Dopamine D1 receptor-expressing neurons activity is essential for locomotor and sensitizing effects of a single injection of cocaine

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Abstract

Dopamine D1 receptors play an important role in the effects of cocaine. Here we investigated the role of neurons, which express these receptors (D1-neurons) in the acute locomotor effects of cocaine and the locomotor sensitization observed after a second injection of this drug, using the previously established two-injection protocol of sensitization. We inhibited D1-neurons using double transgenic mice conditionally expressing the inhibitory Gi-coupled designer receptor exclusively activated by designer drugs (Gi-DREADD) in D1-neurons. Chemogenetic inhibition of D1-neurons by a low dose of clozapine (0.1 mg/kg) decreased the cocaine-induced expression of *Fos* in striatal neurons. It diminished the basal locomotor activity and acute hyper-locomotion induced by cocaine (20 mg/kg). Clozapine 0.1 mg/kg had no effect by itself and did not alter cocaine effects in wild type mice. Inhibition of D1-neurons during the first cocaine administration prevented the sensitization of the locomotor response in response to a second cocaine administration ten days later. On day 11, inhibition of D1-neurons by clozapine stimulation of Gi-DREADD blocked cocaine-induced locomotion including in sensitized mice, whereas on day 12, in the absence of clozapine and D1-neurons inhibition, all mice displayed a sensitized response to cocaine. These results show that chemogenetic inhibition of D1-neurons decreases spontaneous and cocaine-induced locomotor activity. It prevents the induction and expression of sensitization in a two-injection protocol of sensitization but does not alter established sensitization. Our study further supports the central role of D1-neurons in mediating the acute locomotor effects of cocaine and its sensitization.

Keywords: sensitization, dopamine receptor, DREADD, 2-injection protocol of sensitization

Introduction

Psychostimulants such as cocaine enhance locomotor activity and repeated cocaine exposure progressively increases the locomotor responses due to sensitization processes (review in Steketee and Kalivas, 2011). Locomotor sensitization to repeated drug administration is a complex adaptation that involves several neuronal populations and is correlated with aspects of addiction including the excessive pursuit and self-administration of psychostimulant drugs (Vezina, 2004). However, multiple drug exposures can promote the parallel development of tolerance that could interfere with behavioral sensitization (Stewart and Badiani, 1993). Remarkably, a single cocaine administration induces a locomotor sensitization that increases during the first week without repetition (a property sometimes referred to as "incubation") and is long-lasting (Valjent et al., 2005, 2010). This persistent ability of a single injection of cocaine or other drugs to increase locomotor response to a second injection several days later has been reported by several investigators (Jackson and Nutt, 1993; Lin-Chu et al., 1985; O'Neill and Sanger, 1999; Robinson et al., 1982; Vanderschuren et al., 1999, 2001; Weiss et al., 1989) and evaluated in details as the two-injection protocol of sensitization, TIPS (Valjent et al., 2010). TIPS avoids confusing effects of repetition of the manipulations which are investigated and is a simple protocol to explore the signaling mechanisms by which drugs of abuse exert long-lasting effects (Corbillé et al., 2007; Valjent et al. 2005) as well as the role of synaptic long term potentiation in these persistent alterations (Pascoli et al. 2012). It has the advantage to allow simple manipulations during the unique initial injection and test their consequences on subsequent sensitization.

Dopamine D1 receptor (Drd1) plays a major role in the rewarding properties of drugs of abuse including cocaine (Self et al., 1996). Cocaine treatment stimulates the activity of D1-neurons in the striatum *in vivo* (Luo et al., 2011). SCH23390, a Drd1 antagonist, prevents locomotor sensitization (McCreary and Marsden, 1993). In *Drd1* knockout mice spontaneous locomotor activity is either unchanged (Drago et al., 1996; Miner et al., 1995) or increased (Xu et al., 1994, 2000) but in most reports the locomotor response to cocaine is lost (Karlsson et al., 2008; Xu et al., 2000) as well as the ability of cocaine to induce Fos expression in striatal neurons (Drago et al., 1996; Moratalla et al., 1996). *Drd1* knockout mice also show reduced sensitized locomotor responses to cocaine (Karlsson et al., 2008) and amphetamine (Karper et al., 2002). With the TIPS protocol, cocaine sensitization was absent in homozygous *Drd1* knockout mice, and reduced in heterozygous mice (Valjent et al., 2010). However there is a limitation in the interpretation of knockout mice experiments, because the lack of *Drd1* gene affects the expression of other genes (Xu et al., 1994) and developmental alterations cannot be excluded. Targeted mutations in mice also showed the importance in sensitization of dopamine D2 (Drd2) (Solis et al., 2021) and glutamate NMDA (Grin1) receptors (Beutler et al., 2011). These experiments provide evidence about the role of receptors but do not explore the contribution of specific neuronal populations. While local

