTL vs SAP) X region (VO vs LO) interaction effect was also
109 detected ($F_{(1,53)} = 12.42$, $p < 0.01$) (**Figure 1B**). Simple effect analyses confirmed a significant
110 reduction of NA fibers density in both the VO ($F_{(1,53)} = 282.27$, $p < 0.001$) and the LO ($F_{(1,53)} =$
111 232.23 , $p < 0.001$) following SAP injection. By contrast, we found a higher volume of NA fibers
112 in the VO of the control group, as compared to the LO ($F_{(1,53)} = 23.45$, $p < 0.001$), a result
113 consistent with previous findings (Cerpa et al 2019). However, unexpectedly, significant fiber
114 loss was also observed in the medial prefrontal cortex (mPFC; $F_{(1,53)} = 195.57$, $p < 0.001$; **Figure**
115 **1C**).

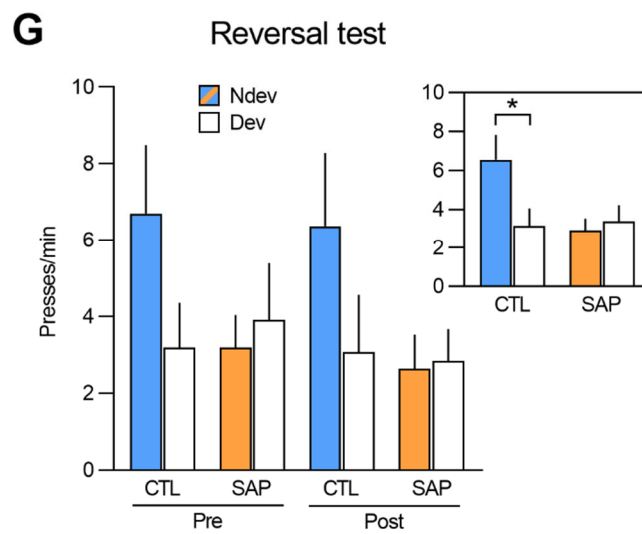
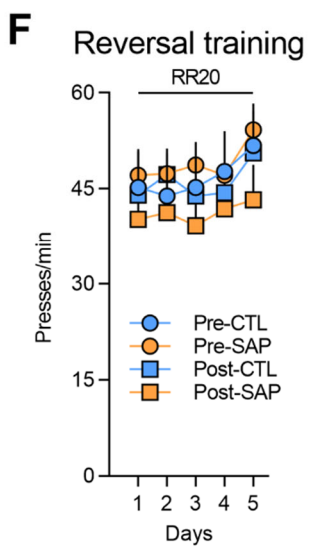
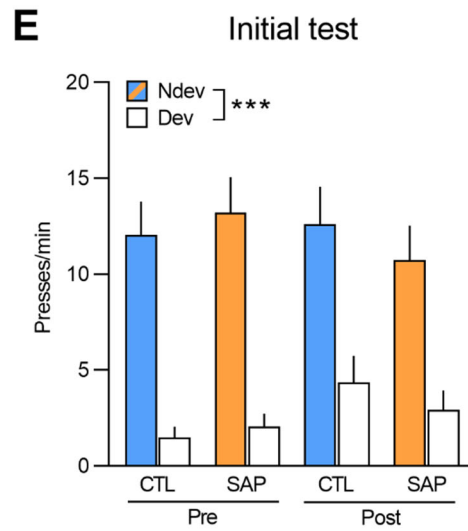
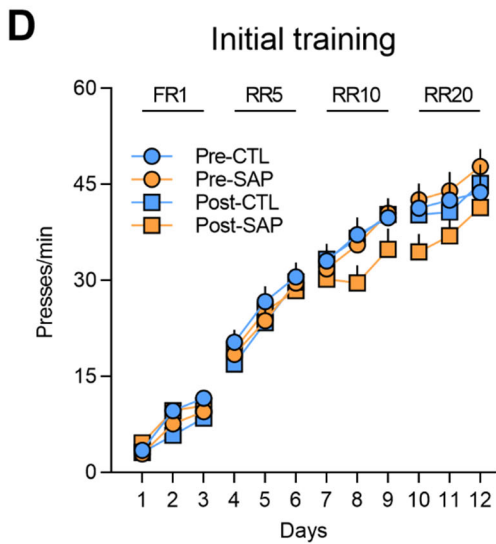
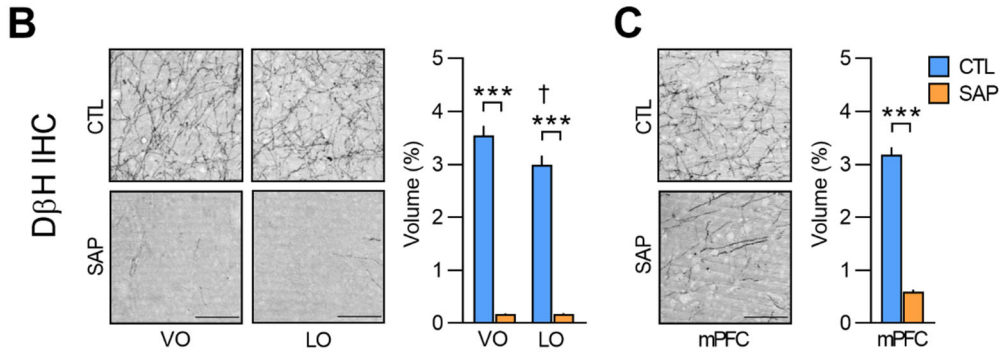
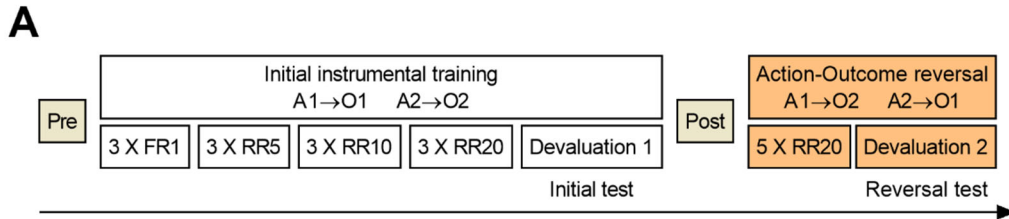
116 We found that all rats acquired the lever pressing response, with their rate of lever pressing
117 increasing across days ($F_{(1,53)} = 508.30$, $p < 0.001$). No differences were found between Pre
118 versus Post groups ($F_{(1,53)} = 0.96$, $p = 0.33$) or between CTL and SAP groups ($F_{(1,53)} = 0.287$, $p =$
119 0.59) and there were no significant interactions (all $F_{(1,53)}$ values < 1.18 , p values > 0.28) (**Figure**
120 **1D**). All groups also showed sensitivity to the change in value during the outcome devaluation

121 test, indicating that rats learned the action-outcome associations and the current value of the
122 outcomes value; i.e., goal-directed behaviour was intact (**Figure 1E**). Indeed, we found a
123 significant effect of devaluation (Ndev vs Dev; $F_{(1,53)}=79.62$, $p < 0.001$) but no effect of group
124 (Pre vs Post; $F_{(1,53)}=0.213$, $p=0.65$) or treatment (CTL vs SAP; $F_{(1,53)}=0.152$, $p=0.70$), and no
125 significant interactions between these factors (all $F_{(1,53)}$ values < 1.78 , p values > 0.18). In
126 addition, when given concurrent access to both outcomes, all groups consumed more of the
127 non-devalued outcome, thereby substantiating the efficacy of the satiety-induced outcome
128 devaluation procedure (**Supp. Figure 1A**). Thus, NA depletion in the OFC and mPFC does not
129 affect the initial learning or expression of goal-directed actions.

130 **NA signaling in the OFC is required to adapt to changes in outcome identity**

131 Next, we tested whether NA depletion affected the ability to encode and express new action-
132 outcome associations using an instrumental outcome-identity reversal paradigm. We found
133 that performance increased across reversal training days ($F_{(1,53)} = 22.73$, $p < 0.001$), but there
134 was no effect of group ($F_{(1,53)} = 0.80$, $p = 0.38$), treatment ($F_{(1,53)} = 0.08$, $p=0.78$), or any
135 significant interactions between these factors (all $F_{(1,53)}$ values < 1.86 , p values > 0.17) (**Figure**
136 **1F**). We then evaluated if the rats were able to use the reversed associations in the outcome
137 devaluation test (**Figure 1G**). While the control groups reduced lever pressing associated with
138 the devalued outcome, NA-depleted animals did not. Statistical analyses confirmed this
139 observation revealing no main effects of group, treatment, or devaluation (all $F_{(1,53)}$ values
140 < 2.88 , p values > 0.10), and no significant devaluation X group interaction ($F_{(1,53)} = 0.007$, $p =$
141 0.93). However, there was a significant devaluation X treatment interaction ($F_{(1,53)} = 5.00$, p
142 < 0.05) and simple effect analyses confirmed that the control groups biased their responding
143 toward the lever associated with the non-devalued outcome ($F_{(1,53)} = 7.35$, $p < 0.01$). Notably,
144 rats in the depleted groups did not ($F_{(1,53)} = 0.15$, $p = 0.70$). Importantly, all groups rejected the

145 devalued food during the consumption test (**Supp. Figure 1B**). These results show that
146 depletion of NA innervation to OFC and mPFC renders rats unable to associate new outcomes
147 to acquired actions. Importantly, this deficit was present in rats that received NA depletion
148 before (group Pre) and after (group Post) learning the initial action-outcome associations,
149 which is consistent with a process of updating established goal-directed actions.



151 **Figure 1. (A)** Experimental timeline for rats injected with anti-D β H saporin in the OFC before
152 (Pre) and after (Post) initial instrumental training and outcome devaluation (Pre-CTL n = 14,
153 Pre-SAP n = 15, Post-CTL n = 13, Post-SAP n = 15). **(B)** Representative microphotographs of
154 noradrenergic depletion and D β H fibre volume in the VO (+3.7 mm from Bregma) and LO (+3.7
155 mm from Bregma) following toxin injection. **(C)** Representative microphotograph of
156 noradrenergic depletion and D β H fibre volume in the mPFC (medial orbitofrontal+A32d+A32v;
157 +4.4 mm from Bregma) following toxin injection. **(D)** Rate of lever pressing across initial
158 training (A1-O1; A2-O2), collapsed across the two actions. **(E)** Initial instrumental test in
159 extinction following satiety-induced devaluation. **(F)** Rate of lever pressing across reversal
160 training (A1-O2; A2-O1), collapsed across the two actions. **(G)** Reversal instrumental test in
161 extinction following satiety-induced devaluation. The inset shows data grouped for CTL (Pre
162 and Post) and SAP groups (Pre and Post). Data are presented as mean + S.E.M. *p <0.05, ***p
163 <0.001, [†]p <0.05 LO vs VO CTL group. Scale bars: 100 μ m.

