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## Synaptic mechanism underlying serotonin modulation of transition to cocaine addiction

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28 Compulsive drug use despite adverse consequences defines addiction. While mesolimbic dopamine  
29 signaling is sufficient to drive compulsion, psychostimulants such as cocaine also boost extracellular  
30 serotonin (5-HT) by inhibiting reuptake. We used SERT Met172 knockin (SertKI) mice carrying a  
31 transporter that no longer binds cocaine to abolish 5-HT transients during drug self-administration  
32 (SA). SertKI mice showed an enhanced transition to compulsion. On the other hand,  
33 pharmacologically elevating 5-HT reversed the inherently high rate of compulsion transition with  
34 optogenetic dopamine self-stimulation. The bidirectional effect on behavior was explained by  
35 presynaptic depression of orbitofrontal cortex to dorsal striatum synapses induced by 5-HT via 5-  
36 HT<sub>1B</sub> receptors. Consequently, in projection-specific 5-HT<sub>1B</sub> receptor knockout mice the fraction of  
37 individuals compulsively self-administering cocaine was elevated.  
38

39 With chronic consumption, about 20% of cocaine users lose control and are eventually diagnosed  
40 as addicted (1). Increasing dopamine (DA) levels is typical of all addictive drugs (2), sufficient to  
41 trigger forms of synaptic plasticity underlying adaptive behaviors (3-5). This is exemplified by  
42 optogenetic DA neuron self-stimulation (oDASS), inducing neuronal adaptations similar to  
43 addictive drugs via selective release of DA from ventral tegmental area (VTA) neurons and yielding  
44 a bimodal distribution of compulsive and non-compulsive individuals (6). Cocaine also inhibits the  
45 serotonin transporter (SERT) causing 5-HT transients in the striatum. While pharmacological  
46 reduction of 5-HT in the entire forebrain can favor compulsive cocaine seeking (7) and differential  
47 efficacy of the 5-HT system may be involved in the vulnerability to drug addiction (8, 9), the  
48 relevant circuits and underlying cellular mechanisms remain elusive. To parse the locus of 5-HT  
49 modulation, we took advantage of SERT Met172 knockin (SertKI) mice carrying a transporter that  
50 does not bind cocaine without altering the basal 5-HT levels (10-12). Genetically encoded 5-HT  
51 sensors (Fig. S1) confirmed the absence of cocaine-evoked transients in the dorsal striatum (DS) of  
52 SertKIs (15 mg/kg i.p., Fig. 1A to C). Mice were trained to press a lever that triggered a cocaine i.v.  
53 infusion (0.5 mg/kg/infusion) accompanied by a cue light, followed by a progressive ratio (PR)  
54 session and four punishment (0.2 mA foot shock) sessions (Fig. 1D and fig. S2A). There was no  
55 difference between the SertKI and WT groups during the acquisition period (Fig. 1E). Facing  
56 punishment, however, some individuals reduced cocaine self-administration (SA), while others  
57 continued unabated (Fig. 1F). An unbiased clustering analysis integrating four behavioral  
58 parameters over the last two punishment sessions yielded two clusters: renouncers and perseverers  
59 (Fig. 1G and H). 14 out of 25 (56%) SertKI mice were classified as perseverers, in stark contrast to  
60 the 3 out of 26 (12%) in WT littermate mice (Fig. 1I). Perseverance was correlated neither to  
61 baseline cocaine SA (Fig. 1J) nor to the break point (fig. S2B), and the success rate accomplishing  
62 increasing break points did not differ between renouncers and perseverers from either genotype  
63 (Fig. 1K). Perseverers and renouncers across genotype perceived pain similarly and hot plate latency  
64 was not affected by cocaine (fig. S2C).

65 Next, we did the converse. We allowed mice to oDASS, which leads to compulsion in more than  
66 half of individuals (6) and pharmacologically elevated 5-HT levels with citalopram (Fig. 2A-C).  
67 Citalopram (10 mg/kg) induced robust 5-HT transients of magnitude comparable to cocaine (fig.  
68 S1D). Active lever presses in mice that expressed ChR2 in VTA DA neurons induced a brief train  
69 of laser stimulations (LS, see methods). All mice readily acquired oDASS (Fig. 2D) regardless of  
70 pharmacological treatment, but major differences emerged when punishment was introduced (Fig.  
71 2E). Clustering analysis as above led to the emergence of perseverers and renouncers in both  
72 treatment groups (Fig. 2F and G). However, only 4 out of 26 (15%) citalopram treated mice were  
73 classified as perseverer, while in the saline treated group 60% were perseverers, a fraction similar  
74 to previous reports (13) (Fig. 2H). Again, perseverance rate was uncorrelated to baseline oDASS  
75 rate (Fig. 2I) or the break point (fig. S2D), and success rate accomplishing each break point did not  
76 differ between renouncers and perseverers across treatment groups (Fig. 2J). For all groups and  
77 condition pain perception was similar (fig. S2E).

78 Given that for oDASS, the synaptic potentiation of afferents from the orbitofrontal cortex (OFC) to  
79 DS drives perseverance (13), we wondered whether this is also the case for cocaine SA. To

80 selectively stimulate the OFC-DS projection, we expressed a red shifted opsin Chrimson in OFC  
81 neurons (Fig 3A) and evoked EPSCs by illuminating the terminals in brain slices of the DS 24-48 h  
82 after the last punishment session. AMPA/NMDA ratio was higher in perseverers than in renouncers  
83 of cocaine SA as well as oDASS, regardless of genotypes and treatment (Fig 3B to E), confirming  
84 that a potentiated OFC-DS pathway reflects perseverance both in oDASS and cocaine SA. In no  
85 condition were the EPSCs rectifying (fig. S3), suggesting a potentiation by an increase of the  
86 number of AMPA receptors without change in subunit composition, akin to the expression  
87 mechanism observed in individuals with compulsive oDASS (13).

88 We next examined the effect of 5-HT on synaptic transmission at the OFC-DS pathway in naïve  
89 mice. Bath application of 5-HT (4  $\mu$ M) induced a presynaptic depression of excitatory transmission  
90 (Fig. 3F and G), which could be blocked by 5-HT<sub>1B</sub> receptor antagonist NAS181 (20  $\mu$ M), but not  
91 5-HT<sub>1A</sub> receptor antagonist WAY100635 (1  $\mu$ M) (Fig. 3F and G). In line with the G<sub>i/o</sub> coupling of  
92 pre-synaptically located 5-HT<sub>1B</sub> receptors, we observed a decreased coefficient variance ( $1/CV^2$ )  
93 and increased paired pulse ratio (PPR) suggesting that the presynaptic depression was expressed by  
94 a reduction of glutamate release probability (Fig. 3H and I). For confirmation, we evoked quantal  
95 events (qEPSC) after replacing extracellular calcium (Ca<sup>2+</sup>) with strontium (Sr<sup>2+</sup>) thus  
96 desynchronize light evoked transmitter release (14) and found that the qEPSC frequency decreased  
97 but the amplitude stayed unchanged (fig. S4A-C). Furthermore, 5-HT induced presynaptic  
98 depression in both D1 positive and negative neurons obtained from D1-tdTomato mice (fig. S4D),  
99 consistent with previous reports (15-16).

100 We next hypothesized that presynaptic depression may reduce the likelihood for LTP at the OFC-  
101 DS synapse, which in turn would prevent the transition to compulsion in cocaine SA. This seems  
102 plausible since chemogenetic reduction of OFC activity also reduced the fraction of perseverers in  
103 oDASS (6). Therefore, to establish a causal link between 5-HT induced presynaptic depression and  
104 compulsive cocaine use, we aimed to abolish 5-HT<sub>1B</sub> receptors selectively in the OFC neurons  
105 targeting the DS. We injected retroAAV-ef1 $\alpha$ -mCherry-IRES-Flpo in the DS of 5-HT<sub>1B</sub> floxed mice  
106 (17). This led to Flpo expression in OFC neurons targeting the DS. AAV9-ef1 $\alpha$ -fDIO-Cre or control  
107 virus was then injected in the OFC to express Cre in OFC cells targeting the DS (Fig. 4A and B),  
108 which then led to recombination and abolishment of 5-HT<sub>1B</sub> receptors. We confirmed the successful  
109 receptor knockout functionally by the inability of 5-HT<sub>1B</sub> agonist CP39129 (2  $\mu$ M) to induce a  
110 presynaptic depression (Fig. 4C and D).

