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Synaptic mechanism underlying serotonin modulation of transition to cocaine addiction

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Abstract

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

One Sentence Summary

Cocaine mediated serotonin transients cause a presynaptic depression in the dorsal striatum that prevents the potentiation of glutamate transmission responsible for compulsion in addicted individuals.

With chronic consumption, about 20% of cocaine users lose control and are eventually diagnosed as addicted (1). Increasing dopamine (DA) levels is typical of all addictive drugs (2), sufficient to trigger forms of synaptic plasticity underlying adaptive behaviors

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

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

Given that for oDASS, the synaptic potentiation of afferents from the orbitofrontal cortex (OFC) to DS drives perseverance (13), we wondered whether this is also the case for cocaine SA. To selectively stimulate the OFC-DS projection, we expressed a red shifted opsin Chrimson in OFC neurons (Fig 3A) and evoked EPSCs by illuminating the terminals in brain slices of the DS 24-48 h after the last punishment session. AMPA/NMDA

ratio was higher in perseverers than in renouncers of cocaine SA as well as oDASS, regardless of genotypes and treatment (Fig 3B to E), confirming that a potentiated OFC-DS pathway reflects perseverance both in oDASS and cocaine SA. In no condition were the EPSCs rectifying (fig. S3), suggesting a potentiation by an increase of the number of AMPA receptors without change in subunit composition, akin to the expression mechanism observed in individuals with compulsive oDASS (13).

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

We next hypothesized that presynaptic depression may reduce the likelihood for LTP at the OFC-DS synapse, which in turn would prevent the transition to compulsion in cocaine SA. This seems plausible since chemogenetic reduction of OFC activity also reduced the fraction of perseverers in oDASS (6). Therefore, to establish a causal link between 5-HT induced presynaptic depression and compulsive cocaine use, we aimed to abolish 5-HT_{1B} receptors selectively in the OFC neurons targeting the DS. We injected retroAAV-ef1α-mCherry-IRES-Flpo in the DS of 5-HT_{1B} floxed mice (17). This led to Flpo expression in OFC neurons targeting the DS. AAV9-ef1α-fDIO-Cre or control virus was then injected in the OFC to express Cre in OFC cells targeting the DS (Fig. 4A and B), which then led to recombination and abolishment of 5-HT_{1B} receptors. We confirmed the successful receptor knockout functionally by the inability of 5-HT_{1B} agonist CP39129 (2 μM) to induce a presynaptic depression (Fig. 4C and D).

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

A synaptic mechanism thus emerges that underlies a modulatory role of 5-HT reducing the likelihood of transition to compulsion and eventually addiction (see fig. S5). In

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

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

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

All data, code, and materials are available on Zenodo at the following address (10.5281/zenodo.5091499).