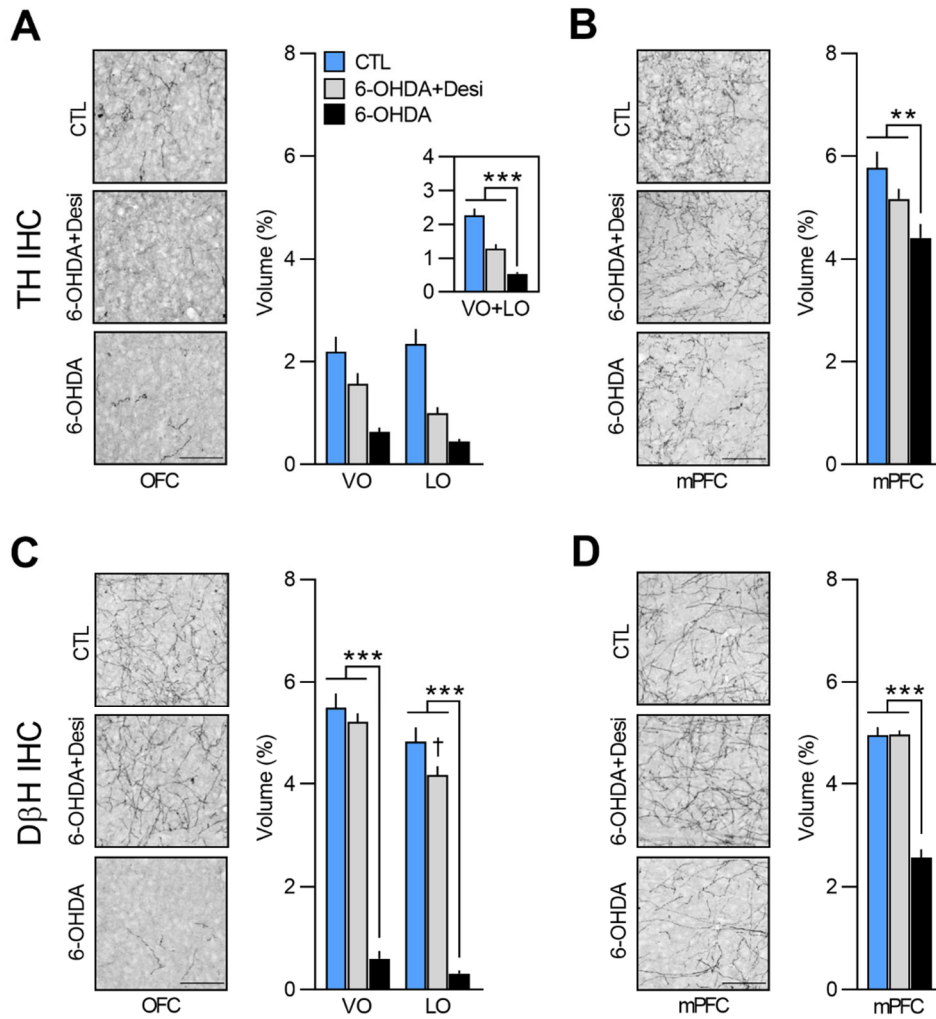
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165

166 **DA signaling in the OFC is not required to adapt to changes in outcome identity**

167 Next, we examined whether dopaminergic (DA) signaling in the OFC is required for updating
168 goal-directed actions. We used the behavioral procedure previously described (see **Figure 1A**)
169 and all rats underwent surgery following initial acquisition and expression of goal-directed
170 behaviour (see **Suppl. Figure 2A-C, Suppl. Figure 3A-C** for behavioral results from this initial
171 phase). We first assessed the impact of full catecholaminergic (CA) deletion on outcome-
172 identity reversal by infusing the CA-targeting toxin 6-OHDA in the OFC (6-OHDA n = 12; control
173 n = 8). We then depleted DA afferents by combining the 6-OHDA infusion with a systemic
174 injection of desipramine (Desi) to protect NA fibers (6-OHDA + Desi, n = 9; control n = 8). In
175 the OFC, DA depletion was effective as assessed by tyrosine hydroxylase (TH)
176 immunoreactivity (**Figure 2A**). We found a significant within-subject effect of region (VO
177 versus LO; $F_{(1,26)} = 8.18$, $p < 0.01$) and fewer TH-positive fibres in group 6-OHDA (n = 12)
178 compared to groups control CTL (n = 8) and 6-OHDA + Desi (n = 9) ($F_{(1,26)} = 41.14$, $p < 0.001$),
179 which also differed ($F_{(1,26)} = 15.7$, $p < 0.01$). There was also a significant interaction between
180 these factors ($F_{(1,26)} = 15.17$, $p < 0.01$) and simple effects revealed that the 6-OHDA + Desi
181 cohort had fewer immunoreactive TH fibres in the LO versus the VO ($F_{(1,26)} = 20.16$, $p < 0.001$).

182 We found no significant difference between LO and VO in the controls (CTL) ($F_{(1,26)} = 1.14$, $p =$
183 0.30) or 6-OHDA ($F_{(1,26)} = 2.74$, $p = 0.11$). Importantly, significantly fewer TH-positive fibres
184 were also present in the mPFC (MO + A32d + A32v) for the 6-OHDA ($F_{(1,26)} = 10.78$, $p < 0.01$),
185 but not 6OHDA+desi cohorts ($F_{(1,26)} = 2.07$, $p = 0.16$) (**Figure 2B**), indicating that, as in the
186 previous experiment, depletions were not restricted to the OFC.

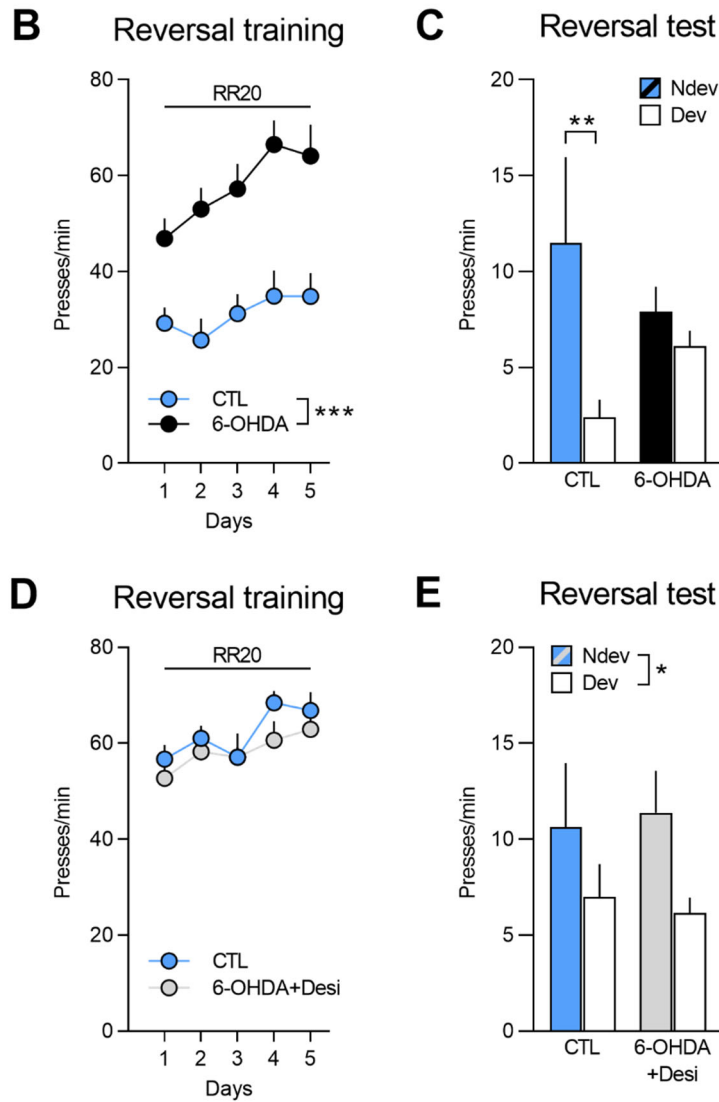
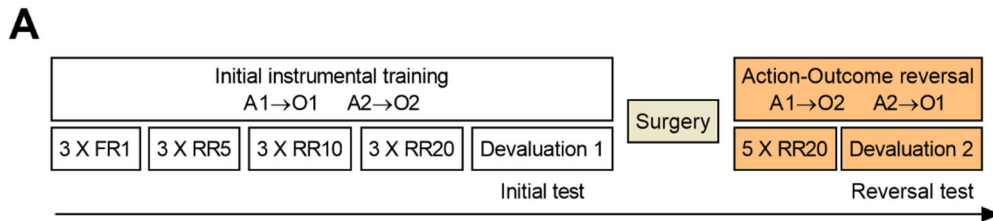
187 The volume of D β H+ fibres in OFC and mPFC also differed. In the OFC, main effects analyses
188 revealed a significant within-subject effect of region (VO versus LO; $F_{(1,26)} = 53.24$, $p < 0.001$)
189 and fewer TH+ fibres in the 6-OHDA group ($n = 12$) compared to CTL ($n = 8$) and 6-OHDA+Desi
190 ($n = 9$) ($F_{(1,26)} = 511.49$, $p < 0.001$), which did not differ ($F_{(1,26)} = 3.24$, $p = 0.08$) (**Figure 2C**). There
191 was also a region x treatment interaction ($F_{(1,26)} = 9.52$, $p < 0.01$) and simple effect analyses
192 revealed that 6-OHDA treatment induced a significant reduction in the volume of NA fibers in
193 both the VO ($F_{(1,26)} = 400.70$, $p < 0.001$) and the LO ($F_{(1,26)} = 459.47$, $p < 0.001$). Significantly fewer
194 D β H+ fibers were also observed for the 6OHDA+Desi group in the LO ($F_{(1,26)} = 6.42$, $p = 0.02$),
195 but not in the VO group ($F_{(1,26)} = 0.83$, $p = 0.37$). In the mPFC, a significant reduction in NA
196 innervation was observed in the 6-OHDA group ($F_{(1,26)} = 65.49$, $p < 0.001$), but not the
197 6OHDA+Desi group ($F_{(1,26)} = 0.55$, $p = 0.47$), an effect that was previously observed (Figure 1C)
198 and further indicates that our depletion was not specific to OFC (**Figure 2D**).



199
 200 **Figure 2. (A, B)** Representative microphotographs and percentage of dopaminergic depletion
 201 in the OFC (VO and LO, +3.7 mm from Bregma) and in the mPFC (MO, A32d and A32v, +4.4
 202 mm from Bregma) following toxin injection. **(C, D)** Representative microphotographs and
 203 percentage of noradrenergic depletion in the OFC (VO and LO, +3.7 mm from Bregma) and in
 204 the mPFC (MO, A32d and A32v, +4.4 mm from Bregma) following toxin injection. Data are
 205 presented as mean + S.E.M. **p <0.01, ***p <0.001, †p <0.05 vs CTL group. Scale bars: 100
 206 μm.

207
 208 Following surgery and recovery, animals were trained with reversed instrumental
 209 associations. We found that rats with 6-OHDA infusions responded more during reversal
 210 training than the controls ($F_{(1,18)} = 14.7$, $p < 0.01$) (**Figure 3B**). There was also an overall increase
 211 in response rate ($F_{(1,18)} = 44.57$, $p < 0.001$) and a significant group x day interaction ($F_{(1,18)} = 7.24$,
 212 $p < 0.05$), indicating that this increase in responding was greater for the 6-OHDA group than

213 for controls. However, during the outcome devaluation test following reversal training (**Figure**
214 **3C**), control rats successfully adjusted their behaviour according to the new action-outcome
215 contingencies, but 6-OHDA group did not. Statistical analysis revealed no main effect of group
216 ($F_{(1,18)} < 0.001$, $p > 0.975$), but a main effect of devaluation ($F_{(1,18)} = 8.72$, $p < 0.01$) and a group
217 x devaluation interaction that approached statistical significance ($F_{(1,18)} = 3.90$, $p = 0.06$). Simple
218 effect analyses confirmed that control rats responded more on the lever associated with the
219 devalued outcome ($F_{(1,18)} = 10.12$, $p < 0.01$), while 6-OHDA rats did not show this preference
220 ($F_{(1,18)} = 0.6$, $p = 0.45$). Importantly, when given access to both rewards, all groups consumed
221 more of the non-devalued food than the devalued food (**Suppl. Figure 2D**).