111 A month after virus injection, the 5-HT<sub>1B</sub> knockout mice learned to self-administer cocaine (Fig.  
112 4E). We observed an acquisition period (Fig. 4F) similar to the one described above (Fig. 1E). Once  
113 punishment was introduced, we again observed persevering and renouncing mice (Fig. 4G and H),  
114 with a higher fraction of perseverers in the projection specific 5-HT<sub>1B</sub> knockout compared to control  
115 group (57% versus 13%) (Fig. 4I). Perseverance was unrelated to baseline performance (Fig. 4J), or  
116 break point in the two groups, in both control and knockout mice (Fig. 4K). The percentage of  
117 perseverers in the 5-HT<sub>1B</sub> knockout group was very close to the fraction of perseverers in the SertKI  
118 group and in saline treated oDASS mice.

119 A synaptic mechanism thus emerges that underlies a modulatory role of 5-HT reducing the  
120 likelihood of transition to compulsion and eventually addiction (see fig. S5). In wildtype mice,  
121 cocaine binds to SERT to block 5-HT reuptake. The elevated extracellular 5-HT activates 5-HT<sub>1B</sub>  
122 receptors and causes presynaptic depression of OFC terminals. This reduces the likelihood of  
123 inducing postsynaptic potentiation at OFC-DS synapses that ultimately drives compulsion. In  
124 SertKI mice, cocaine cannot bind to SERT and extracellular 5-HT remains unaffected by cocaine  
125 infusions (10). An OFC-DS transmission not undergoing presynaptic depression may thus enhance  
126 the likelihood to induce LTP induction and the stochastic process would then show as a higher  
127 fraction of compulsive individuals. In 5-HT<sub>1B</sub> knockout mice, although cocaine still inhibits 5-HT  
128 reuptake the OFC-DS transmission is also not depressed, which again may favor LTP induction.  
129 This interpretation is in line with the report that genome-wide 5-HT<sub>1B</sub> receptor knockout mice are  
130 more impulsive (17). These mice are also more vulnerable to cocaine (18), which raises the  
131 possibility that individual addiction liability may be determined by 5-HT signaling. Variation in 5-  
132 HT synthesis, synaptic release, efficiency of reuptake and extracellular levels could be additional  
133 determinants of overall vulnerability. Here we reveal addiction liability once 5-HT modulation has  
134 been eliminated, which is in line with the general idea that 5-HT opposes DA effects to inhibit  
135 behavior (19). However, this model is challenged by the observation that selective activation of 5-  
136 HT neurons allows to maintain high motivation in complex tasks (20, 21).

137 While our study focused on cocaine, 5-HT may also counteract the transition to compulsion when  
138 other addictive drugs are consumed (8, 9). Amphetamine while having a relatively low SERT  
139 affinity increases non vesicular release of 5-HT and opioids may indirectly activate 5-HT neurons  
140 in the dorsal raphe (22-24). In fact the ratio between DAT and SERT affinity may predict the  
141 addiction liability of emerging drugs (25). This may also apply to natural rewards, such as food and  
142 sex, which however have low addiction liabilities, such that empirical testing will be challenging.  
143 Last but not least, it may also be interesting to explore whether 5-HT modulation levels may not  
144 only prevent the transition to compulsion, but also facilitate regaining control, as suggested by  
145 pharmacological interventions in rodents in a distinct behavioral paradigm. Forebrain 5-HT<sub>2C</sub>  
146 receptors may inhibit compulsive cocaine seeking after compulsion is established (7), possibly via  
147 modulation of acute effects and early adaptive behaviors (26). By contrast, our data show that  
148 pathway specific knockout 5-HT<sub>1B</sub> receptors does not affect acquisition or motivation for cocaine  
149 and specifically modulates compulsion. Three days of 5-HT<sub>1B</sub> agonist (CGS12066B, 10 mg/kg, 30  
150 min i.p. injection before each session) treatment after the last punishment session of oDASS left  
151 perseverance to oDASS intact (fig. S6), confirming that serotonin prevents transition to compulsion  
152 but cannot reverse it, once it is established.

153 The present mechanistic investigation may help to refine approaches to overcome the limitations  
154 and diverging findings on efficacy of 5-HT reuptake blockers in pilot studies with human addicts  
155 (27-29) or design selective agonist complementing to the empirical use of hallucinogens in addiction  
156 treatments (30). In summary, 5-HT emerges as a modulator of the progression to compulsion via  
157 the convergence on key synaptic mechanisms in the framework of the current circuit model for  
158 addiction (5).

159

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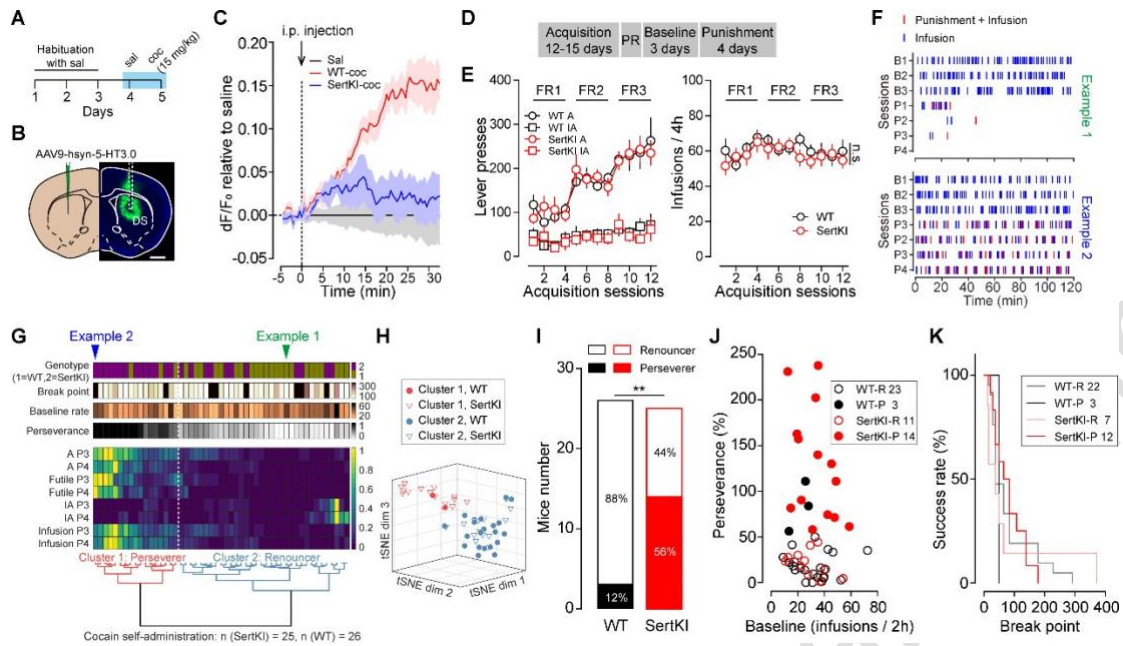
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204 YL, RVZ, JF; Visualization: YL, JF; Reagents: KMN, JXW, FR, YLL, Funding acquisition: CL;  
205 Supervision: CL; Writing: YL, VP, CL; Senior authors: YLL, KMN, VP, CL.

206 **Competing interests:** Authors declare that they have no competing interests.