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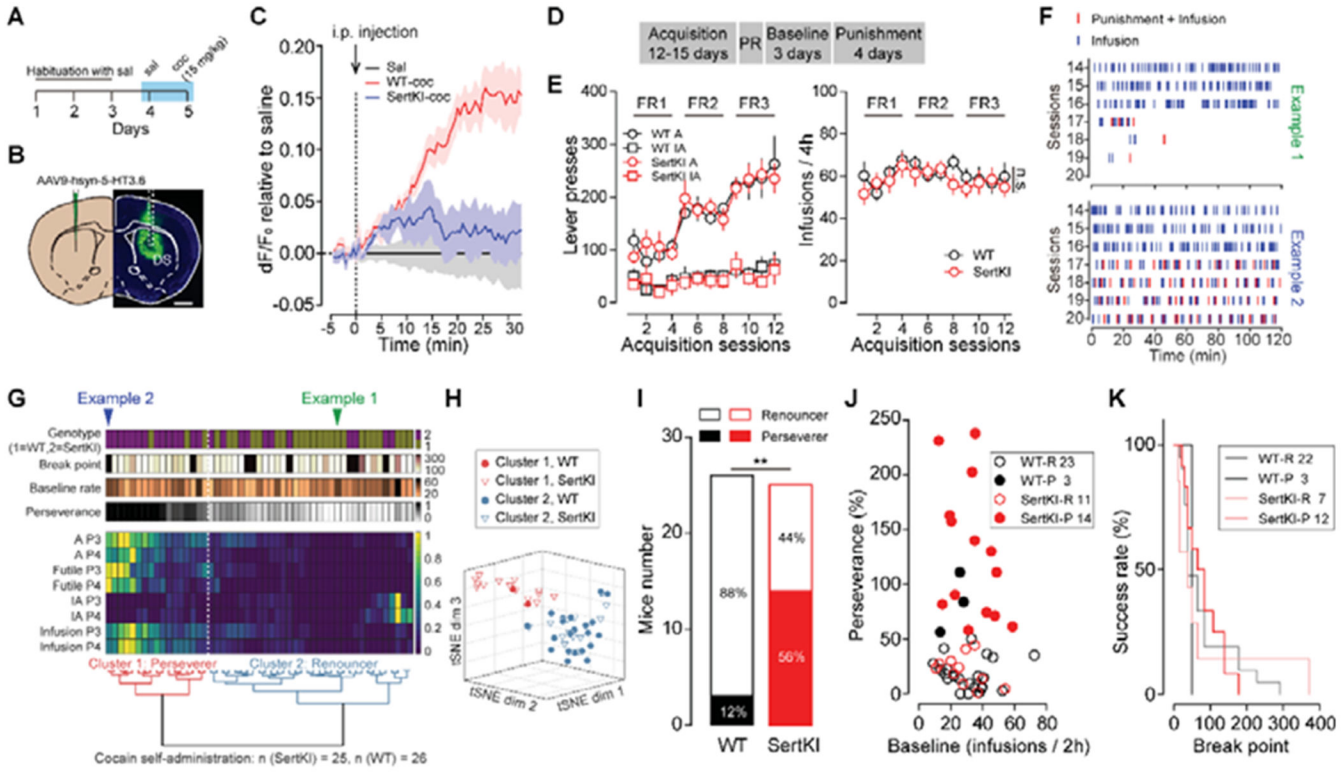

Figure 1. SertKI animals were more compulsive in cocaine self-administration.

(A) Schedule of saline and cocaine injections. **(B)** GRAB 5-HT sensor expression indicated with GFP staining in the DS. Scale bars, 1 mm. **(C)** 5-HT transients in the dorsal striatum induced by saline / cocaine (15 mg/kg) i.p. injection in WT and SertKI mice were detected with a GRAB 5-HT sensor (n = 3 mice for WT and SertKI group, data from saline injected WT and SertKI are pooled). **(D)** Timeline of cocaine self-administration. **(E)** Left, number of active (A) and inactive (IA) lever presses of WT (black) and SertKI (red) animals (n = 26 and 25 for WT and SertKI group) in acquisition sessions. Right, cocaine infusions obtained from WT (black) and SertKI (red) mice in acquisition sessions (two way ANOVA; $F_{1,588}$ = 1.996, P = 0.1583; n = 26 and 25 for WT and SertKI group). **(F)** Raster plots for infusions (blue lines) and punishments (red lines) in baseline and punishment sessions of a renouncer (upper, WT mouse) and a perseverer (lower, SertKI mouse). **(G)** Hierarchical clustering based on tSNE projection of different parameters of punishment sessions 3 and 4 (P3 and P4) of cocaine self-administration. Blue and green arrow heads indicate examples presented in F. Genotype, break point, baseline rate and perseverance were not used for clustering. **(H)** tSNE three dimensional representation of clusters of perseverers (cluster 1) and renouncers (cluster 2) in cocaine self- administration. **(I)** Percentage of perseverers and renouncers among WT and SertKI groups (Fisher's exact test; P = 0.001). **(J)** Perseverance rate as a function of baseline rate (Pearson r = −0.08; P = 0.55). **(K)** The success rate of performance as a function of the last progressive ratio value achieved by the mice (logrank test; P = 0.94). Abbreviations, PR, progressive ratio; FR, fixed ratio; A, active lever presses; IA, inactive lever presses; R, renouncer; P, perseverer; sal, saline; coc, cocaine; WT, wildtype; SertKI, Sert-knockin.