222

223 **Figure 3.** (A) Experimental timeline. After the initial instrumental training and outcome
 224 devaluation testing, rats were injected in the OFC with either vehicle (CTL n = 8; CTL n = 8), 6-
 225 OHDA coupled with desipramine (to specifically target DA neurons, 6-OHDA+Desi n = 9) or 6-
 226 OHDA alone (to target all CA neurons, n = 8). (B, D) Rate of lever pressing across reversal
 227 training, data is presented collapsed across the two actions. (C, E) Reversal instrumental test
 228 in extinction following satiety-induced devaluation. Data are presented as mean + S.E.M. *p
 229 <0.05, **p <0.01, ***p <0.001.

230

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234
235 **Figure 3D** shows responding during reversal training for 6-OHDA + Desi and control groups.
236 Lever pressing increased across reversal training days ($F_{(1,15)} = 15.79$, $p < 0.01$) and there was
237 no main effect of group ($F_{(1,15)} = 0.52$, $p = 0.48$) or group x day interaction ($F_{(1,15)} = 0.157$, $p =$
238 0.70). Moreover, we found that both groups showed goal-directed behaviour and biased their
239 choice towards the action associated with the non-devalued outcome (Figure 3E). Statistical
240 analyses confirmed a main effect of devaluation ($F_{(1,15)} = 5.25$, $p < 0.05$), but no main effect of
241 group ($F_{(1,15)} < 0.001$, $p = 0.975$) or group x devaluation interaction ($F_{(1,15)} = 0.168$, $p = 0.69$).
242 Notably, both groups consumed more of the non-devalued food during the consumption test
243 **(Suppl. Fig 3D)**.

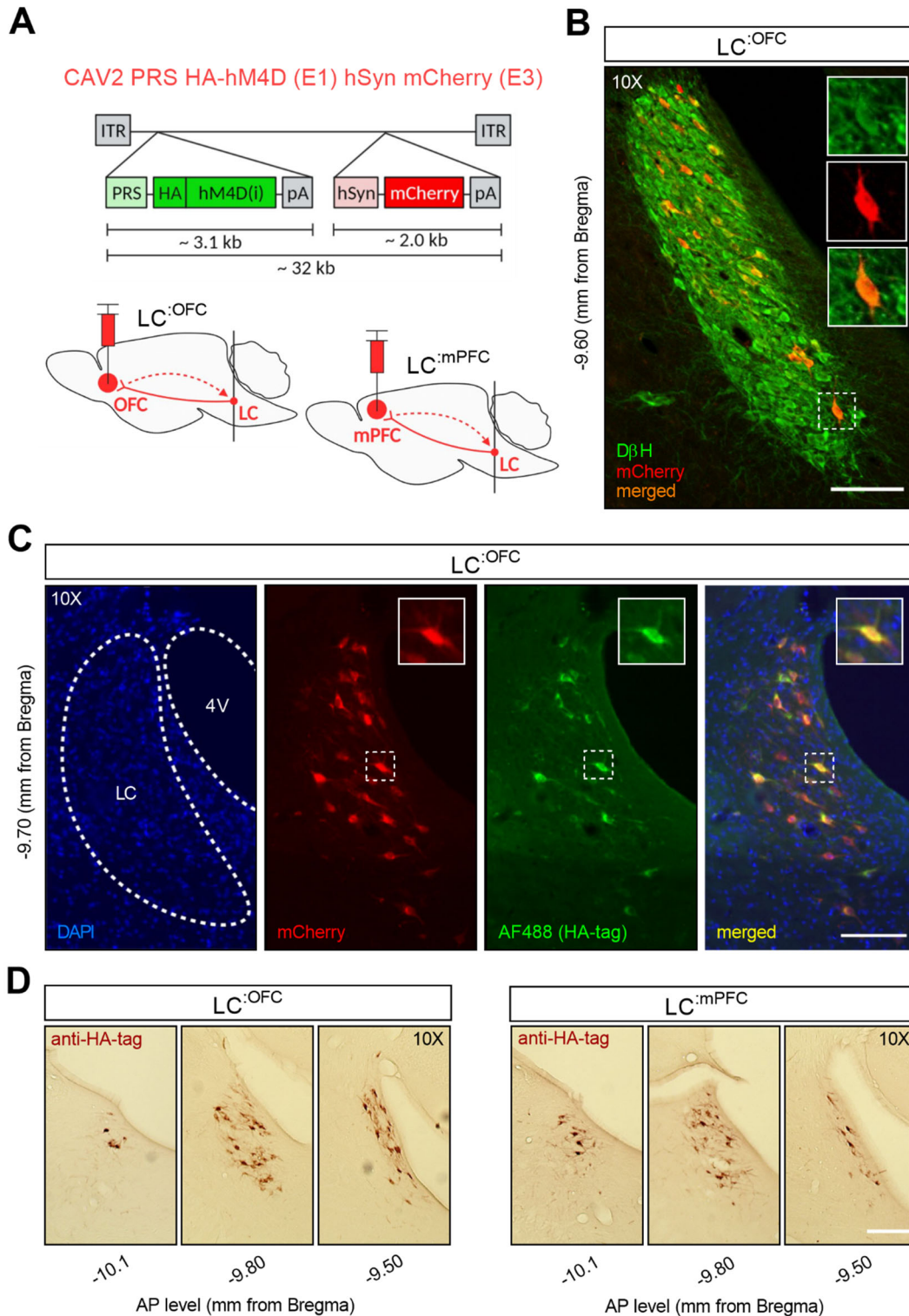
244 These results further support a specific role of NA in updating previously learned goal-directed
245 actions. We show that full CA depletion (DA+NA) in the OFC and mPFC impairs performance
246 in an outcome-identity reversal task, while depletion restricted to DA innervation leaves
247 performance intact.

248

249 **Selective expression of inhibitory DREADDs in LC:^{OFC} or LC:^{mPFC} NA projections**

250 In the previous two approaches, we used pharmacologic ablation to target NA signaling in the
251 OFC. However, injection of anti-DH β -saporin or 6-OHDA in the OFC also caused a significant
252 reduction of fibres in the mPFC, most likely because of NA projections connecting the two
253 cortical areas and/or fibers crossing the OFC before entering the mPFC (Chandler &
254 Waterhouse 2012, 2013, 2014). As such, while we were able to demonstrate that NA but not
255 DA signaling in the prefrontal cortex is necessary to adapt to changes in outcome identity, we
256 could not conclusively attribute our behavioural effects to NA depletion in the OFC. Moreover,

257 given that our approach involved permanent lesions of NA fibres, we were unable to ascertain
258 if NA signaling was required to encode and/or recall the new action-outcome associations.
259 Therefore, to address the regional and temporal specificity of the behavioural effect, we
260 generated CAV2-PRS-hM4D-hSyn-mCherry, a canine adenovirus vector containing PRS, a
261 noradrenergic-specific promoter, driving an HA -tagged hM4D, an inhibitory DREADD, and an
262 mCherry expression cassette. CAV-2 vectors are readily taken up at presynapse and trafficked,
263 via retrograde transport to the soma of projecting neurons CAV2-PRS-hM4D-hSyn-mCherry
264 was infused in either the OFC or the mPFC to targeted either LC:^{OFC} or LC:^{mPFC} NA projections
265 **(Figure 4A).** **Figure 4B** shows retrograde transport of the vector and mCherry in NA cells of the
266 LC following injection of CAV2-PRS-hM4D-hSyn-mCherry in the OFC. The colocalization of
267 mCherry and HA immunoreactivity in the LC indicates a selective expression of HA-hM4D. As
268 expected, while mCherry staining is present at injection sites, reflecting local cortico-cortical
269 connections that are not NA dependent, HA- immunoreactive cell bodies were found
270 exclusively in the LC. These data are consistent with NA-specific expression of the HA-tagged
271 Hm4D due to PRS, and nonselective expression of mCherry expression which is under the
272 control of hSyn, driving pan-neuronal expression **(Figure 4D; Suppl. Figure 4C).**



273

274 **Figure 4. (A)** CAV2-PRS-hM4D-mCherry, a vector bearing a noradrenergic-specific promoter
 275 (PRS) an inhibitory DREADD tagged with HA (hM4Di), an mCherry expression cassette and sites
 276 of injection. **(B)** Immunofluorescent staining for D β H and mCherry in the LC of a representative
 277 rat injected with CAV2-PRS-hM4D-hSyn-mCherry in the OFC. **(C)** High colocalization of
 278 immunofluorescent staining for HA (tag of inhibitory DREADDs) and mCherry in the LC of the
 279 same representative rat injected in the OFC. **(D)** Comparison of antero-posterior DAB staining

280 for HA in two representative rats, one injected in the OFC, the other in the mPFC. Scale bars:
281 100 μ m.

282

283 **Silencing of LC:^{OFC}, but not LC:^{mPFC}, projections impairs adaptation to changes in the action-** 284 **outcome association**

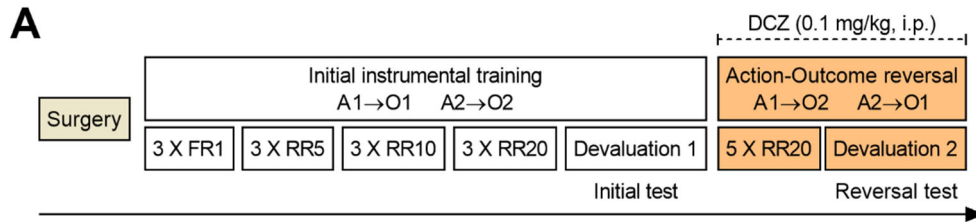
285 Rats were trained and tested as shown in **Figure 5A**. Following initial instrumental training and
286 outcome devaluation testing (**Suppl. Figure 5A-C** for LC:^{OFC} and **Suppl. Figure 6A-C** for LC:^{mPFC}),
287 they were divided in two groups according to their initial performance: one group that would
288 receive vehicle (Veh, -) during reversal training and one group that would receive
289 deschloroclozapine dihydrochloride (DCZ, +), a high affinity and selective agonist for hM4D
290 (Nagai et al., 2020; Nentwig et al., 2021; Oyama et al., 2022).