administration of amphetamine into the VTA is sufficient to induce Drd1-dependent locomotor sensitization (Vezina, 1996), sensitization to systemic amphetamine was prevented in mice by ablation of dorsomedial striatum D2-neurons, but not D1-neurons (Durieux et al., 2012). Metamphetamine sensitization was only delayed in mice by ablation of nucleus accumbens (NAc) shell D1-neurons (Kai et al., 2015). Cocaine locomotor sensitization was markedly decreased in mice with neurotransmission blockade (Hikida et al., 2010) or optogenetic inhibition (Chandra et al., 2013) of NAc D1-neurons. Potentiation of corticostriatal synapses onto NAc D1-neurons is a key component of locomotor sensitization in response to a single injection of cocaine (Pascoli et al., 2012). Chemogenetic inhibition of dorsal striatum nigral projection neurons also impaired amphetamine sensitization (Ferguson et al. 2011). In addition, recent work reveals that striatal cholinergic interneurons also play a role in locomotor responses to cocaine, including sensitization (Hanada et al., 2018; Lewis et al., 2020). Thus, available evidence indicates that locomotor sensitization can be triggered by just a single exposure to psychostimulants and involves several neuronal populations in the striatum.

In the current study we addressed the global role of D1-neurons asking two simple questions: Does inhibition of D1-neurons alter i) the acute locomotor response to cocaine and ii) the induction or expression of sensitization in the TIPS protocol? To address these questions we used chemogenetics with designer receptor exclusively activated by designer drug (DREADDs, Roth BL et al., 2016) and expressed hM4Di, an inhibitory DREADD, referred to below as Gi-DREADD, selectively in Drd1-expressing cells. Since recent work showed that DREADD stimulation with low doses of clozapine (CLZ) is faster and at least as efficient as clozapine-N-oxide (CNO), the commonly used ligand (Gomez et al., 2017), an observation confirmed in our laboratory (Nakamura et al., 2020), we used CLZ to activate hM4Di and inhibit D1-neurons.

Materials and Methods

Animals and treatments

We used heterozygous bacterial artificial chromosome (BAC) *Drd1*-Cre transgenic (Drd1-Cre) mice expressing Cre recombinase under the control of the dopamine D1 receptor gene (*Drd1*) promoter (GENSAT project, EY262 line), bred onto a C57BL/6J genetic background. Gi-DREADD (B6N.129-*Gt(ROSA)26Sor*[tm1(CAG-CHRM4*,-mCitrine)Ute]/J, a.k.a. R26-LSL-hM4Di-DREADDpta-mCitrine or R26-hM4Di/mCitrine) mice were purchased from the Jackson Laboratory (stock #026219). We crossed Drd1-Cre mice with Gi-DREADD mice. A subset of their offspring carried both *Drd1*-Cre and R26-hM4Di/mCitrine alleles, allowing effective removal of the stop cassette and expression of Gi-DREADD (mice referred to as D1-Gi-DREADD below, **Fig. 1A**). Males and females, 10-21-week-old at the beginning of the experiments, were used. Genotypes were determined by polymerase chain reaction (PCR) on mouse tail DNA samples. Cocaine-HCl (Sigma, France) was dissolved in 9 g L^{-1} NaCl solution (saline). CLZ (Sigma-Aldrich) was first dissolved in DMSO, and then diluted in saline

(final DMSO concentration, 0.1 mL L^{-1}). Cocaine (20 mg kg^{-1}) and CLZ (0.1 mg kg^{-1}) were administered by intraperitoneal injection in a volume of 10 mL kg^{-1} body weight. The dose of CLZ was chosen to be efficient for Gi-DREADD stimulation but without other identified pharmacological effects, according to previous reports (Gomez et al., 2017; Nakamura et al., 2020). Mice were housed under a 12-hour light/12-hour dark cycle with free access to food and water. All animal procedures were approved by the *Ministère de l'Education Nationale de l'Enseignement Supérieur de la Recherche* (France, project APAFIS#8196-201704201224850 v1. All methods in this study were performed in accordance with the relevant guidelines and regulations.