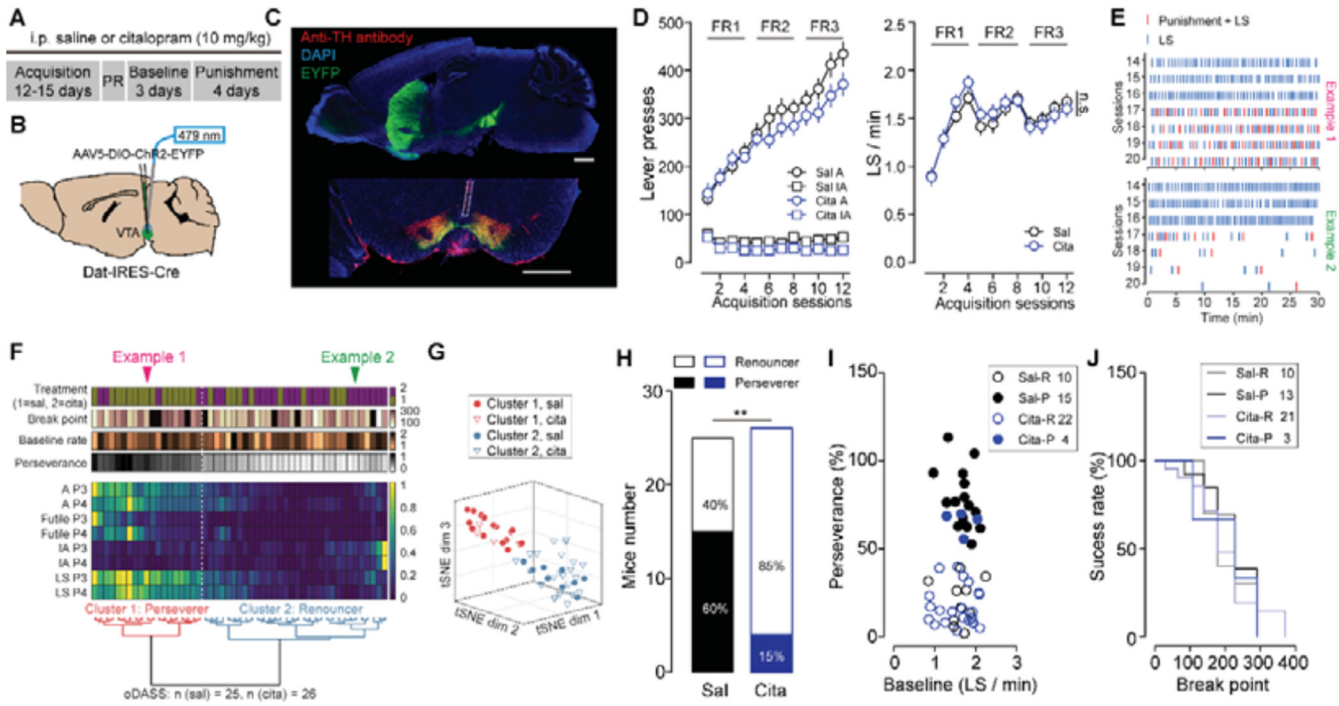

Figure 2. Citalopram decreased compulsive dopamine self-stimulation.

(A) Timeline of oDASS and saline / citalopram treatments. **(B)** Schematic of virus injection sites and optic fiber implantation sites. **(C)** ChR2-EYFP expression in the VTA from a sagittal slice (upper) and a coronal slice co-labeled with TH (lower). Scale bars, 1 mm. **(D)** Left, number of active (A) and inactive (IA) lever presses of saline (black) and citalopram (blue) treated mice (n = 25 and 26 for saline and citalopram group) in acquisition sessions. Right, laser stimulation per minute obtained from saline (black) and citalopram (blue) treated mice in acquisition sessions (two way ANOVA; $F_{1,588}$ = 0.73, P = 0.39; n = 25 and 26 for saline and citalopram group). **(E)** Raster plots for laser stimulations (blue lines) and punishments (red lines) in baseline and punishment sessions of a perseverer (upper, from saline group) and a renouncer (lower, from citalopram group). **(F)** Hierarchical clustering based on different parameters of punishment sessions 3 and 4 (P3 and P4) of oDASS. Red and green arrow heads indicate examples presented in E. Treatment, break point, baseline rate and perseverance were not used for clustering. **(G)** tSNE three dimensional representation of clusters of perseverers (cluster 1) and renouncers (cluster 2) in oDASS. **(H)** Percentage of perseverers and renouncers among saline and citalopram treated groups (Fisher's exact test; P = 0.001). **(I)** Perseverance rate as a function of baseline rate (Pearson r = 0.05; P = 0.71). **(J)** The success rate of performance as a function of the last progressive ratio value achieved by the mice (logrank test; P = 0.95). Abbreviations, PR, progressive ratio; FR, fixed ratio; LS, laser stimulation; A, active lever presses; IA, inactive lever presses; R, renouncer; P, perseverer; sal, saline; cita, citalopram.