291 A linear trend across reversal training sessions was detected for OFC-injected rats ($F_{(1,23)} =$
292 37.44 , $p < 0.001$), but not mPFC-injected rats ($F_{(1,15)} = 0.16$, $p = 0.69$), with no difference
293 between the Veh and the DCZ group for OFC- ($F_{(1,23)} = 0.39$, $p = 0.54$) or mPFC-injected rats
294 ($F_{(1,15)} = 0.01$, $p = 0.92$), and no interactions between these factors (largest $F_{(1,23)}$ value = 2.82 ,
295 $p = 0.11$) (**Figure 5B & D**). All rats then underwent two outcome devaluation tests, once under
296 vehicle injection (-) and once under DCZ injection (+), with the order counterbalanced. This
297 yielded a 2 (between) x 2 (within) factorial design with 4 conditions of interest: vehicle during
298 training and test (-/-), vehicle during training and DCZ during test (-/+), DCZ during training and
299 vehicle at test (+/-), and DCZ during training and test (+/+).

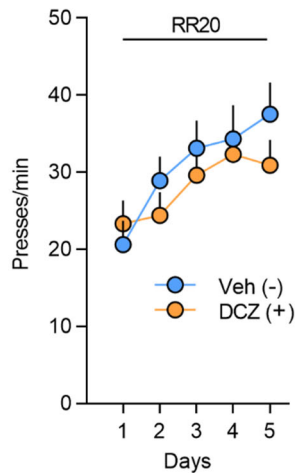
300 We found that only rats in the control group (-/-) showed goal-directed behaviour and
301 performed the action associated with the non-devalued outcome more than the action
302 associated with the devalued outcome (**Figure 5C**). By contrast, rats with bilateral LC:^{OFC} NA
303 projections silenced during the reversal training (+/- and +/+) or the outcome devaluation test
304 (-/+ and +/+) failed to display this preference in responding. The between- and within-subject

305 main effects were not significant (largest $F_{(1,46)}$ value = 1.22, $p = 0.28$), but there was a
306 significant three-way interaction (devaluation X treatment during acquisition X treatment
307 during test: $F_{(1,23)} = 5.45$, $p < 0.05$). Simple effects analyses showed that only rats that received
308 vehicle during both training and test (-/-) biased their choice toward the lever associated with
309 the non-devalued outcome ($F_{(1,23)} = 7.10$, $p < 0.05$), while the -/+ ($F_{(1,23)} = 0.09$, $p = 0.77$), +/-
310 ($F_{(1,23)} = 1.22$, $p = 0.28$), and +/+ groups ($F_{(1,23)} = 0.01$, $p = 0.92$) did not. By contrast, silencing
311 LC:^{mPFC} NA projections left goal-directed behaviour intact (main effect of devaluation:
312 $F_{(1,15)} = 5.763$, $p < 0.05$). We found no main effect of treatment during acquisition ($F_{(1,15)} = 0.35$,
313 $p = 0.56$) or treatment during test ($F_{(1,15)} = 0.53$, $p = 0.47$) and no significant interactions between
314 these factors (largest $F_{(1,15)}$ value = 1.62, p values > 0.21) (**Figure 5E**).

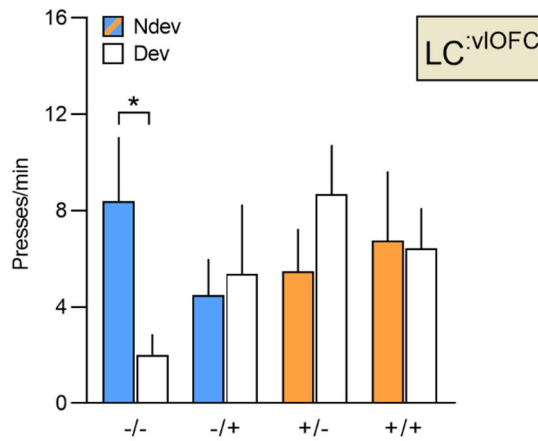
315 Importantly, consumption tests performed immediately after the reversal tests revealed that
316 all groups consumed more of the non-devalued outcome indicating that the satiety-induced
317 devaluation was effective and that DCZ injections did not disrupt the rats' ability to distinguish
318 between devalued and non-devalued rewards (**Suppl. Figure 5D and 6D** for LC:^{OFC} and LC:^{mPFC},
319 respectively). Together, these results led us to conclude that LC NA projections to the OFC,
320 but not to the mPFC, are required to both encode and recall changes in the identity of the
321 expected outcome.



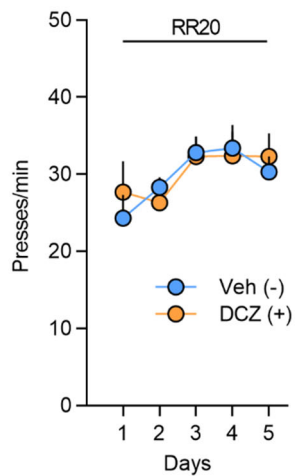
B Reversal training



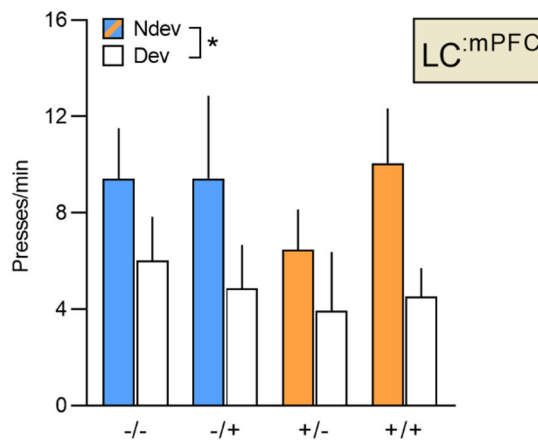
C Reversal test



D Reversal training



E Reversal test



322

323 **Figure 5.** (A) Timeline for rats injected with CAV2-PRS-hM4D-hSyn-mCherry in either the OFC
 324 or the mPFC following reversal training. Each rat was injected with either vehicle (-) or DCZ (+)
 325 during reversal training and was then tested twice, once under DCZ and once under vehicle
 326 with the test order counterbalanced. (B) Reversal training in rats injected in the OFC (Veh n =
 327 12; DCZ n = 13), data is presented collapsed across the two actions (A1-O2; A2-O1). (C)
 328 Reversal instrumental test following satiety-induced devaluation in rats injected in the OFC.
 329 (D) Reversal training in rats injected in the mPFC (Veh n = 8; DCZ n = 9), data is presented
 330 collapsed across the two actions (A1-O2; A2-O1). (E) Reversal instrumental test following
 331 satiety-induced devaluation in rats injected in the mPFC. Data are presented as mean + S.E.M.
 332 *p < 0.05.

333 **DISCUSSION**

334 Goal-directed actions are the expression of learned associations between an action and the
335 outcome it produces. These associations are however flexible, being amenable to updating
336 when the identity of the outcome changes. Our data demonstrate that NA inputs to the
337 orbitofrontal cortex are specifically required for this updating process. This conclusion is based
338 on a body of complementary data. First, we demonstrated that animals with a loss of NA
339 inputs in the OFC can initially learn and express action–outcome contingencies, but are
340 impaired when the identity of the outcome has been modified. Importantly, such deficits were
341 also observed when NA depletion occurred immediately before the encoding of the new
342 action-outcomes contingencies. We then showed that this impairment was selective to NA
343 inputs to the OFC, because combined depletions of DA and NA, but not of DA alone, induced
344 a profound deficit in outcome reversal. Finally, we investigated the temporal and anatomical
345 specificity of this effect using a selective NA retrograde virus carrying inhibitory DREADDs to
346 selectively target either LC:^{OFC} or LC:^{mPFC} pathways. We found that silencing LC:^{OFC}, but not
347 LC:^{mPFC}, projections impaired the rats' ability to acquire and express the reversed instrumental
348 contingencies. Taken together, these results reveal the NA regulation of goal-directed
349 behaviour.

350

351 **NA inputs into the OFC, but not the mPFC, are required for action-outcome updating**

352 Depletion of NA inputs was achieved using neurochemical toxins (anti-DβH saporin or 6-
353 OHDA). Both toxins led to a dramatic decrease in NA fiber density in the ventral and lateral
354 OFC, and in the medial prefrontal cortex. This pattern is unlikely due to an overlap between
355 LC projections to the mPFC and the OFC, given that divergent prefrontal projections from the
356 LC exist but are limited (Chandler et al. 2013, Foote et al. 1983, Fuxe et al. 1968, Levitt &

357 Moore 1978, Lewis & Morrison 1989, Morrison et al. 1978, Uematsu et al. 2017). It is therefore
358 more likely that the depletion in the mPFC results from the crossing of the OFC by NA fibers
359 *en route* to the mPFC, as recently suggested (Cerpa et al. 2019). In the present study, a high
360 degree of anatomical selectivity was achieved by using a CAV-2 vector carrying the
361 noradrenergic promoter PRS to target either the LC:^{mPFC} or the LC:^{OFC} pathways (Hayat et al;
362 2020; Hirschberg et al., 2017).

363 A key finding is that NA inputs to the OFC are specifically required for updating the association
364 between an action and its outcome. Indeed, a similar impairment in goal-directed behaviour
365 resulted from NA depletion performed either prior to initial training or prior to reversal
366 training, which indicates that the reversal period is critically reliant on NA inputs. In addition,
367 chemogenetic silencing of the LC:^{OFC} pathway before the reversal training, or before testing
368 also produced similar impairments, which further demonstrates that OFC NA inputs are
369 required for both the encoding and the recall of new action-outcome associations. These
370 results are consistent with recent views on the role of the OFC in goal-directed behaviour
371 (Parkes et al., 2018; Panayi & Killcross, 2018; Cerpa et al., 2021).

372 In contrast to NA inputs to the OFC, our results show that NA input to the mPFC is not required
373 for responding based on initial or reversed instrumental contingencies. These data add to the
374 current literature indicating a major dissociation in the role of NA inputs to different prefrontal
375 regions (Robbins & Arnsten 2009). Indeed, NA input to mPFC is required for attentional
376 regulation since lesioning NA inputs (McGaughy et al 2008, Newman et al 2008), or
377 chemogenetic inhibition of NA-mPFC (Cope et al 2019) alter attentional set-shifting, while NA
378 recapture inhibition via atomoxetine improves it (Newman et al 2008). Similar systematic
379 investigation is not yet available but OFC NA depletion can alter cue-outcome reversal, but
380 not dimensional shift, in an attentional set-shifting task (Mokler et al 2017).

381 **NA but not DA inputs to the OFC are required for action-outcome updating**

382 Using a strategy which allows for a differential depletion of DA and/or NA fibers, we found
383 that NA-dependent mechanisms are required during the encoding and recall of new action-
384 outcome. The role of cortical DA-dependent mechanisms in goal-directed behaviour remains
385 poorly understood. However, we previously demonstrated that dopaminergic signaling in the
386 mPFC plays a critical role in the detection of contingency degradation (Naneix et al 2009, 2012,
387 2013). Such detection is likely to involve the processing of non-expected rewards which
388 induces, at the level of mPFC, a DA-dependent reward prediction error signal (Montague et al
389 2004, Schultz & Dickinson 2000). These results therefore raise the intriguing possibility that
390 the coordination of goal-directed behaviour under environmental changes might depend on
391 a DA-mPFC system to adapt to causal contingencies and NA-OFC system to adapt to changes
392 in outcome identity (Cerpa et al., 2021).