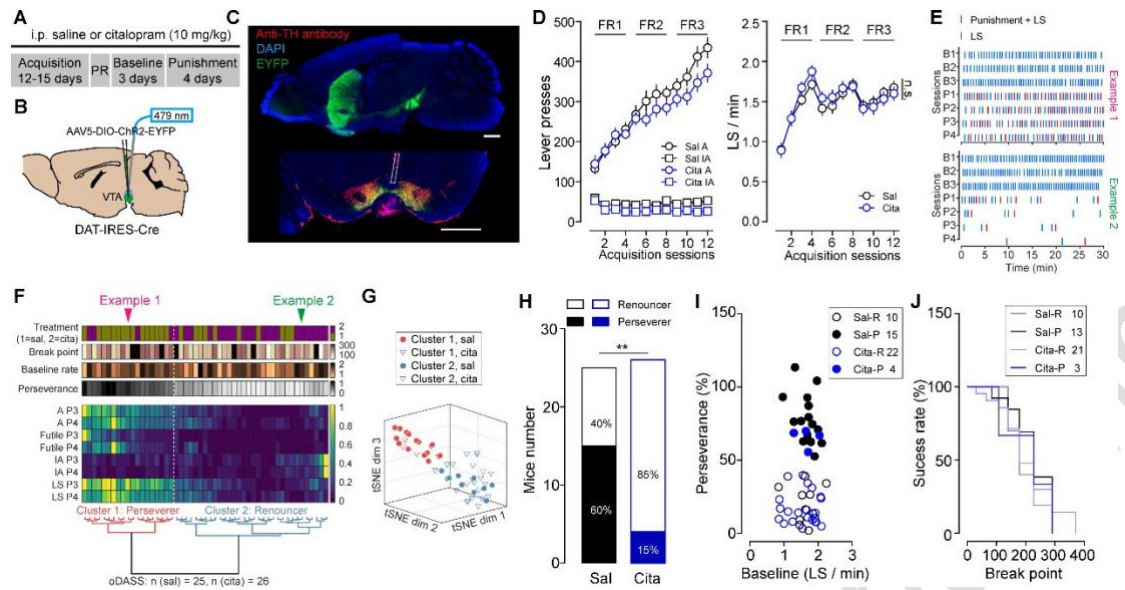
207 **Data and materials availability:** All data, code, and materials are available on Zenodo at the  
208 following address (10.5281/zenodo.5026115).

209



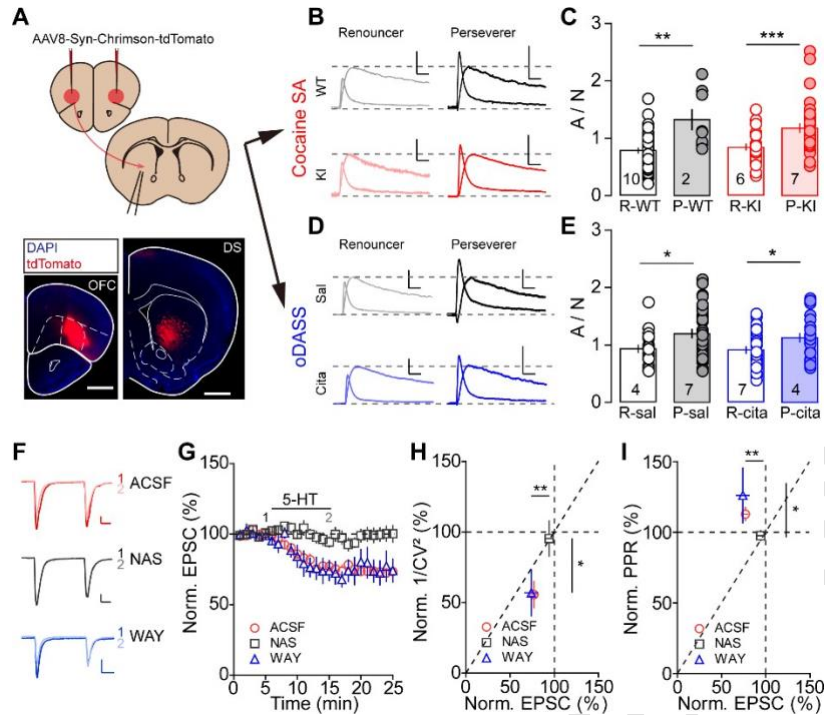
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210 **Fig. 1. SertKI animals are more compulsive for cocaine self-administration.** (A) Schedule of  
 211 saline and cocaine injections. (B) GRAB 5-HT sensor expression indicated with GFP staining in the  
 212 DS. Scale bars, 1 mm. (C) 5-HT transients in the DS induced by saline / cocaine (15 mg/kg) i.p.  
 213 injection in WT and SertKI mice ( $n = 3$  mice for WT and SertKI group, data from saline injected  
 214 WT and SertKI are pooled). (D) Timeline of cocaine SA. (E) Left, number of active (A) and inactive  
 215 (IA) lever presses of WT (black) and SertKI (red) animals ( $n = 26$  and  $25$  for WT and SertKI group)  
 216 in acquisition sessions. Right, cocaine infusions obtained from WT (black) and SertKI (red) mice  
 217 in acquisition sessions (two way ANOVA;  $F_{1,588} = 1.996$ ,  $P = 0.1583$ ;  $n = 26$  and  $25$  for WT and  
 218 SertKI group). (F) Raster plots for infusions (blue lines) and punishments (red lines) in baseline and  
 219 punishment sessions of a renouncer (upper, WT mouse) and a perseverer (lower, SertKI mouse).  
 220 (G) Hierarchical clustering based on tSNE projection of different parameters of punishment sessions  
 221 3 and 4 (P3 and P4) of cocaine SA. Blue and green arrow heads indicate examples presented in F.  
 222 (H) tSNE three dimensional representation of clusters of perseverers (cluster 1) and renouncers  
 223 (cluster 2) in cocaine self-administration. (I) Percentage of perseverers and renouncers among WT  
 224 and SertKI groups (Fisher's exact test;  $P = 0.001$ ). (J) Perseverance rate as a function of baseline  
 225 rate (Pearson  $r = -0.08$ ;  $P = 0.55$ ). (K) The success rate of performance as a function of the last  
 226 progressive ratio value achieved by the mice (logrank test;  $P = 0.95$ ). Abbreviations, PR, progressive  
 227 ratio; FR, fixed ratio; A, active lever presses; IA, inactive lever presses; R, renouncer; P, perseverer;  
 228 sal, saline; coc, cocaine; WT, wildtype; SertKI, SERT Met172 knockin. Data presented as means  $\pm$   
 229 SEM.  
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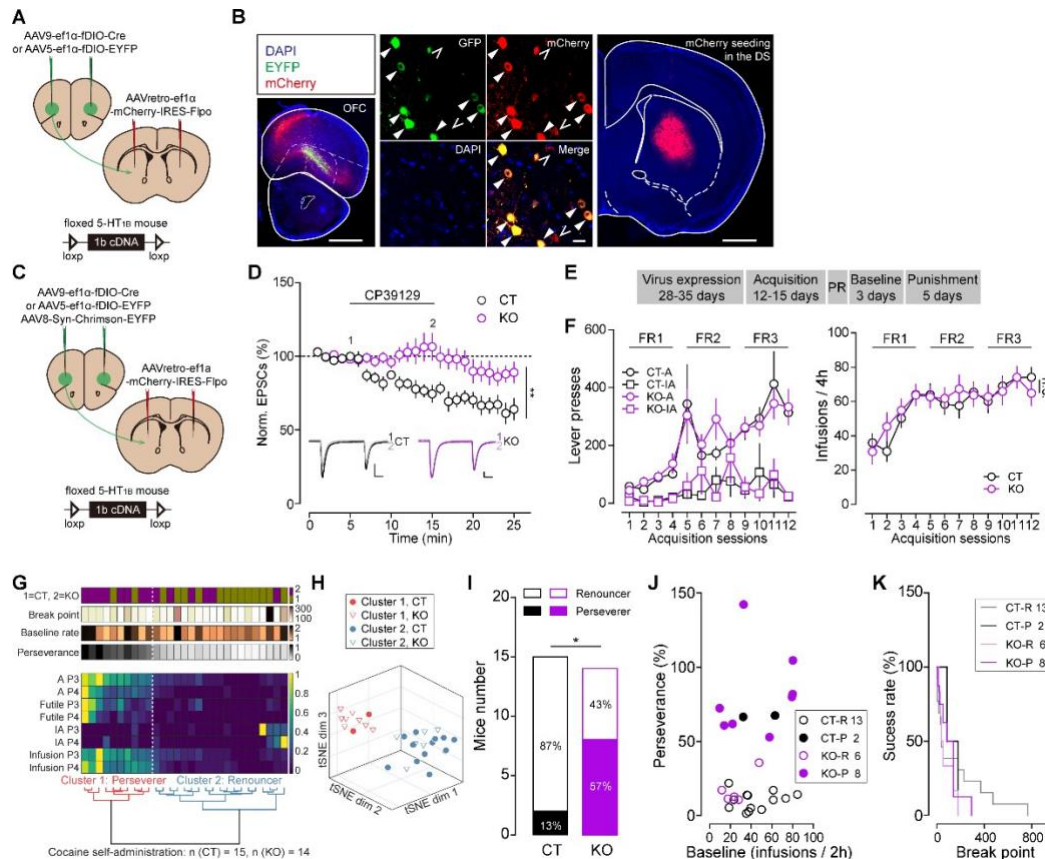
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232 **Fig. 2. Citalopram decreases compulsive dopamine self-stimulation.** (A) Timeline of oDASS  
 233 and saline / citalopram treatments. (B) Schematic of virus injection sites and optic fiber implantation  
 234 sites. (C) ChR2-EYFP expression in the VTA from a sagittal slice (upper) and a coronal slice co-  
 235 labeled with TH (lower). Scale bars, 1 mm. (D) Left, number of active (A) and inactive (IA) lever  
 236 presses of saline (black) and citalopram (blue) treated mice ( $n = 25$  and  $26$  for saline and citalopram  
 237 group) in acquisition sessions. Right, laser stimulation per minute obtained from saline (black) and  
 238 citalopram (blue) treated mice in acquisition sessions (two way ANOVA;  $F_{1,588} = 0.73$ ,  $P = 0.39$ ;  $n$   
 239  $= 25$  and  $26$  for saline and citalopram group). (E) Raster plots for laser stimulations (blue lines) and  
 240 punishments (red lines) in baseline and punishment sessions of a perseverer (upper, from saline  
 241 group) and a renouncer (lower, from citalopram group). (F) Hierarchical clustering based on  
 242 different parameters of punishment sessions 3 and 4 (P3 and P4) of oDASS. Red and green arrow  
 243 heads indicate examples presented in E. (G) tSNE three dimensional representation of clusters of  
 244 perseverers (cluster 1) and renouncers (cluster 2) in oDASS. (H) Percentage of perseverers and  
 245 renouncers among saline and citalopram treated groups (Fisher's exact test;  $P = 0.001$ ). (I)  
 246 Perseverance rate as a function of baseline rate (Pearson  $r = 0.05$ ;  $P = 0.71$ ). (J) The success rate of  
 247 performance as a function of the last progressive ratio value achieved by the mice (logrank test;  $P$   
 248  $= 0.95$ ). Abbreviations, LS, laser stimulation; cita, citalopram. Data presented as means  $\pm$  SEM.  
 249