Behavioral analysis

Locomotor activity was measured in a circular corridor with four infrared beams placed at every 90° angles (Imetronic, Pessac, France) in a low luminosity environment (locomotor activity box, LA-box). Counts were incremented by consecutive interruptions of two adjacent beams. Locomotor sensitization induced by a single cocaine injection was studied as in Valjent et al. (2010), as descibed in **Fig. 1B-C**. Mice were habituated to handling, test apparatus, and procedures for 3 consecutive days (Days -3 to -1). In this habituation period, mice received every day a first saline injection and were placed in the LA-box (T0 min). Thirty minutes later, they received a second saline injection (T30 min) and were put back in the LA-box for 1 h. On experimental days (Day 0, Days 10-12), mice were handled in the same manner, except that they were injected with the solutions indicated in **Fig. 1B** at T0 min and T30 min, respectively.

Tissue preparation and immunofluorescence

For the experiment in which the brains were used for immunohistofluorescence, D1-Gi-DREADD mice different from those used for sensitization experiments were treated as above on Day 0 but killed 90 min after cocaine injection (**Fig. 2A**) with a lethal dose of pentobarbital (500 mg kg^{-1}, i.p., Sanofi-Aventis, France) and perfused transcardially with 40 g L^{-1} paraformaldehyde in 0.1 M sodium phosphate buffer (**Fig. 2A**). Brains were post-fixed overnight at 4°C, cut into free-floating sections (30 μm) with a vibrating microtome (Leica) and kept at -20°C in a solution containing 300 mL L^{-1} ethylene glycol, 300 mL L^{-1} glycerol in 0.1 M sodium phosphate buffer. Immunolabeling procedures were as previously described (Valjent et al., 2000). Primary antibodies were rabbit Fos antibodies (1:1000, Santa Cruz, #sc-52) and mouse hemagglutinin (HA) antibodies (1:50, Sigma, #A-2095). Secondary antibodies were anti-rabbit A488-labeled antibody (1:400, Invitrogen, #A-21206) and anti-mouse A555-labeled antibody (1:400; Invitrogen, #A-31570). Images were acquired using a Leica SP5-II confocal microscope at the *Institut du Fer à Moulin Tissue and Cell Imaging Facility*.

Statistical analysis

When normal distribution was not rejected by d'Agostino-Pearson and Shapiro-Wilk tests, data were analyzed with Student's t test or paired t-test or two-way ANOVA followed by post hoc comparisons

as indicated in the figure legends. When normal distribution was rejected, analysis was done with non-parametric tests. All statistical analyses were performed using Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA). A threshold value for significance of $p < 0.05$ was used throughout the study.

Results

D1-Gi-DREADD stimulation prevents cocaine-induced hyperlocomotion and Fos expression in the striatum

We first assessed the functionality of Gi-DREADD by testing the ability of CLZ to alter cocaine-induced locomotion and prevent cocaine-induced expression of Fos, a marker of activated neurons. D1-Gi-DREADD mice were treated with CLZ (0.1 mg/kg) or vehicle and 30 min later with cocaine (**Fig. 2A**). Cocaine markedly increased locomotion in the group of vehicle-pretreated mice whereas CLZ-pretreated mice displayed little locomotor activity (**Fig. 2B**). Brains were collected 90 min after cocaine injection and we quantified the percentage of cells expressing Gi-DREADD, revealed by HA immunoreactivity, a peptide tag of Gi-DREADD, which were also positive for Fos (**Fig. 2C**). We analyzed this percentage in the dorsolateral and dorsomedial striatum and in the NAc core and shell regions (**Fig. 2D**). In both vehicle- and cocaine-treated mice, most Fos-positive cells, but not all, co-expressed HA (and thus Gi-DREADD), indicating that Fos was mainly induced in D1-neurons as previously reported (Bertran-Gonzalez et al., 2008; Guez-Barber et al., 2011; Zhang et al., 2006). As compared with vehicle, CLZ pretreatment decreased Fos-positive/HA-positive ratio in the NAc shell, dorsomedial, and dorsolateral striatum (**Fig. 2E**). These results indicated that stimulating Gi-DREADD with CLZ inhibited the cocaine-induced response of striatal D1-neurons.