393 **Updating goal-directed behaviour**

394 When trained on reversed contingencies, animals encode the new action-outcome
395 associations (Fresno et al 2019, Parkes et al 2018). Under similar experimental conditions, past
396 research has shown that reversal learning performance is the result of updating prior existing
397 action-outcome contingencies without unlearning the initial ones (Bradfield & Balleine 2017).
398 In other words, the animals build a partition between a state for the new contingencies and
399 the initial state of old contingencies (Hart & Bradfield, 2020). Current research (McDannald et
400 al 2011, Sadacca et al 2016, Wikenheiser & Schoenbaum 2016) has proposed that the OFC is
401 critically involved in the partition of information when task states change without explicit
402 notice (Wilson et al 2014). Consistent with this view, chemogenetic inhibition of the OFC
403 (ventral and lateral) impairs goal-directed responding following identity reversal (Parkes et al
404 2018; Howard & Kahnt, 2021). Here, we found a similar deficit following lesion of NA inputs

405 to the OFC. Given that the deficit in goal-directed behavior was restricted to the reversal
406 phase, including both reversal training and the test based on reversed contingencies, it is likely
407 that NA-OFC is involved in both creating new states and in the “online” use of the information
408 included in this new state. Such a proposal is in accordance with popular theories of LC-NA
409 system which suggest that a rise in NA activity allows for behavioral flexibility when a change
410 in contingencies is detected (Aston-Jones et al 1997, Bouret & Sara 2005, Sadacca et al 2016).

411

412 **Conclusion**

413 Our results confirm the involvement of ventral and lateral OFC in the updating and use of new
414 action-outcome associations, and further demonstrate that NA input to the OFC is critical for
415 these learning processes. Recent research has revealed a remarkable parcellation of cortical
416 functions in goal-directed action (Parkes & Coutureau, 2019; Turner & Parkes, 2020). The
417 current study provides a clear basis for an in-depth understanding of the cortical coordination
418 involved in executive functions.

419 **DECLARATION OF INTERESTS**

420 The authors declared that there is no actual or potential conflict of interest about this article.

421

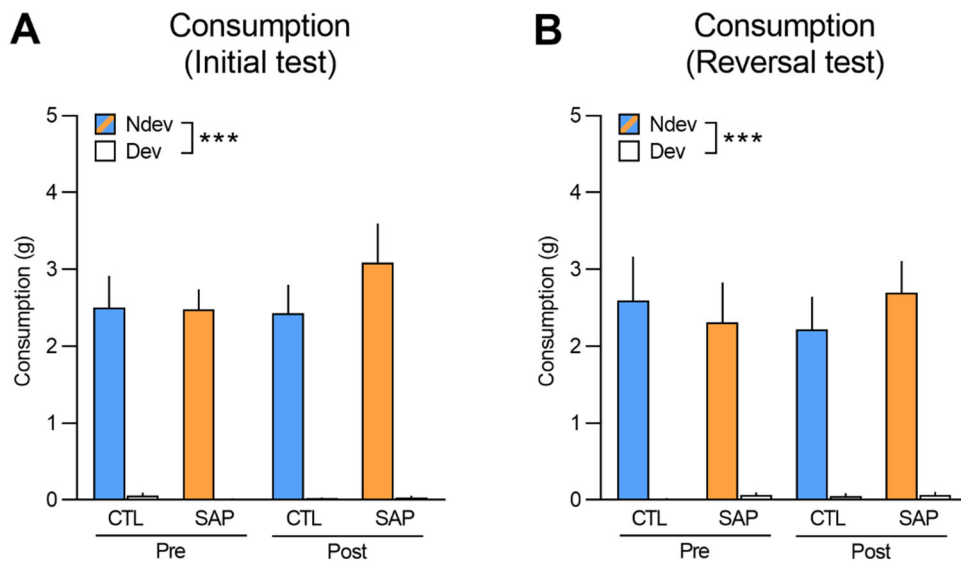
422 **ACKNOWLEDGEMENTS**

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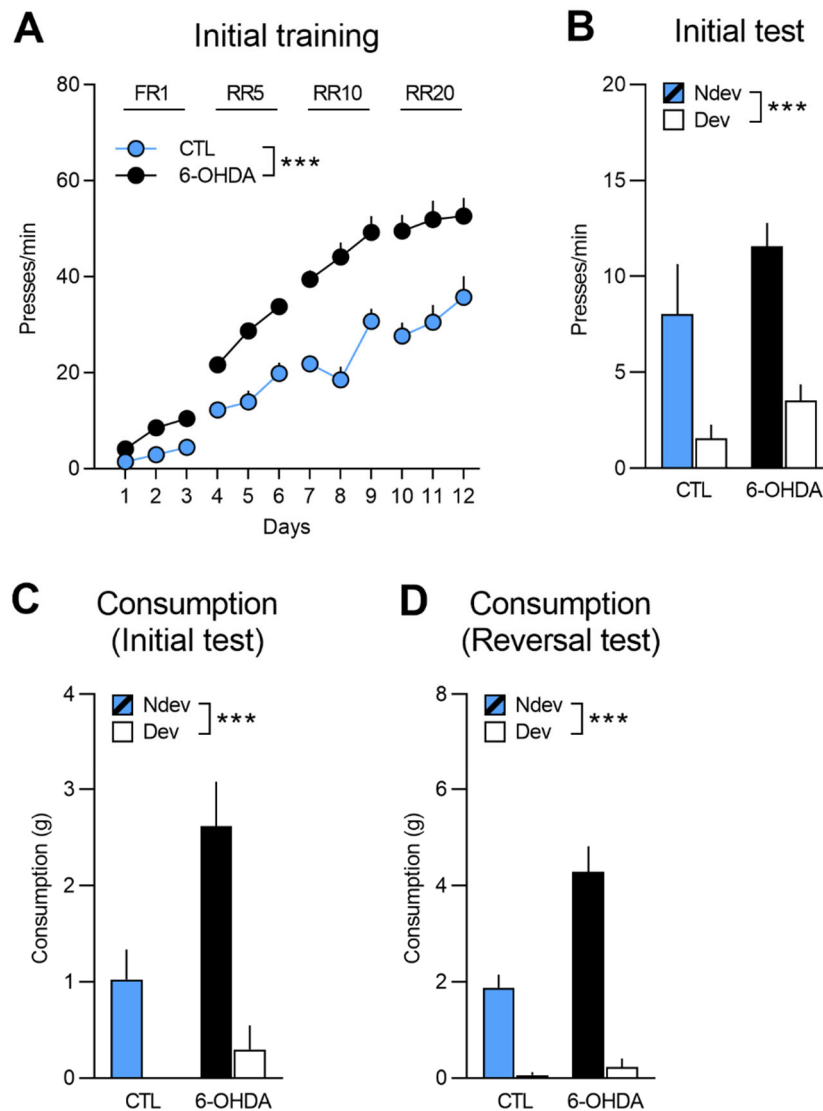
434 **AUTHORS CONTRIBUTIONS**

435 All authors had full access to all the data in the study and took responsibility for the integrity
436 of the data and the accuracy of the data analysis. Conceptualization: E.C. & S.P.; Methodology:
437 J-C C., E. K., M.L., M.W. & A.P.; Investigation: J-C. C., M.D. & A.P.; Formal Analysis: A.P., J-C C.,
438 E.C. & S.P.; Resources: E.C., E. K., M.W. & S.P.; Writing: S.P., A.P., J-C C., M.W. & E.C;
439 Supervision: E.C., M.W. & S.P.; Funding EK & E.C.



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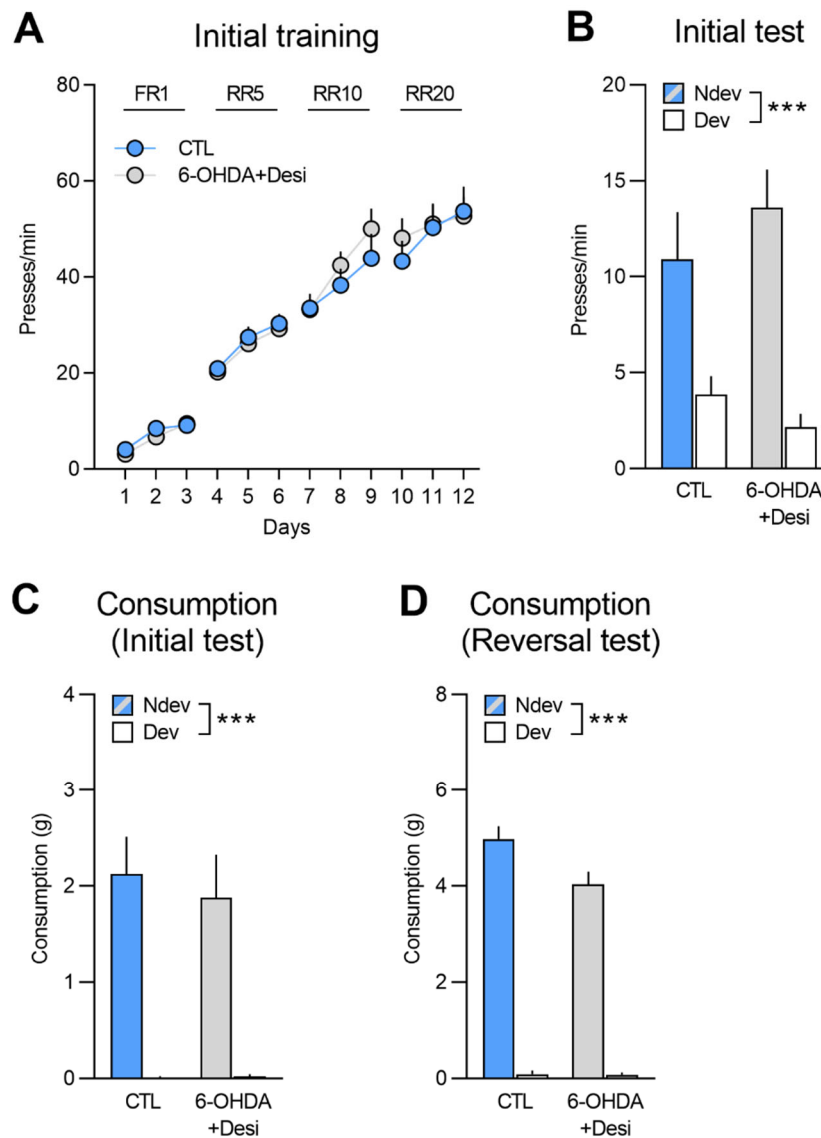
443 **Suppl. Figure 1.** Consumption tests performed immediately after the initial (A) and reversal
 444 (B) instrumental tests in extinction. Rats were given access to both food rewards (10 g each)
 445 for 10 min. Statistics revealed a within-subjects effect of devaluation for both the initial
 446 ($F_{(1,53)}=168.94$, $p < 0.001$) and reversal tests ($F_{(1,53)}=97.25$, $p < 0.001$) and no main effects of
 447 group, treatment, or interactions between these factors (largest $F_{(1,53)}$ value=0.57, $p=0.45$).
 448 Data are presented as mean + S.E.M. *** $p < 0.001$.