250

251 **Fig. 3. The OFC-DS pathway is modulated by 5-HT.** (A) Upper, Schematic of virus injection and  
 252 recording sites. Lower, Chromson-tdTomato expressing cell bodies in the OFC (left) and terminals  
 253 in the DS (right). Scale bars, 1 mm. (B) AMPA and NMDA currents at +40 mV of a renouncer (left)  
 254 and a perseverer (right) from WT (upper) and SertKI (lower) group after cocaine SA. Scale bars,  
 255 200 pA, 15 ms. (C) Average A/N of WT and KI renouncers and perseverers after cocaine SA (Mann  
 256 Whitney test;  $U = 74$ ,  $P = 0.003$ ;  $n = 53$  and 8 cells from 10 and 2 mice for renouncer and perseverer  
 257 in WT group;  $U = 252$ ,  $P = 0.0001$ ;  $n = 32$  and 35 cells from 6 and 7 mice for renouncer and  
 258 perseverer in SertKI group). (D) AMPA and NMDA currents at +40 mV of a renouncer (left) and a  
 259 perseverer (right) from saline (upper) and citalopram (lower) treated group after oDASS. Scale bars,  
 260 200 pA, 15 ms. (E) Average A/N of saline and citalopram treated renouncers and perseverers after  
 261 oDASS (two tailed  $t$  test;  $t_{54} = 2.54$ ,  $P = 0.01$ ;  $n = 21$  and 35 cells from 4 and 7 mice for renouncer  
 262 and perseverer in saline treated group;  $t_{55} = 2.38$ ,  $P = 0.02$ ;  $n = 34$  and 23 cells from 7 and 4 mice  
 263 for renouncer and perseverer in citalopram treated group). (F) Traces before and after bath  
 264 application of 5-HT in the presence of ACSF (red), NAS181 (gray), and WAY100635 (blue). Scale  
 265 bars, 200 pA, 10 ms. (G) Average traces of EPSC before, during and after bath application of 5-HT  
 266 in the presence of ACSF (red), NAS181 (gray), and WAY100635 (blue) ( $n = 13$  and 14 cells from  
 267 3 mice for ACSF and NAS181 group, and 7 cells from 4 mice for WAY100635 group). (H)  
 268 Normalized coefficient of variation ( $1/CV^2$ ) versus normalized EPSC (one way ANOVA;  $F_{2,31} =$   
 269  $3.682$ ,  $P = 0.0367$  for Norm.  $1/CV^2$ ;  $F_{2,31} = 7.948$ ,  $P = 0.0016$  for Norm. EPSC;  $n = 13$  and 14 cells  
 270 from 3 mice for ACSF and NAS181 group, and 7 cells from 4 mice for WAY100636 group). (I)  
 271 Normalized pair pulse ratio (PPR) versus normalized EPSC (Kruskal-Wallis test;  $P = 0.041$  for  
 272 Norm. PPR;  $n = 13$  cells from 3 mice for ACSF group, 10 and 6 cells from 2 mice for NAS181 and  
 273 WAY100636 group). Data presented as means  $\pm$  SEM.



274

275 **Fig. 4. Knocking out 5-HT<sub>1B</sub> receptors promotes compulsive cocaine self-administration.** (A)  
 276 Schematic of virus injections for cocaine SA. (B) Left and middle, EYFP co-expressing with  
 277 mCherry in the OFC. Right, mCherry seeding in the DS. Scale bars, 1 mm (left and right), and 20  
 278  $\mu$ m (middle). Arrows indicate DS projectors in the OFC expressing both EYFP and mCherry, open  
 279 arrows indicate DS projectors expressing mCherry not infected by AAV-fDIO-EYFP. (C)  
 280 Schematic of virus injections for patch clamp recording. (D) Average traces of EPSC before, during  
 281 and after bath application of 5-HT<sub>1B</sub> receptor agonist CP39129 in control (CT, black) and pathway  
 282 specific knockout 5-HT<sub>1B</sub> receptor (KO, violet) groups (compared Norm. EPSC recorded on last 5  
 283 minutes; two tailed *t* test;  $t_{25} = 2.86$ ,  $P = 0.008$ ;  $n = 15$  and 12 cells from 3 mice for CT and KO  
 284 group). Scale bars, 200 pA, 10 ms. (E) Timeline of virus injection and cocaine SA experiments. (F)  
 285 Left, number of active (A) and inactive (IA) lever presses of CT (black) and KO (violet) mice in  
 286 acquisition sessions. Right, cocaine infusions obtained by CT (black) and KO (violet) mice in  
 287 acquisition sessions (two way ANOVA;  $F_{1,324} = 0.198$ ,  $P = 0.66$ ;  $n = 15$  and 14 for CT and KO  
 288 group). (G) Hierarchical clustering based on different parameters of punishment sessions 3 and 4  
 289 (P3 and P4) of cocaine SA. (H) tSNE three dimensional representation of clusters of perseverers  
 290 (cluster 1) and renouncers (cluster 2) in cocaine SA. (I) Percentage of perseverers and renouncers  
 291 among CT and KO groups (Fisher's exact test;  $P = 0.02$ ). (J) Perseverance rate as a function of  
 292 baseline rate (Pearson  $r = 0.20$ ;  $P = 0.30$ ). (K) The success rate of performance as a function of the  
 293 last progressive ratio value achieved by the mice (logrank test;  $P = 0.84$ ). Data presented as means  
 294  $\pm$  SEM.

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## Supplementary Materials for

### **Synaptic mechanism underlying serotonin modulation of transition to cocaine addiction**

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#### **This PDF file includes:**

Materials and Methods  
Figs. S1 to S5  
References

## 25 **Materials and Methods**

### 26 **Animals**

27 DAT-IRES-Cre (B6.SJL-Slc6a3<sup>tm1.1(cre)Bkmm</sup>/J) mice were used for oDASS experiments.  
28 SERT Met172 knockin mice (10) on a C57BL/6J background (31) from Dr. Randy D.  
29 Blakely lab were used for cocaine self-administration experiments. 5-HT<sub>1B</sub> floxed mice  
30 from Dr. Katherine M. Nautiyal lab (17) were used for 5-HT<sub>1B</sub> receptor knock out  
31 experiments. D1-tdTomato (B6.Cg-Tg(Drd1a-tdTomato)6Calak/J) and C57BL/6J mice  
32 were used for electrophysiology studies. SERT-Cre mice (B6.129(Cg)-  
33 Slc6a4<sup>tm1(cre)Xz</sup>/J) were used for optogenetic activation of DR 5-HT neurons. Both male  
34 and female mice at the age of 6-20 weeks were used. Mice were food deprived 12 hours  
35 before the first session of oDASS and cocaine self-administration. 5-HT<sub>1B</sub> floxed mice  
36 were mildly food restricted (body weight kept above 90% of baseline) during all  
37 sessions of cocaine self-administration. In other conditions, food and water were  
38 provided ad libitum. Mice were single housed after surgery. All mice were housed at a  
39 constant temperature and humidity with a 12-h light/dark cycle. All procedures were  
40 approved by the Institutional Animal Care and Use Committee of the University of  
41 Geneva and by the animal welfare committee of the Canton of Geneva.