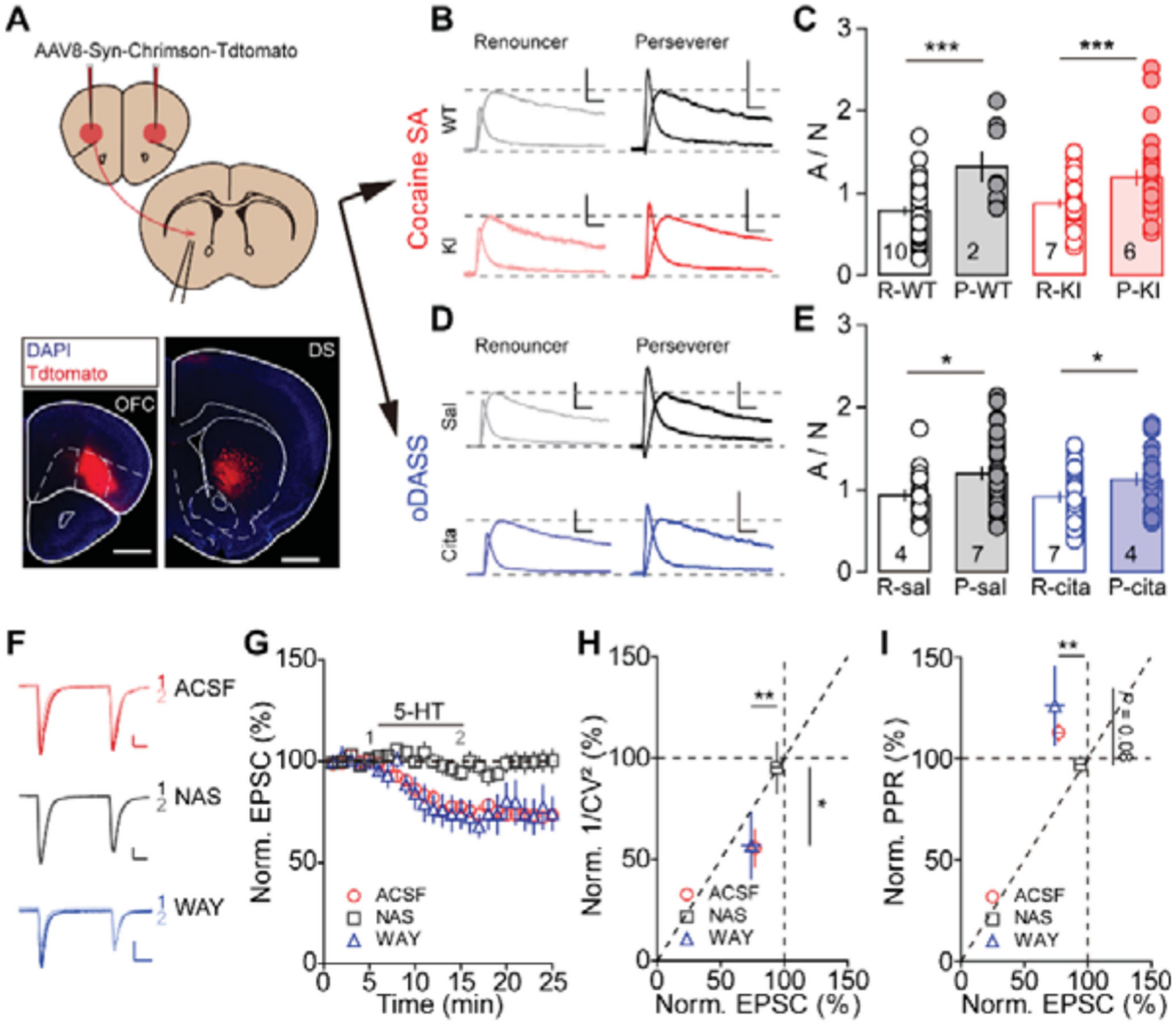

Figure 3. The OFC-DS pathway was modulated by 5-HT.