Inhibition of D1-neurons decreases basal and cocaine-induced locomotor activity

Next, using different groups of mice, we analyzed how inhibition of D1-neurons affected spontaneous locomotion and locomotor responses after the first and second cocaine injection (see **Fig. 1B-C**). On Day 0, during the 30-min period after CLZ pretreatment (groups 3-4) the basal locomotor activity of mice was decreased as compared with vehicle-pretreated mice (groups 1-2, **Fig. 3A-B**). In contrast CLZ pretreatment had no effect in wild type mice (WT, **Fig. 3B**, groups 5-8). During the 60 min after cocaine injection, locomotor activity was dramatically decreased in CLZ-pretreated D1-Gi-DREADD mice (group 4) as compared to vehicle-pretreated mice (group 2, **Fig. 3A, C**), whereas CLZ injection did not alter cocaine-induced hyper-locomotion in WT mice (**Fig. 3C**, group 8). Thus, inhibition of Gi-DREADD-expressing D1-neurons by CLZ decreased the spontaneous locomotor activity and cocaine-induced hyper-locomotion. Although sex-dependent differences in the response to cocaine have been reported (Festa et al. 2006, Rivera et al. 2021, Kokane and Perrotti, 2020), the sex of the mice, which was evenly distributed in all groups of mice used in these experiments, did not modify the responses (**Figs. 1B, 3D**).

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Inhibition of D1-neurons impairs the induction of cocaine locomotor sensitization

We then investigated the effects of chemogenetic inhibition of D1-neurons during the first injection of cocaine on locomotor sensitization. To test this effect we used the TIPS protocol which avoids confusing effects due to repeated drugs administration, and provides a robust assessment of sensitization (Valjent et al., 2010). On Day 10, we injected cocaine to all D1-Gi-DREADD mice (**Fig. 3E**). All groups displayed an increased locomotor activity after cocaine injection (**Fig. 3E**). In the groups that had not received CLZ on Day 0 (groups 1-2), locomotor response to cocaine on Day10 was significantly increased in the mice injected with cocaine for the second time (group 2) as compared to those that had previously received saline (group 1, **Fig. 3E-F**). This result confirmed the locomotor sensitization induced in the TIPS protocol. In contrast, in the mice pretreated with CLZ on Day 0 (groups 3-4), the responses to cocaine were not significantly different whether or not they had received cocaine a week before. Thus sensitization was blunted in group 4 mice that had received cocaine on Day 0 in the presence of CLZ (**Fig. 3E-F**). In WT mice (groups 5-8) the locomotor response to the second injection of cocaine was increased (groups 6, 8) as compared to mice previously injected with saline (groups 5, 7), whether they also had received on Day 0 CLZ (group 8) or vehicle (group 6, **Fig. 3F**).

Inhibition of D1-neurons blocks previously sensitized cocaine-induced locomotion

To assess the acute effects of D1-neuron inhibition on locomotor activity in mice previously exposed to cocaine, we injected on Day 11 the same mice as in Fig. 3 with CLZ, followed 30 min later by cocaine (**Fig. 4A**). In the four D1-Gi-DREADD groups (1-4), the locomotor responses (30-90 min) were lower than those observed the day before in the absence of CLZ (Day 10, compare **Fig. 3 E-F** and **Fig. 4A-B**) and there was no significant difference between groups on Day 11 (**Fig. 4B**). In contrast, in all groups of WT mice injected with CLZ (groups 5-8), cocaine induced a clear hyper-locomotion (**Fig. 4B**), ruling out a non-specific pharmacological effect of CLZ. Paired analysis showed that locomotor responses to cocaine on Day 11 were significantly decreased compared with those on Day10 in the four D1-Gi-DREADD groups (**Fig. 4C**), including group 2 which was clearly sensitized on Day 10 (**Fig. 3D-E**). These results showed that inhibition of D1-neurons strongly decreased the cocaine-induced hyper-locomotion, including in mice with already established cocaine-sensitized locomotion (group 2).