### 42 **Stereotaxic injections**

43 6-8 weeks old mice were deeply anesthetized using 5% Isoflurane (w/v) and placed in  
44 a stereotaxic apparatus (Angle One). Anesthesia was maintained with 2% Isoflurane.  
45 Lacryvisc (Alcon, Switzerland) was applied to prevent the eyes from drying. Lidocaine  
46 was applied on the surface of the epicranium. An incision was made to expose the  
47 bregma and lambda point of the skull. The skull above the target area was thinned with  
48 a dental drill and carefully removed. Viruses were injected with glass pipette at a rate  
49 of 50 nl/min. The amount of virus per injection site was 350-500 nl. After injection, the  
50 pipette was left in the place for 10 min to allow diffusion of the virus. The skin was  
51 sutured and disinfected after the injection. 500 µl saline was i.p. injected. Paracetamol  
52 (2 mg/ml in the water bottle) was given orally for the next 2-4 days.

53 To express opsins in the VTA, AAV5-ef1 $\alpha$ -DIO-ChR2(H134R)-EYFP (UNC) was  
54 injected with the coordinates: anterior-posterior (AP) -3.3; medial-lateral (ML) +0.9  
55 with a 10° angle; dorsal-ventral (DV) -4.3. To express opsins in the OFC, AAV8-hsyn-  
56 Chrimson-tdTomato (UNC) was injected bilaterally with the coordinates: AP +2.6, ML  
57  $\pm$ 1.65, DV -2.25. To express GRAB 5-HT sensor in the DS, AAV9-hSyn-5-HT3.0 from  
58 Yu-Long Li lab was injected unilaterally with the coordinate: AP +0.8; ML -1.8; DV -  
59 3.3. To express opsins in the DRN, AAV8-syn-Flex-Chrimson-tdTomato (UNC) were  
60 injected with the coordinate: AP -4.2, ML +0.6 with a 10° angle, DV -3.05. To knock  
61 down 5-HT<sub>1B</sub> receptors in the OFC-DS pathway, retroAAV-ef1 $\alpha$ -mCherry-IRES-Flpo  
62 (RRID: Addgene\_55634) was injected in the DS and AAV9-ef1 $\alpha$ -fDIO-Cre (RRID:  
63 Addgene\_121675) was injected in the OFC.

64 For oDASS and fiber photometry experiments, optic ferrules were placed 0.2 mm above  
65 the virus injection sites in the VTA or DS immediately after virus injection. Two screws  
66 and dental cement were used to secure the implantation.

### 67 **Catheter implantation**

68 Mice were anesthetized using a mixture of ketamine (80-100 mg/kg) and xylazine (10  
69 mg/kg) by i.p. injection. Lacryvisc (Alcon, Switzerland) was applied to prevent the eyes  
70 from drying. The right lateral part of the neck was disinfected and a small incision of  
71 about 5 mm was made above the vein. A small incision was made on the upper back  
72 between the shoulders, and the surrounding skin subcutaneous fat was detached. The  
73 catheter (model MIVSA, CamCaths) was filled with heparin (Heparin Bichsel®) and  
74 its tip was brought under the skin from the back to the incised neck area. The neck vein  
75 was carefully isolated and regularly rinsed with saline. A small hole was punched on  
76 the vein with a needle, and the tip of the catheter was placed into the vein through this  
77 hole. The successful placement was checked by withdrawing blood from the vein. The  
78 catheter was stabilized with cotton threads and glue. The catheter was put under the  
79 skin and all incisions on the neck and back were sutured and disinfected. 500 µl saline  
80 was i.p. injected. Paracetamol (2 mg/ml in the water bottle) was given orally for the  
81 next 2-4 days. Antibiotics (Amikin, 1 mg/kg) was injected s.c. for five days to prevent  
82 potential infections. Mice were single housed after catheter implantation.

### 83 **Optogenetic dopamine self-stimulation (oDASS)**

84 The mice were subjected to oDASS protocol adapted from previous publication (6, 13)  
85 4 weeks after recovery from the surgery. Operant chambers (ENV307A-CT, Med  
86 Associates) situated in sound-attenuating boxes (Med Associates) were used for operant  
87 tasks. The apparatus was controlled and data were captured using MED-PC IV software  
88 (Med Associates).

#### 89 Acquisition:

90 Mice were food deprived 12 h before the first session. After that the mice had food and  
91 water access ad libitum. Each session lasted for 1 h, and started with insertion of two  
92 levers. Active lever presses induced a train of laser stimulation following a delay of 5 s  
93 predicted with 10 s cue light right above the lever. The laser stimulation composed of  
94 30 bursts separated by 250 ms (each burst consisted of 5 laser pulses of 4-ms pulse  
95 width at 20 Hz). Inactive lever presses had no result. Each active lever press leading to  
96 laser stimulation was followed by a 20 s time out during which laser stimulation was  
97 no longer available. Acquisition consisted of 4-6 sessions with FR1, 4 sessions with  
98 FR2, and 4 sessions with FR3 (FR $n$  means the mice have to press the active lever  $n$   
99 times to get one laser stimulation).

#### 100 Progressive ratio:

101 After the acquisition, the mice underwent one session of progressive ratio to measure  
102 the motivation for oDASS. The session lasted for a maximum of 4 h. The breakpoint  
103 was considered to be the last reached reinforced schedule after 40 min without receiving  
104 any laser stimulation. The reinforced schedules were: 1, 3, 5, 8, 12, 16, 22, 29, 38, 50,  
105 65, 84, 108, 139, 178, 228, 291, 371, 473, 603, 767, 977, 1243 and 1582.

#### 106 Punishment:

107 After the progressive ratio, mice were subjected to 3 sessions of baseline and 4-5  
108 sessions of punishment to measure compulsivity. The baseline sessions were the same  
109 as acquisition sessions with FR3 except for restricted access for 0.5 h. The punishment  
110 sessions were the same as baseline sessions except the mice received a mild foot shock  
111 (0.2 mA, 500 ms) every 3 laser stimulations, that was predicted with a house light cue.  
112 Perseverance rate was calculated by dividing the average infusions from the last two  
113 punishment sessions by the average infusions during baseline.

#### 114 **Cocaine self-administration**

115 The mice underwent catheter implantation 3 weeks after virus injections, and were  
116 subjected to cocaine self-administration 1 week after recovery from the surgery.

117 The apparatus and procedure were similar to oDASS except that active lever presses  
118 resulted to an i.v. infusion of 0.5 mg/ml cocaine (provided by the pharmacy of the  
119 Geneva University Hospital) dissolved in saline. Each acquisition session lasted for 4  
120 h, and each baseline and punishment session lasted for 2h.

#### 121 **Hot plate test**

122 The hot plate apparatus was heated to  $55^{\circ}\text{C} \pm 0.2$ , after which the mice were placed on  
123 the surface of the hot plate equipped with a cylindrical animal restrainer at 25 cm height.  
124 The hot plate latency was set as the latency to the first jump / hind paw withdrawal /  
125 paw tremble. The cut off was set to 30 s to avoid tissue damage. The baseline was tested  
126 as the hot plate latency without any treatment. Citalopram (10 mg/kg) or cocaine (15  
127 mg/kg) were i.p. injected immediately after baseline test, and the second test was  
128 conducted 30 min after the injections.

#### 129 **Electrophysiology recording ex vivo**

130 To obtain acute brain slices, the mice were anesthetized with 5% isoflurane. The mice  
131 were decapitated and the brain was quickly removed and placed in cold oxygenated  
132 artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 119, KCl 2.5, MgCl<sub>2</sub>  
133 1.3, CaCl<sub>2</sub> 2.5, Na<sub>2</sub>HPO<sub>4</sub> 1.0, NaHCO<sub>3</sub> 26.2 and glucose 11. 220 μm coronal slices  
134 containing the DS were prepared with a vibratome (Leica, VT1200). The slices were  
135 allowed to recover in 30 °C oxygenated ACSF for 15 min and maintained at room  
136 temperature.