450

451 **Suppl. Figure 2.** Initial training and test for rats to be injected with 6-OHDA (n = 12) and control
 452 rats (n = 8). **(A)** Training data is presented collapsed across the two actions (A1-O1; A2-O2).
 453 There was a main effect of group ($F_{(1,18)}=35.33$, $p < 0.001$), devaluation ($F_{(1,18)}=190.66$, $p < 0.001$)
 454 and group x devaluation interaction ($F_{(1,18)}=8.8$, $p < 0.01$). Simple effects confirmed that
 455 responding increased in both group 6-OHDA and group control ($F_{(1, 18)}=175.85$, $p < 0.001$ and
 456 $F_{(1,18)}=48.8$, $p < 0.001$, respectively). **(B)** Statistical analyses on the outcome devaluation test
 457 revealed an overall effect of devaluation ($F_{(1,18)}=39.37$, $p < 0.001$), no effect of group
 458 ($F_{(1,18)}=2.84$, $p=0.11$), and no group x devaluation interaction ($F_{(1,18)}=0.46$, $p=0.51$). **(C)** During
 459 the initial consumption test, both groups consumed more of the non-devalued outcome as
 460 indicated by a significant effect of devaluation ($F_{(1,18)}=18.69$, $p < 0.001$) and group ($F_{(1,18)}=9.6$,
 461 $p < 0.01$) but no group x devaluation interaction ($F_{(1,18)}=2.79$, $p=0.11$). **(D)** The results from the
 462 reversal consumption test revealed a significant main effect of devaluation ($F_{(1,18)}=94.72$, p
 463 < 0.001) as well as a significant main effect of group ($F_{(1,18)}=10.0$, $p < 0.01$) and a significant
 464 group x devaluation interaction ($F_{(1,18)}=13.86$, $p < 0.01$), indicating that the difference between

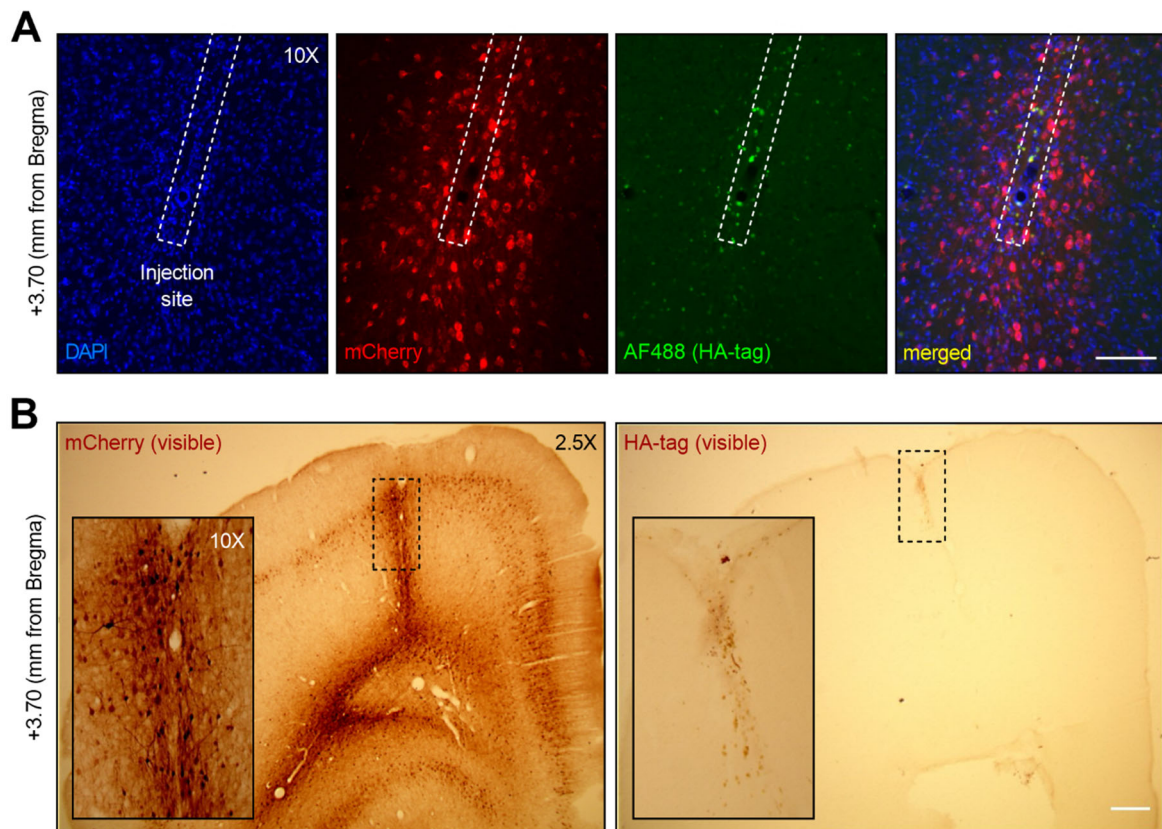
465 consumption of the non-devalued versus devalued food was actually greater for group 6-
466 OHDA (devalued mean = 0.23; non-devalued mean = 4.29) than for group control (devalued
467 mean = 0.06; non-devalued mean = 1.86). Nevertheless, simple effects analyses confirmed
468 that both 6-OHDA and control groups consumed more of the non-devalued food than the
469 devalued food ($F_{(1,18)}=15.05$, $p < 0.01$ and $F_{(1,18)}=113.16$, $p < 0.001$, respectively). Data are
470 presented as mean + S.E.M. *** $p < 0.001$.



472

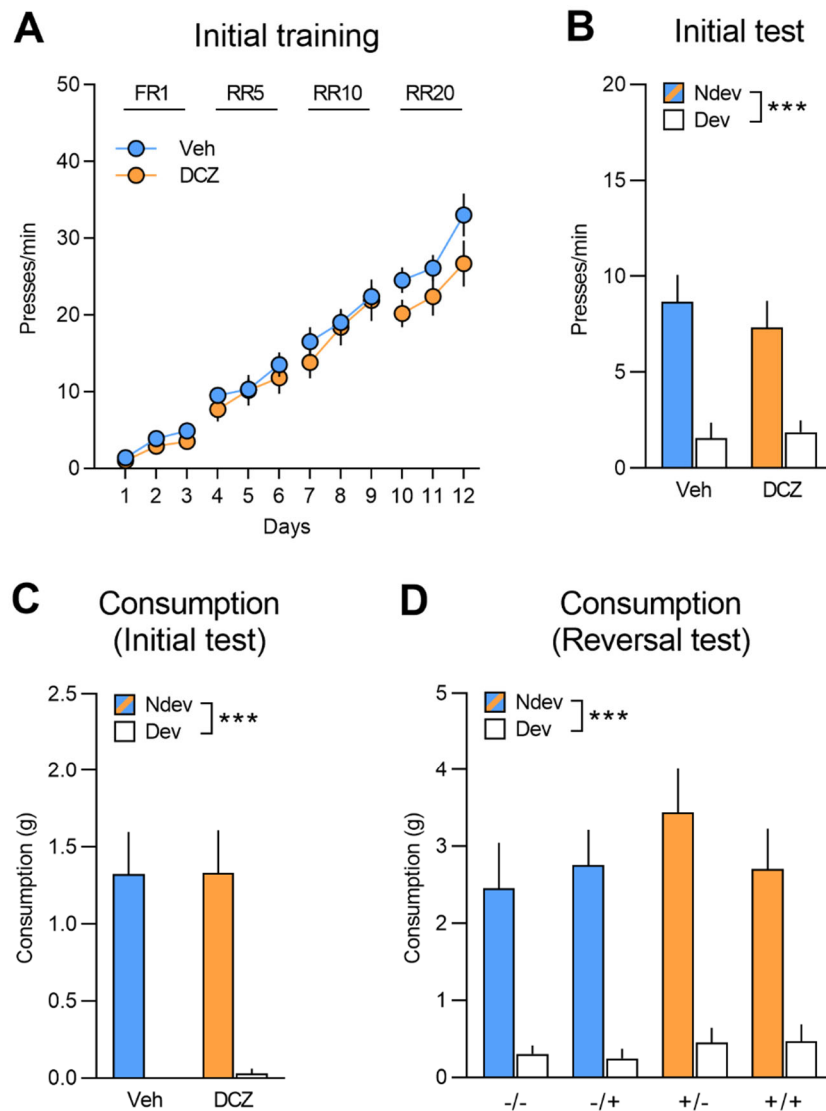
473 **Suppl. Figure 3.** Initial training and test for rats to be injected with 6OHDA+Desi (n = 9) and
 474 control rats (n = 8). **(A)** Training data is presented collapsed across the two actions (A1-O1; A2-
 475 O2). There was a main effect of devaluation ($F_{(1,15)}=220.45$, $p < 0.001$) but no effect of group
 476 ($F_{(1,15)}=0.06$, $p=0.81$) or group x devaluation interaction ($F_{(1,15)}=0.31$, $p=0.59$). **(B)** Statistical
 477 analyses on the outcome devaluation test revealed an overall effect of devaluation
 478 ($F_{(1,15)}=44.36$, $p < 0.001$), no effect of group ($F_{(1,15)}=0.07$, $p=0.79$), and no group x devaluation
 479 interaction ($F_{(1,15)}=2.5$, $p=0.13$). **(C)** During the initial consumption test, both groups consumed
 480 more of the non-devalued outcome as indicated by a significant effect of devaluation
 481 ($F_{(1,15)}=45.57$, $p < 0.001$) but no effect of group ($F_{(1,15)}=0.15$, $p=0.70$) or group x devaluation
 482 interaction ($F_{(1,15)}=0.19$, $p=0.67$). **(D)** During the reversal consumption test both groups still
 483 consumed more of the non-devalued food ($F_{(1,15)}=466.43$, $p < 0.001$). Data are presented as
 484 mean + S.E.M. *** $p < 0.001$.