Inhibition of D1-neurons on Day 11 does not alter established cocaine sensitization on Day 12

Because cocaine TIPS is protein synthesis- and context-dependent (Valjent et al., 2010) and context memory retrieval in the presence of a protein synthesis inhibitor can disrupt locomotor sensitization in rats (Bernardi et al., 2007), we asked whether exposure of mice to the LA-box and cocaine in the presence of CLZ could have persistent effects on cocaine locomotor responses. We tested whether D1-neuron inhibition on Day 11 by CLZ in D1-Gi-DREADD mice affected the locomotor response to cocaine the next day (Day 12). On Day 12, all groups were injected with vehicle and 30 min later

with cocaine. There was no significant difference in locomotor activity (30-90 min) between the 4 D1-Gi-DREADD groups (**Fig. 4D, E**). These results were similar to those observed in WT mice receiving the same treatment on Day 12 (groups 5-8, **Fig. 4E**). Paired analysis showed that the locomotor response to cocaine (30-90 min) on Day 12 was significantly increased in Groups 1, 3, and 4, as compared to Day 10 (**Fig. 4F**), indicating the existence of a sensitization step. No significant difference between Day 10 and 12 was observed in group 2 (**Fig. 4F**), suggesting that sensitization had reached a plateau in this group, in agreement with previous report showing that cocaine sensitization to the 2nd cocaine injection is time-dependent and becomes maximum about 7 days after the first cocaine injection (Valjent et al., 2010). None of the groups displayed any decreased cocaine-induced locomotor activity on Day 12 as compared to Day 10, showing that inhibition of D1-neurons during cocaine exposure in paired context on Day 11 did not diminish previously established sensitization.

Discussion

In this study, we used double transgenic mice with conditional expression of Gi-DREADD in neurons that express Drd1 and showed that inhibition of these neurons decreased spontaneous locomotor activity and acute cocaine-induced hyper-locomotion. Inhibition of D1-neurons during the first cocaine administration blunted sensitization to a cocaine challenge ten days later. In addition, acute inhibition of D1-neurons prevented cocaine-induced locomotor activity in previously sensitized mice. However, inhibition of D1-neurons concomitant with cocaine injection in the drug-associated environment did not diminish previously acquired sensitization.

For pharmacological activation of Gi-DREADD, we used low doses of CLZ instead of commonly used CNO. CNO induces cellular DREADD-mediated effects with different time-courses in the brain and peripheral organs (Alexander et al., 2009). The effects of systemic administration of CNO on DREADD-expressing neurons appear to result mainly from the action of CLZ produced by its metabolic degradation, which crosses the blood brain barrier more efficiently and has a higher affinity for DREADDs (Gomez et al., 2017). In a recent fMRI study of DREADD-induced activation of D1-neurons, we confirmed a more rapid and efficient effect of low doses of CLZ as compared to CNO (Nakamura et al., 2020). Here low doses of CLZ (0.1 mg/kg, i.p.) had clear effects only in mutant mice expressing Gi-DREADD. Although CLZ has numerous endogenous targets at higher doses including serotonin, dopamine, and muscarinic acetylcholine receptors (Bonaventura et al., 2019; Schotte et al., 1993), in our experiments we did not observe any effect of the low dose of CLZ used in WT mice, ruling out off-target effects.

Cell-type-specific technologies allow better characterization of cell populations involved in the effects of psychostimulants (Durieux et al., 2011; Yager et al., 2015). Optogenetic inhibition of striatal D1- but not D2-neurons inhibited the expression of cocaine-induced locomotor sensitization, (Lee et al., 2017). Repeated association of optogenetic inhibition of NAc D1-neurons with cocaine

injection decreased sensitization (Chandra et al. 2013). Chemogenetic inhibition of dorsal striatum dynorphin-neurons (corresponding to D1) but not enkephalin-neurons (D2) impaired behavioral sensitization although it did not alter spontaneous activity or acute response to amphetamine (Ferguson et al., 2011). In our study, we observed a strong effect of inhibiting D1-neurons on spontaneous locomotor activity and acute response to cocaine. These stronger effects may result from the wider inhibition of D1-neurons excitability throughout the brain, including in dorsal striatum, NAc and cerebral cortex, in contrast to the restricted inhibition in the dorsomedial striatum in the study of Ferguson et al. In addition, it is possible that the efficacy of CLZ to activate DREADD is higher and more rapid than that of CNO, as suggested by Gomez et al. (2017).