137 For patch clamp recordings, slices were kept at 30 °C in a recording chamber perfused  
138 with 2.5 ml/min ACSF. Neurons were visualized with a fluorescent microscope  
139 (Olympus BX50WI). Signals were amplified (Multiclamp 700B, Axon Instruments),  
140 filtered at 5 kHz and digitized at 20 kHz (National Instruments Board PCI-MIO-16E4,  
141 Igor, Wave Metrics). Data were rejected if the access resistance changed more than  
142 20%. EPSCs were evoked with 2 ms orange LED (ThorLabs), and recorded in the  
143 presence of PTX (100 μM, Tocris).

144 AMPA/NMDA (A/N) ratio and rectification index (RI) were recorded with an internal  
145 solution containing (in mM): CsCl 130, NaCl 4, creatine phosphate 5, MgCl<sub>2</sub> 2,  
146 Na<sub>2</sub>ATP 2, Na<sub>3</sub>GTP 0.6, EGTA 1.1, HEPES 5, QX-314 5, and spermine 0.1. A/N ratio  
147 was calculated by dividing AMPA currents isolated with AP5 (50 μM) to NMDA  
148 currents obtained by subtracting AMPA component from recordings at +40 mV. RI was  
149 calculated as the ratio of the chord conductance calculated at -70 mV divided by chord  
150 conductance at +40 mV. Serotonin induced LTD were recorded with an internal  
151 solution contained (in mM): potassium gluconate 140, MgCl<sub>2</sub> 2, KCl 5, Na<sub>2</sub>ATP 4,  
152 Na<sub>3</sub>GTP 0.3, creatine phosphate 10, HEPES 10 and EGTA 0.2. Strontium mediated  
153 qEPSCs were measured by replacing Ca<sup>2+</sup> with Sr<sup>2+</sup> in the ACSF. Unitary events were  
154 recorded between 10 ms to 100 ms after the light pulse with the internal solution  
155 containing (in mM): potassium gluconate 140, MgCl<sub>2</sub> 2, KCl 5, Na<sub>2</sub>ATP 4, Na<sub>3</sub>GTP  
156 0.3, creatine phosphate 10, HEPES 10 and EGTA 0.2. All recordings were conducted  
157 with PTX (100 μM, Tocris) in the bath.

## 158 **Immunostaining**

159 Brains were fixed with 4% paraformaldehyde (PFA) for at least 24 h and sliced with  
160 vibratome (Leica, VT1200). 50 μm thick slices were washed with 3 times PBS for 5  
161 min and blocked with 10% bovine serum albumin (BSA) dissolved in 0.5% TritonX-  
162 100 for 1 hour at room temperature. Slices were incubated with primary antibodies  
163 dissolved in blocking buffer overnight at 4 °C, followed by 4 times 15 min wash with  
164 PBS at room temperature. After that, slices were incubated with fluorescent-conjugated  
165 secondary antibodies dissolved in blocking buffer for 2 h at room temperature followed  
166 by 4 times 15 min wash with PBS. Last, slices were mounted using mounting medium  
167 containing DAPI (Fluoroshield, Abcam). Primary and secondary antibodies are listed  
168 below:

169 Rabbit anti GFP (1:500, Invitrogen, A11122, RRID: AB\_221569); Rabbit anti 5-HT  
170 (1:1000, Immunostar, 20080, RRID: AB\_572263); Mouse anti TH (1:500, SIGMA,  
171 T2928, RRID: AB\_477569); Goat anti Rabbit 488 (1:500, Invitrogen, A11008, RRID:  
172 AB\_143165); Donkey anti Mouse Cy3 (1:500; Jackson, 715-165-150, RRID:  
173 AB\_2340813).

## 174 **Fiber photometry**

### 175 Recordings

176 Fiber photometry was performed similar to before (32). Briefly, 5-HT3.0 sensor was  
177 excited using two excitation sources corresponding to 470 nm wavelength (M470F3,  
178 Thorlabs) and 405 nm wavelength (M405FP1, Thorlabs) LED light. Both LED lights  
179 were sinusoidally modulated at 211 and 531 Hz (470 nm and 405 nm light, respectively)  
180 and light was passed through excitation filters (FMC4\_AE(405)\_E(450-490)\_F(500-  
181 550)\_S) onto an optic fiber patch cable (MFP\_400/430/1100-0.48\_4 m\_FC-ZF2.5,  
182 Doric Lenses) that was connected to the chronically implanted fiber (MFC\_400/430-  
183 0.48\_6mm\_ZF2.5(G)\_FLT, Doric Lenses). Light intensity at the tip of the patch cable  
184 was around 0.25 mW. 5-HT3.0 sensor emission light travelled back through the same  
185 fibers onto a photo-receiver (Newport 2151, Doric Lenses), after which it was digitized,  
186 demodulated and stored using a signal processor (RZ5P, Tucker Davis Technologies).  
187 The sample rate was 101.725 Hz, signals were low-pass filtered online at 3 Hz.

188 For recording DS 5-HT transients elicited by cocaine and citalopram in Fig.1A to C and  
189 figS1D, mice were habituated with handling and saline i.p. injection in a circular  
190 corridor for three days. On the next days, 5-HT dynamics were recorded 10 min  
191 baseline, followed by an i.p. injection of saline or cocaine (15 mg/kg) or citalopram (10  
192 mg/kg), and 40 min recordings afterward.

193 For recording DS 5-HT transients induced by optogenetic activation of DR 5-HT  
194 neurons in figS1A to C, mice were placed in a circular corridor and connected to orange  
195 laser (593 nm) as well as the fiber photometry set up. Master-8 was used to control the  
196 laser and send markers to the fiber photometry signal processor simultaneously. 5-HT  
197 dynamics were recorded before, during, and after the laser stimulation (5-ms pulse  
198 width at 20 Hz for 10 s).

### 199 Analysis

200 Fiber photometry signals were analyzed offline in Matlab (Mathworks). To calculate  
201  $dF/F_0$ , the signal originating from the 405 nm excitation light was linearly regressed to  
202 the signal originating from the 470 nm excitation light. It was then subtracted to create  
203 a  $dF/F_0$  using the following formula: (470 nm signal – fitted 405 nm signal)/fitted 405  
204 signal. The average  $dF/F_0$  signal in the baseline periods before experimental  
205 intervention was then subtracted to normalize the signal to baseline. Finally, the  
206 normalized signal was binned into appropriate time bins in the graphs and analyses.

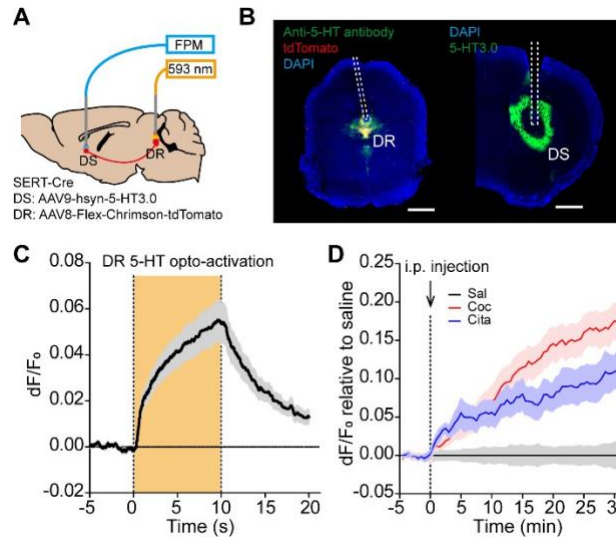
### 207 **Clustering analysis**

208 Clustering analysis was performed with Matlab (Mathworks). Prior to the clustering,  
209 the variables during the punished sessions (P3 and P4) of each mouse were normalized  
210 to the mean signal during the baseline sessions (B1 to B3). The variables used depended  
211 on the experiment: cocaine self-administration (active lever presses, futile lever presses,  
212 inactive lever presses, infusions) and oDASS (active lever presses, futile lever presses,  
213 inactive lever presses, laser stimulations). Futile lever presses reflecting impulsion were  
214 defined as the active lever presses during the 20 s time out following the active lever

215 presses leading to laser stimulations or cocaine infusions. A dimension reduction  
216 (Matlab function 'tsne' with option algorithm = exact, distance = seucclidean,  
217 numDimension = 3) was applied to the variables followed by a hierarchical clustering  
218 method (Matlab functions 'pdist', 'linkage' and 'cluster' with a metric = seucclidean and  
219 linkage = ward). Since the tsne is a stochastic method, the dimension reduction and  
220 clustering were ran 1000 times and the best tree was taken based on the clustering  
221 robustness, namely the mean silhouette score was the highest for two clusters (>0.75  
222 over 1, Matlab function 'silhouette' for clustering accuracy) and the Cophenetic  
223 distance (>0.75 over 1, Matlab function 'cophenet' for tree construction accuracy).  
224 Finally, other relevant variables were sorted according to the obtained clustering  
225 (genotype or treatment, break point, baseline rate and perseverance).