(A) Upper, Schematic of virus injection and recording sites. Lower, Chrimson-Tdtomato expressing cell bodies in the OFC (left) and terminals in the DS (right). Scale bars, 1 mm. **(B)** Representative traces of AMPA and NMDA currents recorded at +40 mV of a renouncer (left) and a perseverer (right) from WT (upper) and SertKI (lower) group after cocaine self-administration. Scale bars, 200 pA, 15 ms. **(C)** Average A/N of WT and KI renouncers and perseverers after cocaine self-administration (two tailed *t* test; $t_{59} = 4.26$, $P < 0.0001$; $n = 53$ and 8 cells from 10 and 2 mice for renouncer and perseverer in WT group; $t_{65} = 3.48$, $P = 0.0009$; $n = 37$ and 30 cells from 7 and 6 mice for renouncer and perseverer in SertKI group). **(D)** Representative traces of AMPA and NMDA currents recorded at +40 mV of a renouncer (left) and a perseverer (right) from saline (upper) and citalopram (lower) treated group after oDASS. Scale bars, 200 pA, 15 ms. **(E)** Average A/N of saline and citalopram treated renouncers and perseverers after oDASS (two tailed *t* test; $t_{54} = 2.54$, $P = 0.01$; n

= 21 and 35 cells from 4 and 7 mice for renouncer and perseverer in saline treated group; t_{55} = 2.38, P = 0.02; n = 34 and 23 cells from 7 and 4 mice for renouncer and perseverer in citalopram treated group). **(F)** Representative traces before and after bath application of 5-HT in the presence of ACSF (red), NAS181 (gray), and WAY100635 (blue). Scale bars, 200 pA, 10 ms. **(G)** Average traces of EPSC before, during and after bath application of 5-HT in the presence of ACSF (red), NAS181 (gray), and WAY100635 (blue) (n = 13, 14 and 7 cells from 3 mice for ACSF, NAS181, and WAY100635 group). **(H)** Normalized coefficient of variation ($1/CV^2$) versus normalized EPSC (one way ANOVA; $F_{2,31}$ = 3.682, P = 0.0367 for Norm. $1/CV^2$; $F_{2,31}$ = 7.948, P = 0.0016 for Norm. EPSC; n = 13, 14 and 7 cells from 3 mice for ACSF, NAS181, and WAY100636 group). **(I)** Normalized pair pulse ratio (PPR) versus normalized EPSC (one way ANOVA; $F_{2,26}$ = 2.807, P = 0.08 for Norm. PPR; $F_{2,31}$ = 7.948, P = 0.0016 for Norm. EPSC; n = 13, 14 and 7 cells from 3 mice for ACSF, NAS181, and WAY100636 group). Abbreviations, SA, self-administration; R, renouncer; P, perseverer; sal, saline; cita, citalopram; WT, wildtype; KI, Sert-knockin.