485 **Suppl. Figure 4**



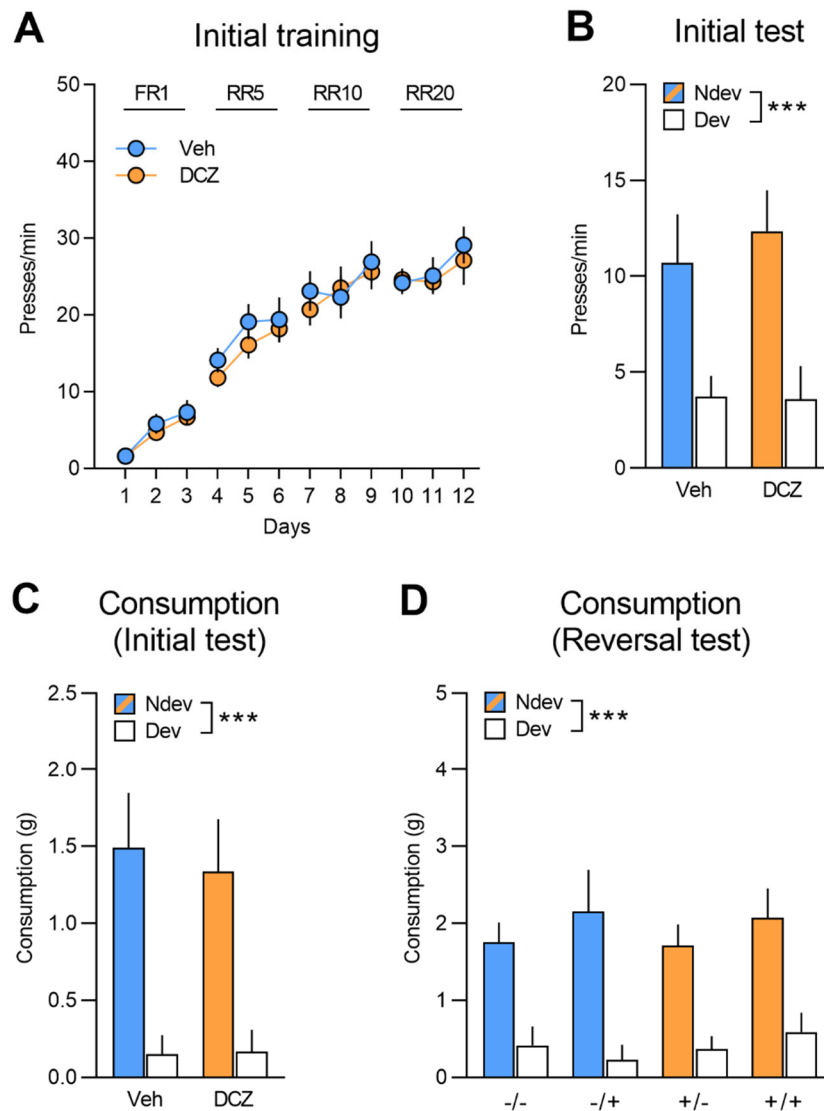
486

487 **Suppl. Figure 4. (A)** Immunofluorescent staining for mCherry and HA (tag of inhibitory
488 DREADDs) in one injection site of a representative rat injected with CAV2-PRS in the OFC. **(B)**
489 DAB staining for mCherry and HA in one injection site of the same representative rat injected
490 with CAV2-PRS in the OFC. In both cases, although mCherry-stained cell bodies can be seen all
491 around the injection site (and above), there are no HA-stained cells. Scale bar **(A)**: 100 μm ;
492 scale bar **(B)**: 400 μm .



494

495 **Suppl. Figure 5. (A)** Initial training for rats injected with CAV2-PRS in the OFC that would be
 496 allocated to group vehicle (Veh; n = 12) and group DCZ (n = 13) for the subsequent reversal
 497 training, data is presented collapsed across the two actions. Both groups acquired the
 498 instrumental response ($F_{(1,23)}=236.06$, $p < 0.001$) and there was no difference between groups
 499 ($F_{(1,23)}=0.53$, $p=0.47$) or interaction ($F_{(1,23)}=0.45$, $p=0.51$). **(B)** Initial instrumental test following
 500 satiety-induced devaluation. Statistics revealed a main effect of devaluation ($F_{(1,23)}=32.61$, p
 501 < 0.01) but no effect of group ($F_{(1,23)}=0.16$, $p=0.69$) or a significant interaction between these
 502 factors ($F_{(1,23)}=0.30$, $p=0.59$). **(C, D)** All groups consumed more of the non-devalued food than
 503 the devalued food during the consumption tests performed immediately after the initial **(C)**
 504 ($F_{(1,23)}=42.93$, $p < 0.001$) and reversal instrumental tests **(D)** ($F_{(1,23)}=74.19$, $p < 0.001$) with no
 505 main effects of group or treatment or interactions between these factors (largest F value=2.76,
 506 $p=0.11$). Data are presented as mean + S.E.M. *** $p < 0.001$.



508

509 **Suppl. Figure 6.** (A) Initial training for rats injected with CAV2-PRS in the mPFC that would be
 510 allocated to group vehicle (Veh n = 8) and group DCZ (n = 9) for the subsequent reversal
 511 training, data is presented collapsed across the two actions. Both groups acquired the
 512 instrumental response ($F_{(1,15)}=278.73$, $p < 0.001$) and there was no difference between groups
 513 ($F_{(1,15)}=1.37$, $p=0.26$) or interaction ($F_{(1,15)}=0.16$, $p=0.70$). (B) Initial instrumental test following
 514 satiety-induced devaluation. Statistics revealed a main effect of devaluation ($F_{(1,15)}=12.57$,
 515 $p=0.01$) but no effect of group ($F_{(1,15)}=0.22$, $p=0.65$) or a significant interaction between these
 516 factors ($F_{(1,15)}=0.16$, $p=0.70$). (C, D) All groups consumed more of the non-devalued food than
 517 the devalued food during the consumption tests performed immediately after the initial (C)
 518 ($F_{(1,15)}=19.92$, $p < 0.001$) and reversal (D) instrumental tests ($F_{(1,15)}=51.18$, $p < 0.001$) with no
 519 main effects of group or treatment or interactions between these factors (largest F value=1.1,
 520 $p=0.31$). Data are presented as mean + S.E.M. *** $p < 0.001$.

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653 Detailed methods are provided in the online version of this paper and include the following:

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670 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-D β H	Merck Millipore	MAB308
Mouse monoclonal anti-TH	Merck Millipore	MAB318
Goat polyclonal anti-mouse (Biotin-conjugated)	Jackson ImmunoResearch	115-065-062
Rabbit polyclonal anti-RFP	MBL International Corporation	PM005
Goat polyclonal anti-rabbit (Biotin-conjugated)	Jackson ImmunoResearch	111-065-003
Goat polyclonal anti-mouse (FITC-conjugated)	Jackson ImmunoResearch	115-095-003
Goat polyclonal anti-rabbit (TRITC-conjugated)	Jackson ImmunoResearch	111-025-003
Rabbit monoclonal anti-HA	Cell Signaling Technology	C29F4 (#3724)
Goat polyclonal anti-rabbit (AF488-conjugated)	Jackson ImmunoResearch	111-545-003
Chemicals		
Anti-D β H-saporin	Advanced Targeting Solutions	KIT-03
Anti-IgG-saporin (inactive)	Advanced Targeting Solutions	KIT-03
Avidin-biotin-peroxydase (ABC kit)	ThermoFisher Scientific	32020
Diaminobenzidine (DAB)	Sigma-Aldrich	D5905
Deschloroclozapine (DCZ)	MedChemExpress	HY-42110
6-OHDA hydrochloride	Sigma Aldrich	H4381
Desipramine	Sigma-Aldrich	D3900
Bacterial and virus strains		
CAV2-PRS	https://plateau-igmm.pvm.cnrs.fr/?vector=cav-prs-ha-hm4di	CAV PRS HA-hM4D(Gi)

671

672

673 **RESOURCE AVAILABILITY**

674 **Lead contact**

675 Further information and requests for resources should be directed to and will be fulfilled by
676 the lead contact, Etienne Coutureau (etienne.coutureau@u-bordeaux.fr).

677

678 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

679 **Animals and housing**

680 A total of 136 male Long-Evans rats, aged 2-3 months, were obtained from the Centre
681 d'Élevage Janvier (France). Rats were housed in pairs with *ad libitum* access to water and
682 standard lab chow prior to behavioural experiments. Rats were handled daily for 3 days prior
683 to the beginning of the experiments and were put on food restriction 2 days before behaviour
684 to maintain them at approximately 90% of their *ad libitum* feeding weight. The facility was
685 maintained at 21 ± 1 °C on a 12 hr light/dark cycle (lights on at 8:00 am). Environmental
686 enrichment was provided by tinted polycarbonate tubes and nesting material, in accordance
687 with current French (Council directive 2013-118, February 1, 2013) and European (directive
688 2010-63, September 22, 2010, European Community) laws and policies regarding animal
689 experimentation. The experiments received approval from the local Bordeaux Ethics
690 Committee (CE50).

691

692 **METHODS DETAILS**

693 **Stereotaxic surgery**

694 For all experiments, rats were anesthetized with 5% inhalant isoflurane gas with oxygen and
695 placed in a stereotaxic frame with atraumatic ear bars (Kopf Instruments) in a flat skull
696 position. Anaesthesia was maintained with 1.5% isoflurane and complemented with a

697 subcutaneous injection of ropivacaine (a bolus of 0.1 mL at 2 mg/mL) at the incision site. After
698 each injection, the injector was kept in place for an additional 10 min before being removed.
699 Rats were given 4 weeks to recover following surgery. Injection sites were confirmed
700 histologically after the completion of behavioural experiments.

701 In the first experiment (n = 57), we used a toxin selective for noradrenergic neurons (saporin;
702 SAP) to target and deplete noradrenergic terminals in the OFC. For half of the rats ('Pre'
703 groups, n = 29), surgery was performed before the initial instrumental training and testing
704 phase, for the other half surgery was performed after the initial training and testing ('Post'
705 groups, n = 28). Intracerebral injections were made using repeated pressure pulses delivered
706 via a glass micropipette connected to a pressure injector (Picospritzer III, Parker). For SAP
707 groups (Pre n = 15; Post n = 15), 0.1 μ L of anti-D β H saporin (0.1 μ g/ μ L) was bilaterally injected
708 at one site targeting the OFC, while control (CTL) rats (Pre n = 14; Post n = 13) received 0.1 μ L
709 of inactive anti-IgG saporin (0.1 μ g/ μ L). Injection coordinates (in mm from Bregma) were
710 determined from the atlas of Paxinos and Watson (2014): +3.5 antero-posterior (AP), \pm 2.2
711 medio-lateral (ML) and -5.4 dorso-ventral (DV).

712 We then used a toxin selective for catecholaminergic neurons (6-OHDA hydrochloride) and a
713 noradrenaline uptake-blocker (desipramine; Desi) to target and deplete dopaminergic
714 neurons in the OFC. All rats underwent surgery after the initial instrumental training phase.
715 Rats were then allocated to the full catecholaminergic depletion condition (group 6-OHDA n
716 = 12; control n = 8) or the specific DA depletion condition (6OHDA+Desi n = 9; control n = 8).
717 6-OHDA (4 μ g/ μ L) was dissolved in vehicle solution containing 0.9% NaCl and 0.1% ascorbic
718 acid. A volume of 0.2 μ L of 6-OHDA was bilaterally injected in the OFC at the same coordinates
719 as for the first experiment. Animals in the CTL group received injections of the vehicle solution.

720 Thirty minutes before the surgical procedure, animals in the 6-OHDA+Desi group received a
721 systemic (i.p.) injection of desipramine (25 mg/mL) at a volume of 1 mL/kg.

722 In the chemogenetic experiments (n = 42), we employed a canine adenovirus type 2 (CAV2)
723 vector equipped with a noradrenergic-selective synthetic promoter (PRS) and inhibitory
724 DREADDs (hM4Di) to specifically target LC:OFC and LC:mPFC noradrenergic projections. This
725 viral construct was designed and controlled at the Plateforme de Vectorologie de Montpellier,
726 Institute of Molecular Genetics, Montpellier, [https://plateau-igmm.pvm.cnrs.fr/?vector=cav-
727 prs-ha-hm4di](https://plateau-igmm.pvm.cnrs.fr/?vector=cav-prs-ha-hm4di). All rats underwent surgery before the initial instrumental training phase. All
728 animals received 1.0 µL bilateral injections of the adenovirus (titer 3.5×10^{12} pp/mL), which
729 were performed using a 10 µL Hamilton syringe and a stereotax-mounted injection pump
730 (World Precision Instruments) at a flow rate of 100 nL/min. To target both ventral and lateral
731 regions of the OFC, rats (n = 25) were injected at the following coordinates (mm from Bregma):
732 +3.7 AP, ±2.0 ML, -5.0 DV and +3.2 AP, ±2.8 ML, -5.2 DV (2 injection sites per hemisphere). To
733 target the mPFC, rats (n = 17) were injected at the following coordinates (mm from Bregma):
734 +3.2 AP, ±0.6 ML, -3.6 DV (1 injection site per hemisphere).