## 226 **Data analysis**

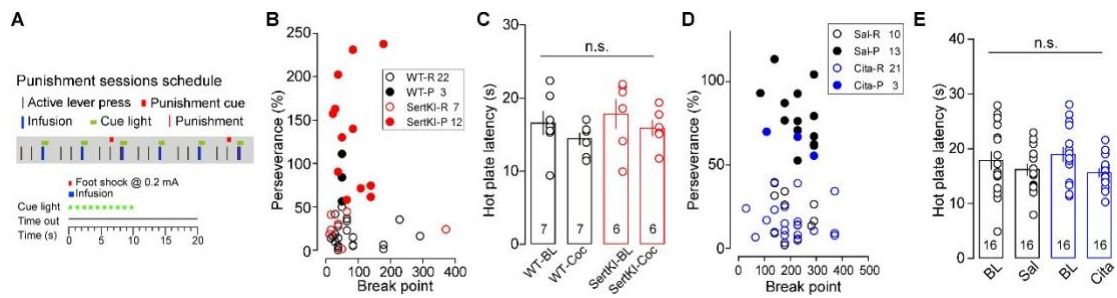
227 Statistical analyses were performed with GraphPad Prism 6. One-way ANOVA, two-  
228 way ANOVA, or two tailed *t* tests were used to analyze data when applicable.  
229 Nonparametric test were used if data don't meet Gaussian distribution. All of the  
230 statistical details were presented in the figure legends. Data were presented as means ±  
231 standard errors of the means (SEM). Significance was defined as \**P* < 0.05, \*\**P* < 0.01,  
232 and \*\*\**P* < 0.001. Sample size were chosen according to previous publications (6, 13).  
233 Mice were randomly assigned to treatments and conditions. Investigators were blind to  
234 genotypes and behavioral outcomes. Electrophysiology data were replicated by 3  
235 investigators in the lab. Behavioral data were replicated at least 3 batches of animal.  
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237

238 **Fig. S1. Validation of GRAB 5-HT sensor.** (A) Schematic of virus injection,  
 239 stimulation, and recording sites. (B) Left, Chrimson-tdTomato (red) expression co-  
 240 labeled with 5-HT (green) in the dorsal raphe (DR). Right, GRAB 5-HT sensor  
 241 expression in the dorsal striatum (DS). Scale bars, 1 mm. (C) 5-HT transients recorded  
 242 in the DS while opto-activating DRN serotonergic neurons ( $n = 3$  mice). (D) 5-HT  
 243 transients in the DS induced by saline, cocaine, and citalopram i.p. injection in WT  
 244 mice ( $n = 6, 6$  and  $5$  mice for saline, cocaine and citalopram group).

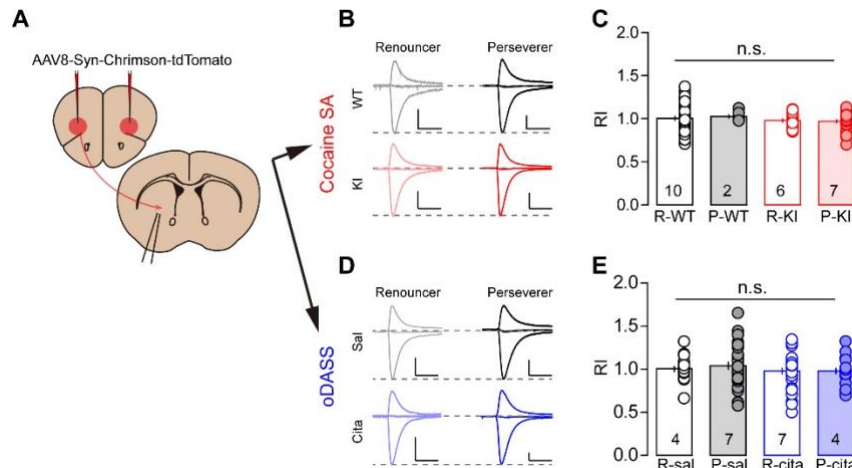
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246

247 **Fig. S2. Lack of correlation between perseverance and breakpoint; no effect on**  
 248 **pain perception in cocaine SA and oDASS (A)** Schedule of punishment sessions of  
 249 cocaine self-administration. **(B)** Perseverance rate as a function of break point in  
 250 cocaine self-administration (Pearson  $r = -0.01$ ;  $P = 0.93$ ). **(C)** Hot plate latency of WT  
 251 and SertKI group before (BL) and after cocaine (15 mg/kg) i.p. injection (one way  
 252 ANOVA;  $F_{3,22} = 1.031$ ,  $P = 0.398$ ;  $n = 7$  and  $6$  for WT and KI group). **(D)** Perseverance  
 253 rate as a function of break point in oDASS (Pearson  $r = 0.02$ ;  $P = 0.92$ ). **(E)** Hot plate  
 254 latency before (BL) and after saline or citalopram (10 mg/kg) i.p. injection (Kruskal-  
 255 Wallis test;  $P = 0.28$ ;  $n = 16$  for each group).

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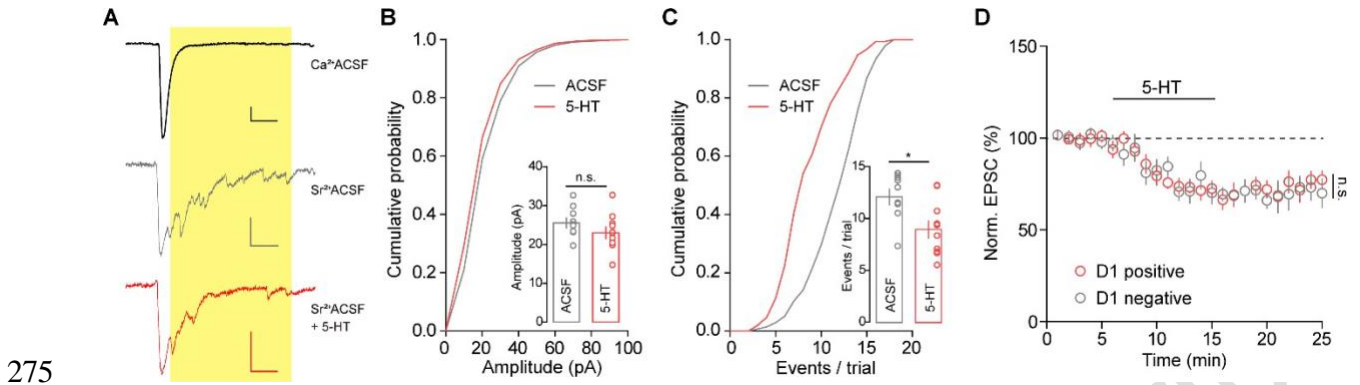
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258 **Fig. S3. Absence of Rectification of AMPA Epscs in renouncers and perseverers.**

259 (A) Schematic of virus injection and recording sites. (B) Representative traces of  
 260 AMPA current holding at -70 mV, 0 mV and +40 mV of a renouncer (left) and a  
 261 perseverer (right) from WT (upper) and SertKI (lower) treated group of cocaine self-  
 262 administration. Scale bars, 200 pA, 20 ms. (C) Average rectification index (RI) of  
 263 renouncers and perseverers in cocaine self-administration (WT group: Mann Whitney  
 264 test;  $U = 138$ ,  $P = 0.42$ ;  $n = 49$  and 7 cells from 10 and 2 mice for renouncer and  
 265 perseverer; SertKI group: two tailed  $t$  test;  $t_{58} = 0.44$ ,  $P = 0.66$ ;  $n = 27$  and 33 cells from  
 266 6 and 7 mice for renouncer and perseverer). (D) Representative traces of AMPA current  
 267 holding at -70 mV, 0 mV and +40 mV of a renouncer (left) and a perseverer (right)  
 268 from saline (upper) and citalopram (lower) treated group of oDASS. Scale bars, 200  
 269 pA, 20 ms. (E) Average RI of renouncers and perseverers in oDASS (saline treated  
 270 group: Mann Whitney test;  $U = 320$ ,  $P = 0.53$ ;  $n = 21$  and 34 cells from 4 and 7 mice  
 271 for renouncer and perseverer; citalopram treated group: two tailed  $t$  test;  $t_{54} = 0.004$ ,  $P$   
 272  $= 0.996$ ;  $n = 33$  and 23 cells from 7 and 4 mice for renouncer and perseverer).