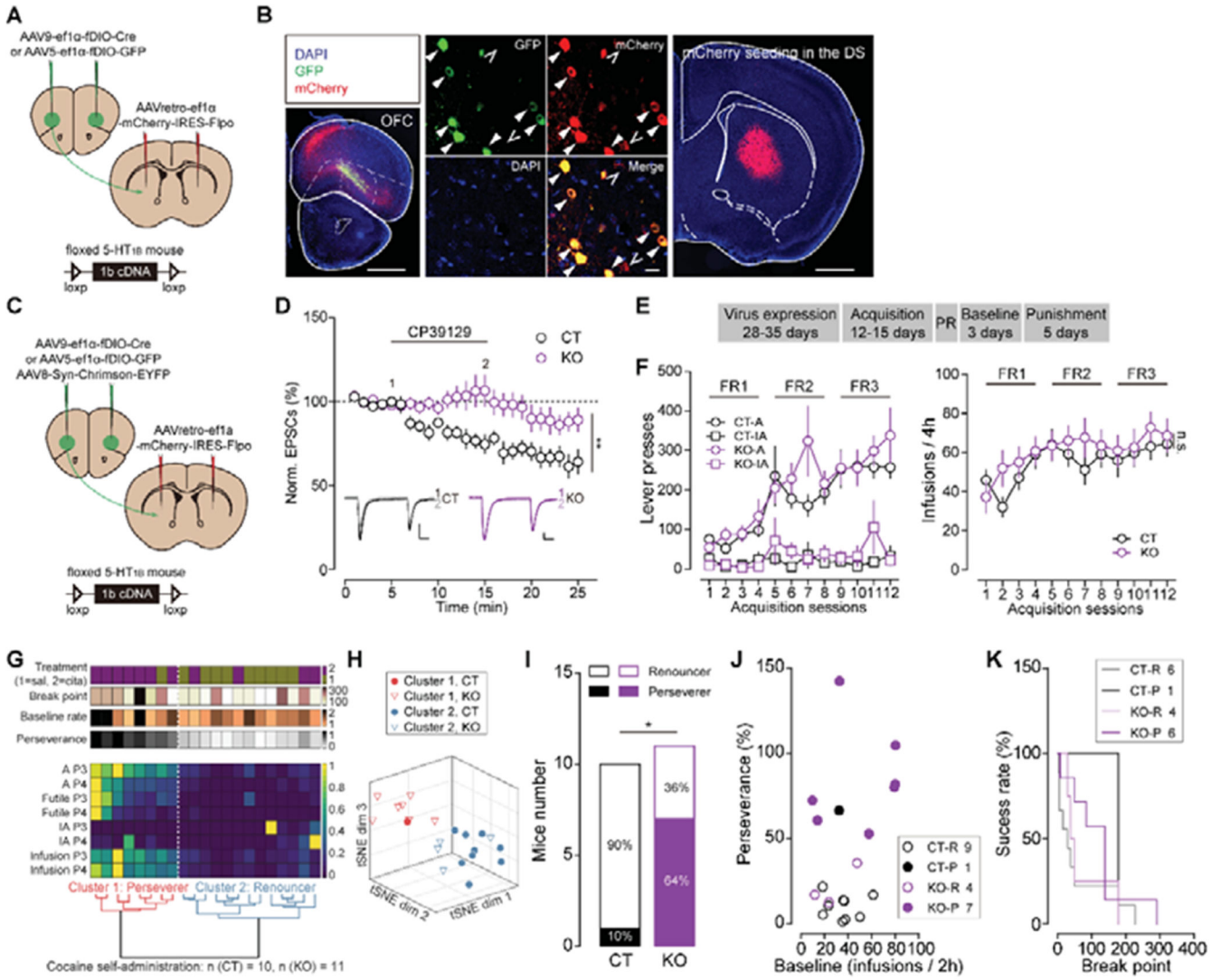

Figure 4. Knockout 5-HT$_{1B}$ receptors promoted compulsive cocaine self-administration.

(A) Schematic of virus injections for cocaine self-administration. **(B)** Left and middle, GFP co-expressing with mCherry in the OFC. Right, mCherry seeding in the DS. Scale bars, 1 mm (left and right), and 20 μm (middle). **(C)** Schematic of virus injections for patch clamp recording. **(D)** Average traces of EPSC before, during and after bath application of 5-HT$_{1B}$ receptor agonist CP39129 in control (CT, black) and pathway specific knockout 5-HT$_{1B}$ receptor (KO, violet) groups (compared Norm. EPSC recorded on last 5 minutes; two tailed *t* test; $t_{25} = 2.86$, $P = 0.008$; $n = 15$ and 12 cells from 3 mice for CT and KO group). Scale bars, 200 pA, 10 ms. **(E)** Timeline of virus injection and cocaine self-administration experiments. **(F)** Left, number of active (A) and inactive (IA) lever presses of CT (black) and KO (violet) mice in acquisition sessions. Right, cocaine infusions obtained by CT (black) and KO (violet) mice in acquisition sessions (two way ANOVA; $F_{1,288} = 2.53$, $P = 0.06$; $n = 10$ and 11 for CT and KO group). **(G)** Hierarchical clustering based on different parameters of punishment sessions 3 and 4 (P3 and P4) of cocaine self-administration. Treatment, break point, baseline rate and perseverance were not used for clustering. **(H)**

tSNE three dimensional representation of clusters of perseverers (cluster 1) and renouncers (cluster 2) in cocaine self-administration. (I) Percentage of perseverers and renouncers among CT and KO groups (Fisher's exact test; $P = 0.02$). **(J)** Perseverance rate as a function of baseline rate (Pearson $r = 0.36$; $P = 0.11$). **(K)** The success rate of performance as a function of the last progressive ratio value achieved by the mice (logrank test; $P = 0.6$). Abbreviations, PR, progressive ratio; FR, fixed ratio; A, active lever presses; IA, inactive lever presses; R, renouncer; P, perseverer; CT, control; KO, pathway specific knockout.