735

736 **Behavioural apparatus**

737 For all behavioural experiments, training and testing was conducted in 8 identical operant
738 chambers (40 cm width x 30 cm depth x 35 cm height, Imetronic, Pessac, France) individually
739 enclosed in sound and light resistant wooden chambers (74 x 46 x 50 cm). Each chamber was
740 equipped with 2 pellet dispensers that delivered grain (Rodent Grain-Based Diet, 45mg, Bio-
741 Serv) or sugar (LabTab Sucrose Tablet, 45 mg, TestDiet) pellets into a food port when
742 activated. For instrumental conditioning, two retractable levers were located on each side of
743 the food port. Each chamber had a ventilation fan producing a background noise of 55 dB.

744 During the session, the chamber was illuminated by four LEDs in the ceiling. Experimental
745 events were controlled and recorded by a computer located in the room and equipped with
746 the POLY software (Imetronic).

747

748 **Behavioural protocol**

749 ***Initial training and test***

750 The training procedure was adapted from Parkes et al (2018). On days 1-3, rats were trained
751 to retrieve food pellets from the food port. During each daily session, 40 sugar and 40 grain
752 pellets were delivered pseudo-randomly every 60 s, on average. Following food port training,
753 rats received 12 daily sessions of instrumental training, during which they were required to
754 learn initial action-outcome (A-O) associations. During these sessions, each lever, in
755 alternation, was presented twice for a maximum of 10 min or until 20 outcomes were earned.
756 The inter-trial interval between lever presentations was 2.5 min (i.e., the session could last up
757 to 50 min and the rats could obtain a maximum of 80 food pellets). The A-O associations and
758 the order of lever presentations were counterbalanced between rats and days. During the first
759 three sessions, lever pressing was continuously reinforced with a fixed ratio (FR) 1 schedule.
760 Then, the probability of receiving an outcome was reduced, first with a random ratio (RR) 5
761 schedule (days 4-6), then with an RR10 (days 7-9,) and an RR20 schedule (days 10-12).
762 Outcome devaluation tests were performed one day after the last instrumental training
763 session. First, to induce sensory-specific satiety (Rolls 1986), rats received access to one of the
764 two outcomes (20 g) for 1 hr in a set of plastic feeding cages to which they were previously
765 habituated. Immediately after the satiety procedure, rats were returned to the operant
766 chambers where they were given a choice test in extinction (i.e. unrewarded) with both levers
767 available for 10 min. The devalued (sated) food was counterbalanced between rats. Following

768 the extinction test, animals were returned to the plastic feeding cages and given a
769 consumption test of satiety-induced devaluation, during which they received 10 min
770 concurrent access to both types of food pellets (10 g of each). The amount consumed of each
771 pellet type was measured to confirm that the satiety-induced devaluation was effective and
772 that rats were able to distinguish between the sensory features of the different food pellets.

773 ***Reversal training and test***

774 Following the initial phase, rats were trained on reversed A-O associations with a procedure
775 adapted from previous studies from our laboratory (Fresno et al 2019, Parkes et al 2018).
776 Specifically, the identity of outcomes was switched so that rats had to update previously
777 established A-O associations, always keeping a RR20 schedule of reinforcement. Following
778 reversal training, outcome devaluation tests were conducted in the same manner as
779 previously described.

780

781 **Chemogenetics**

782 The DREADD agonist deschloroclozapine (DCZ) was dissolved in dimethyl sulfoxide (DMSO) to
783 a final volume of 50 mg/mL, aliquoted in small tubes (50 μ L) and stored at -80°C (stock
784 solution). For behavioural experiments, our stock solution was diluted in physiological saline
785 to a final injectable volume of 0.1 mg/kg and administered systemically (i.p.) 40-45 min prior
786 to testing at a volume of 10 mL/kg. Fresh injectable solutions were prepared from stock
787 aliquots on the day of the usage. DCZ was always handled in dim light conditions.

788

789 **Histology**

790 At the end of all behavioral experiments, rats were injected with a lethal dose of sodium
791 pentobarbital (Exagon® Euthasol) and perfused transcardially with 60 mL of saline followed by

792 260 mL of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Brains were removed
793 and post-fixed in the same PFA 4% solution overnight and then transferred to a 0.1 M PB
794 solution or to a 0.1 M PB with 30% saccharose solution (6-OHDA experiment). Subsequently,
795 40 μ m coronal sections were cut using a VT1200S Vibratome (Leica Microsystems) or freezing
796 microtome for the 6-OHDA experiment. Every fourth section was collected to form a series.
797 DAB staining was performed for D β H (for the saporin and the 6-OHDA experiments), TH (6-
798 OHDA experiment), HA and mCherry (chemogenetic experiments).
799 Free-floating sections were first rinsed (4 x 5 min) in 0.1 M phosphate buffer saline (PBS)
800 containing 0.3% Triton X-100 (PBST) and then incubated in PBST containing 0.5% (for mCherry)
801 or 1% (for D β H and TH) hydrogen peroxide solution (H₂O₂) for 30 min in the dark. Further
802 rinses (4 x 5 min) in PBST and a 1 hr incubation in blocking solution (PBST containing 3% goat
803 serum) followed. Sections were then incubated with the primary antibody (mouse monoclonal
804 anti-DBH, 1/10000; mouse monoclonal anti-TH, 1/2000; rabbit monoclonal anti-HA, 1/1000;
805 rabbit polyclonal anti-RFP, 1/2000) diluted in blocking solution for 24 hr (for mCherry) or 48
806 hr (for D β H and TH) at 4°C. After rinses (4 x 5 min) in PBS (for D β H and TH) or PBST (for
807 mCherry), sections were placed in a bath containing the secondary antibody (biotinylated goat
808 anti-mouse, 1/1000; biotinylated goat anti-rabbit, 1/1000) diluted in PBS (for D β H and TH) or
809 PBST containing 1% goat serum (for mCherry) for 2 hr at room temperature. Following rinses
810 (4 x 5 min) in PBS (for D β H and TH) or PBST (for mCherry), they were then incubated with the
811 avidin-biotin-peroxydase complex (1/200 in PBS for D β H and TH; 1/500 in PBST for mCherry)
812 for 90 min in the dark at room temperature. H₂O₂ was added to the solution before the final
813 staining with DAB was made (10 mg tablet dissolved in 50 mL of 0.1 M Tris buffer). Stained
814 sections were finally rinsed with 0.05 M Tris buffer (2 x 5 min) and 0.05 M PB (2 x 5 min),

815 before being collected on gelatin-coated slides using 0.05 M PB, dehydrated (with xylene for
816 D β H and TH), mounted and cover-slipped using the Eukitt mounting medium.

817 Immunofluorescence staining was also performed for D β H, mCherry and HA (chemogenetic
818 experiments). Free-floating sections were first rinsed in PBS (4 x 5 min) and PBST (3 x 5 min),
819 before being incubated in blocking solution for 1 hr at room temperature. Sections were then
820 incubated with the primary antibody (mouse monoclonal anti-D β H, 1/1000; rabbit polyclonal
821 anti-RFP, 1/1000; rabbit monoclonal anti-HA, 1/1000) diluted in blocking solution for 24 hr at
822 4°C. Following rinses in PBS (4 x 5 min), they were then incubated with the secondary antibody
823 (goat polyclonal anti-mouse FITC-conjugated, 1/400; goat polyclonal anti-rabbit TRITC-
824 conjugated, 1/200; with goat polyclonal anti-rabbit, 1/1000) diluted in PBS for 2 hr in the dark
825 at room temperature. Stained sections were finally rinsed with PSB (4 x 5 min), before being
826 collected on gelatin-coated slides using 0.05 M PB, dehydrated, mounted and cover-slipped
827 using Fluoroshield with DAPI mounting medium.

828

829 **QUANTIFICATION AND STATISTICAL ANALYSIS**

830 **Fiber loss**

831 To measure fiber density in the saporin and 6-OHDA experiments, we used the protocol
832 described in Cerpa et al (2019). We examined sections at +4.4, +3.7 and +3.0 (mm from
833 Bregma) using a Nanozoomer slide scanner with a 20X lens (Hamamatsu Photonics). Digital
834 microphotographs of regions of interest (ROI, square windows of 300 x 300 μ m, 1320 x 1320
835 pixels) in each hemisphere were examined under a 20X virtual lens with the NDP.view 2
836 freeware (Hamamatsu Photonics). Each ROI was outlined according to Paxinos and Watson
837 (2014). Quantification of D β H- and TH-positive fibers was performed using an automated
838 method developed in the laboratory with the ImageJ software (Cerpa et al 2019). Specifically,

839 a digitized version of the microphotograph was converted to black and white by combining
840 blue, red and green channels (weights 1, -0.25 and -0.25), subjected to a median filter (radius
841 3 pixels) in order to improve the signal-to-noise ratio, smoothed with a Gaussian filter (radius
842 8), and subtracted from the previous picture to isolate high spatial frequencies. Large stains
843 were further eliminated by detecting them in a copy of the image. The picture was then
844 subjected to a fixed threshold (grey level 11) to extract stained elements, and the relative
845 volume occupied by fibers estimated by the proportion of detected pixels in the ROI. As a
846 control for poor focus, the same images were analysed a second time while allowing lower
847 spatial frequencies (Gaussian filter radius 20). The ratio between the proportions of pixels
848 detected by the two methods was used as a criterion to eliminate blurry images.

849

850 **Experimental design and data analysis**

851 Each rat was assigned a unique identification number that was used to conduct blind testing
852 and statistical analyses. Behavioural data and fibre volume were analysed using sets of
853 between and within orthogonal contrasts controlling the per contrast error rate at alpha =
854 0.05 (Hays, 1963). Simple effects analyses were conducted to establish the source of
855 significant interactions. Statistical analyses were performed using PSY Statistical Program
856 (<http://www.psy.unsw.edu.au/research/research-tools/psy-statistical-program>; Kevin Bird,
857 Dusan Hadzi-Pavlovic, and Andrew Issac © School of Psychology, University of New South
858 Wales) and graphs were created using GraphPad Prism.

859 All experiments employed a between- x within-subjects behavioural design. In the first
860 experiment, the between-subject factors were group (Pre versus Post) and treatment (control
861 versus saporin) and the within-subject factors were training day (acquisition data) or
862 devaluation (responding on lever associated with non-devalued or devalued outcome) for the

863 test data. In the 6-OHDA experiments, the between-subject factor was group (control vs 6-
864 OHDA or control vs 6OHDA+Desi) and the within-subject factor was training day (acquisition
865 data) or devaluation (test data). To analyse D β H and TH fibre volume, the between-subject
866 factor was group (experiment 1: control versus saporin; experiment 2: control, 6OHDA+Desi,
867 or 6OHDA) and the within-subject factor was region (VO versus LO) for the OFC. There was no
868 within-subject factor for the quantification of fibres in the mPFC. In the final chemogenetics
869 experiment, the between-subject factor was treatment during reversal acquisition (vehicle
870 versus DCZ) and the within-subject factors were training day (acquisition data) or treatment
871 during reversal test (vehicle versus DCZ) and devaluation (test data).