Decreasing activity of the medial prefrontal cortex afferent to the NAc did not impair amphetamine sensitization (Kerstetter et al., 2016). In contrast, increasing activity of striatopallidal neurons (D2-neurons) with Gs-DREADD prevented amphetamine sensitization (Farrell et al., 2013). This is in agreement with the results on mice lacking Drd2, a receptor exerting an inhibitory effect on D2-neurons, in which acute response to cocaine or to a Drd1 agonist (SKF81297) was blunted and locomotor sensitization to cocaine and amphetamine attenuated (Solis et al. 2021). Combined with our current findings, these reports underline the importance of the balance between D1- and D2-neurons in regulating sensitization.

Although Drd1 is predominantly expressed in a subset of striatal projection neurons (Gerfen et al., 1990), it is also found at lower levels in some neurons of other regions such as prefrontal cortex (Santana et al. 2009) and hippocampus (Gangarossa et al., 2012) and some non-neuronal cells in and outside the brain (Amenta et al., 1997, Corkrum et al., 2020). These cells were potentially expressing Gi-DREADD and could have responded to CLZ stimulation, but not necessarily by an inhibition, as suggested for hippocampal astrocytes (Nam et al. 2019). We therefore cannot formally exclude that the effects of CLZ were also involving cells outside the striatum.

Drug addiction is a complex disorder in which pathological memories play a critical role in the maintenance of addictive behaviors and relapse to drug seeking (reviews in Hyman et al., 2006; Robbins et al., 2008; Tronson and Taylor, 2013). Several rodent studies aimed to erase or weaken these drug-associated memories by inducing targeted disruption of memory reconsolidation processes. Memory reconsolidation is the process by which memories become labile under certain conditions of reactivation (retrieval) before being stabilized again and persistently stored in the brain (review in Lee et al., 2017). Drug-induced conditioned place preference was attenuated or erased by combining re-exposure to the drug-paired stimuli and/or the drug itself with inhibition of protein synthesis (Valjent et al., 2006; Dunbar and Taylor., 2016), ERK signaling (Valjent et al., 2006), D1 receptors (Yan et al., 2014, Marion-Poll et al., 2019) or knockdown of the immediate early gene *zif268/Egr1* (Lee et al., 2005, 2006; Théberge et al., 2010). Locomotor sensitization may undergo protein synthesis-dependent reconsolidation when the animals are briefly exposed to the

drug-associated context without drug (Bernardi et al. 2007). Locomotor sensitization induced by a single injection of cocaine was shown to involve potentiation of excitatory synapses onto nucleus accumbens D1-neurons and was erased by optogenetic depotentiation of cortical inputs to these neurons (Pascoli et al., 2012). Complete DREADD-induced inhibition of D1 neurons in the current study did not erase sensitization however, presumably because it did not modify synaptic input weights.

In conclusion, our results show that specific inhibition of D1-neurons strongly decreases spontaneous and cocaine-induced locomotor activity and alters locomotor sensitization induced by a single injection of cocaine. Our study suggests that selective manipulation of the excitability of these neurons is an interesting therapeutic approach in the context of addiction.

Competing Interests

The authors declare no competing interest.

Authors contributions

Yukari N carried out experiments, analyzed results and wrote the manuscript, SL and Yuki N carried out experiments and analyzed results, AN analyzed results, JAG supervised the work, DH, JAG, and Yuki N planned and conceived experiments, analyzed results and wrote the manuscript.

Data accessibility

Original data are accessible upon request

Abbreviations

CLZ: clozapine
CNO: clozapine-N-oxide
D1-neurons: dopamine D1 receptor-expressing neurons
Drd1: dopamine D1 receptor
Drd1-Cre mice: *Drd1-Cre* transgenic mice
DREADD: designer receptor exclusively activated by designer drugs
NAc: nucleus accumbens
TIPS: two-injection protocol of sensitization