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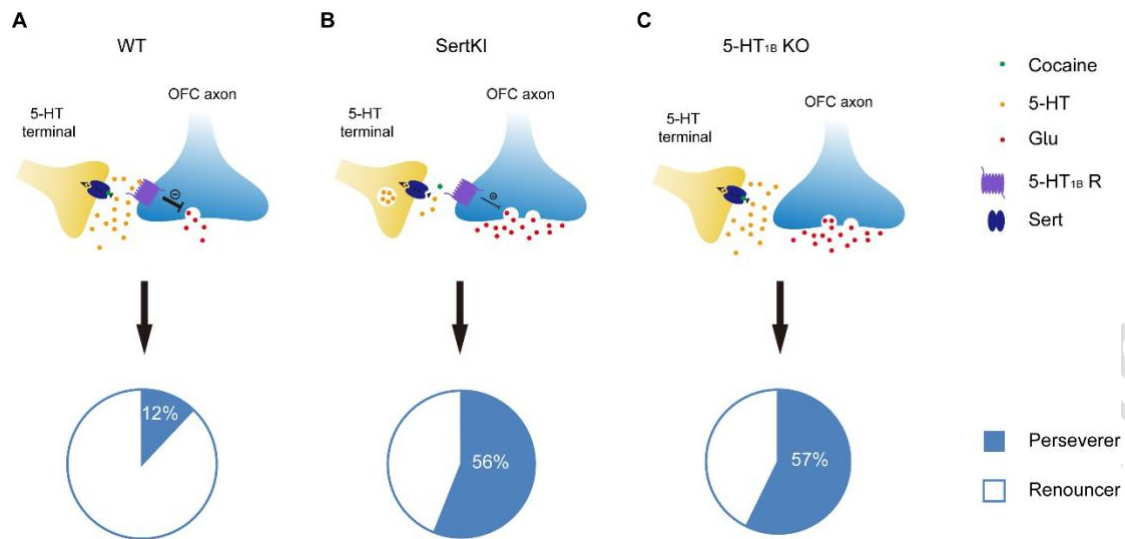
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276 **Fig. S4. Modulation of OFC-DS pathway by 5-HT.** (A) Representative traces of  
 277 evoked EPSC (upper, black) in calcium based ACSF and qEPSC with (middle, gray)  
 278 and without (lower, red) 5-HT application in strontium based ACSF. Scale bars, 100  
 279 pA, 20 ms. (B) Cumulative probability of qEPSC amplitude (two tailed  $t$  test;  $t_{17} =$   
 280 1.278,  $P = 0.218$ ;  $n = 9$  and 10 cells from 3 mice). (C) Cumulative probability of qEPSC  
 281 events/trial (two tailed  $t$  test;  $t_{17} = 2.762$ ,  $P = 0.013$ ;  $n = 9$  and 10 cells from 3 mice).  
 282 (D) Average traces of EPSC before, during and after bath application of 5-HT in D1  
 283 positive (red) and negative (gray) cells (compared Norm. EPSC recorded on last 5  
 284 minutes; two tailed  $t$  test;  $t_{23} = 0.33$ ,  $P = 0.75$ ;  $n = 13$  and 12 cells from 5 mice for D1  
 285 positive and negative groups).

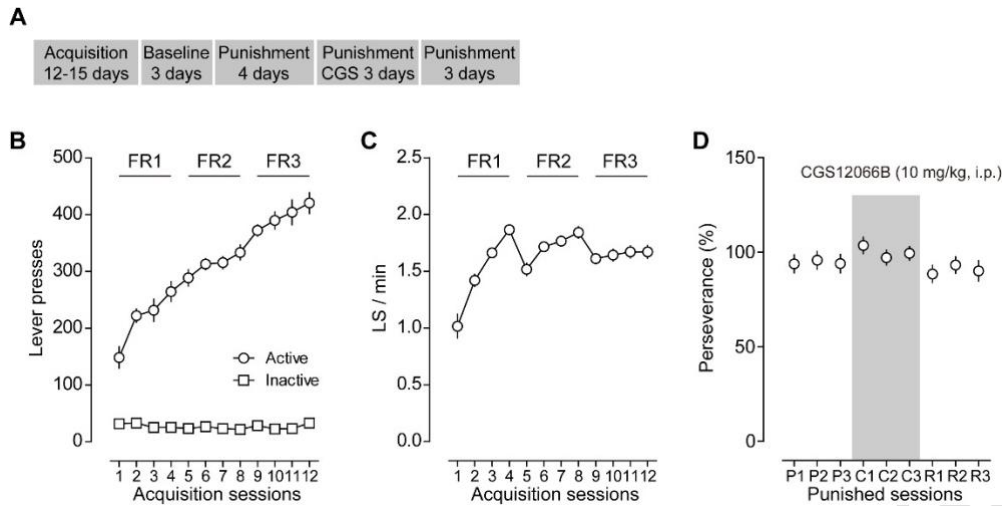
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287

288 **Fig. S5. Schematics of working model.** (A) In WT mice, when cocaine binds to 5-HT  
 289 transporter (SERT), 5-HT reuptake is inhibited and extracellular 5-HT level is elevated.  
 290 Elevated 5-HT strongly inhibits glutamate release from OFC terminals within the DS  
 291 through activating presynaptic G<sub>i/o</sub> coupled 5-HT<sub>1B</sub> receptors. Weakened OFC-DS  
 292 synapse results in low fraction of mice to compulsively self-administrate cocaine  
 293 despite punishment. (B) In SertKI mice, in which cocaine cannot bind to SERT, 5-HT  
 294 level are not elevated by cocaine infusions during cocaine self-administration. The  
 295 excitatory neural transmission between OFC and DS in SertKI mice is more efficient  
 296 than in WT mice, leading to higher fraction of perseverers. (C) In 5-HT<sub>1B</sub> KO mice,  
 297 although cocaine still inhibits 5-HT reuptake and induce elevated extracellular 5-HT  
 298 level, 5-HT cannot inhibit OFC-DS transmission because of a lack of presynaptic 5-  
 299 HT<sub>1B</sub> receptors. The transmission between OFC and DS is strong and promotes  
 300 transition to compulsive cocaine self-administration.

301



302

303 **Fig. S6. 5-HT<sub>1B</sub> agonist treatment in persevering mice after punishment left**  
 304 **oDASS unaffected.** (A) Time line of oDASS and 5-HT<sub>1B</sub> agonist (CGS12066B, 10  
 305 mg/kg, i.p.) treatment. (B) Number of active and inactive lever presses in acquisition  
 306 sessions of oDASS ( $n = 15$  mice). (C) Laser stimulation per minute in acquisition  
 307 sessions ( $n = 15$  mice). (D) Perseverance rate in the last 3 punished sessions (P1-3),  
 308 punished CGS12066B treatment sessions (C1-3), and additional punished recovery  
 309 sessions after the treatment (R1-3) (repeated one way ANOVA followed by Dunnett's  
 310 test; for three consecutive comparisons:  $q_{14} = 3.04$ ,  $P = 0.02$ ;  $q_{14} = 0.89$ ,  $P = 0.88$ ;  $q_{14}$   
 311  $= 1.66$ ,  $P = 0.37$ ; and  $q_{14} = 2.02$ ,  $P = 0.20$ ;  $q_{14} = 0.44$ ,  $P = 0.99$ ;  $q_{14} = 1.48$ ,  $P = 0.49$  for  
 312 punished versus punished CGS sessions and punished versus punished recovery  
 313 sessions, respectively;  $n = 15$  mice).

314

315 **References**

316

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