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Figure legends

Figure 1. Experimental design of Gi-DREADD inhibition of D1 neurons in cocaine two-injection protocol of sensitization (TIPS). A) Gi-DREADD (hM4Di) expression in Drd1-expressing cells (D1-cells) of double transgenic Drd1-Cre x Gi-DREADD mice (referred to as D1-Gi-DREADD mice). Following Cre-mediated removal of an upstream floxed-STOP cassette, the HA-tagged Gi-DREADD-mCitrine mRNA is expressed in D1-cells. HA, hemagglutinin epitope tag, PTA, porcine teschovirus cleavage site. **B**) Groups of mutant and wild type mice used in the study. The number of mice and sex ratio is indicated for each group. **C**) Schematic schedule of behavioral experiments. Cocaine, 20 mg/kg, clozapine, CLZ, 0.1 mg/kg.
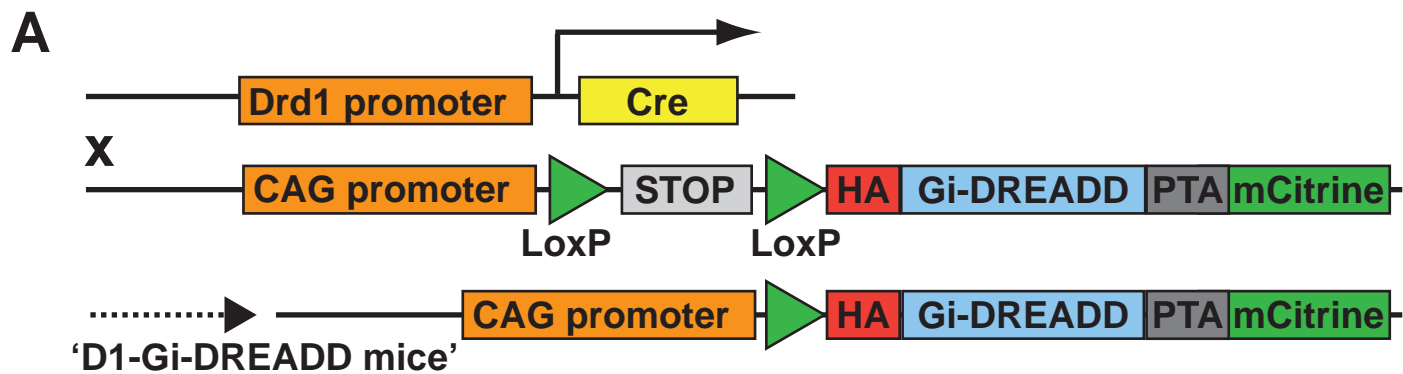
Figure 2. CLZ decreases cocaine-induced locomotor activity and Fos expression in Gi-DREADD-expressing striatal neurons. A) Schematic experimental design. Vehicle or CLZ was injected 30 min before cocaine administration. The locomotor activity box is a circular corridor. Mice were killed 90 min after cocaine administration for immunofluorescence. **B**) Inhibition of D1-neurons by stimulating Gi-DREADD with CLZ prevents cocaine-induced hyper-locomotion. Locomotor activity was measured during 60 min after cocaine injection in mice pretreated with vehicle or CLZ as indicted. Data were collected in 5-min bins and shown for each mouse. Bars indicate means + SEM, n = 5 mice per group. Mann-Whitney's U = 0, p = 0.0079, ** p<0.01. **C**) Fos- and HA-immunoreactivity 90 min after cocaine administration (20 mg kg^{-1} i.p.) were visualized by immunofluorescence and confocal microscopy. Representative images of double immunolabeling of Fos (green) and HA (red) in NAc shell. Merged image demonstrates colocalization (arrows) of HA-Gi-DREADD and Fos. Scale bar, 50 µm. **D**) The position of the striatal regions used for quantification (388×388 µm) is indicated by red squares on a mouse brain coronal section at 0.98 mm antero-posterior coordinate (after Paxinos and Franklin, 2001): dorsomedial (**DM**) and dorsolateral (**DL**) striatum, NAc core (**C**) and shell (**S**). **E**) The percentage of Fos-positive neurons among HA-positive neurons was analyzed in the 4 regions indicated in D. Each data point corresponds to the mean results of the right and left side for each region in one mouse (5 mice per group), with bars indicating means + SEM. Mann-Whitney's test, NAc core, U = 26, p<0.075, NAc shell, U = 18.5, p = 0.016, DM, U = 5, p = 2 10^{-4}, DL, U = 18, p = 0.067. *p<0.05, *p<0.01, ***p<0.001.

Figure 3. Gi-DREADD-induced inhibition of D1-neurons decreases acute cocaine-induced hyper-locomotion and cocaine sensitization in the TIPS protocol. A-D) On day 0, D1-Gi-DREADD mice (D1-DREADD, groups 1-4) and wild type mice (WT, groups 5-8, independent experiments) were injected with vehicle (Veh, groups 1, 2, 5, 6) or CLZ (0.1 mg/kg, groups 3, 4, 7, 8) and placed in the locomotor activity box (circular corridor). Thirty min later, they were injected with saline (Sal, groups 1, 3, 5, 7) or cocaine (Coc, 20 mg/kg, groups 2, 4, 6, 8). (**A**) Time-course of locomotor activity measured in 5-min bins in D1-Gi-DREADD mice. Repeated measures 2-way ANOVA, interaction, $F_{(33, 407)} = 3.25$, p<10^{-4}, time, $F_{(11, 407)} = 5.16$, p<10^{-4}, groups, $F_{(3, 37)} = 25.92$, p<10^{-4}. (**B**) Basal locomotor activity during the 30 min before cocaine injection, in D1-Gi-DREADD and WT mice after vehicle or CLZ pretreatment as indicated. CLZ decreased basal locomotion in transgenic mice (pooled groups 1 and 2 vs pooled

groups 3 and 4, Mann-Whitney test, U = 21, $p<10^{-4}$) but not in WT mice (pooled groups 5 and 6 vs pooled groups 7 and 8, Mann-Whitney test, U = 654, NS). **C**) Total locomotor activity during the 60 min after cocaine injection. D1-Gi-DREADD mice (groups 1-4), Kruskal-Wallis test value = 25.2, $p<10^{-4}$, Dunn's multiple comparisons test, 1 vs 2, $p<0.05$. Wild type mice (groups 5-8, independent experiment) Kruskal-Wallis test value = 50.8, $p<10^{-4}$, Dunn's multiple comparisons test, 5 vs 6 and 7 vs 8, $p<10^{-4}$. **D**) Same data as in C) but shown separately for males (M) and females (F). **E-F**) On day 10, all mice were injected with vehicle, put in the locomotor activity box and injected with cocaine (20 mg/kg) 30 min later. **E**) Time-course of locomotor activity measured in 5-min bins in D1-Gi-DREADD mice. Repeated measures 2-way ANOVA, interaction, $F_{(33, 407)}$ = 1.93, p = 0.002, time, $F_{(11, 407)}$ = 17.21, $p<10^{-4}$, groups, $F_{(3, 37)}$ = 7.29, p = 6 10^{-4}. **F**) Total locomotor activity during the 60 min after cocaine injection. D1-Gi-DREADD mice (groups 1-4), 2-way ANOVA, interaction, $F_{(1, 37)}$ = 5.93, p = 0.02, pretreatment on day 0, $F_{(1, 37)}$ = 2.06, NS, treatment on day 0, $F_{(1, 37)}$ = 13.62, p = 7 10^{-4}, Sidak's multiple comparisons test, 1 vs 2, $p<0.001$, WT mice (groups 5-8, independent experiment), 2-way ANOVA, interaction, $F_{(1, 75)}$ = 0.24, NS, pretreatment on day 0, $F_{(1, 75)}$ = 1.83, NS, treatment on day 0, $F_{(1, 75)}$ = 16.92, $p<10^{-4}$, Sidak's multiple comparisons test, 5 vs 6, $p<0.05$, 7 vs 8, $p<0.01$. In A and E each point correspond to a group mean ± SEM. In B, C, D, and F, each point correspond to a mouse, bars indicate means + SEM. NS, not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<10^{-4}$.

Figure 4. Gi-DREADD-induced inhibition of D1-neurons blocks cocaine-induced locomotion in previously sensitized mice without persistent effect on its further expression. A-C) On day 11, the same D1-Gi-DREADD and WT mice as in Fig. 3 were all injected with CLZ, placed in the locomotor activity box, and 30 min later, injected with cocaine (20 mg/kg). The time-course of locomotor activity was measured in 5-min bins. **A**) D1-Gi-DREADD mice, repeated measures 2-way ANOVA, interaction, $F_{(33, 407)}$ = 1.39, NS, time, $F_{(11, 407)}$ = 7.29, $p<10^{-4}$, groups, $F_{(3, 37)}$ = 1.20, NS. **B**) Total locomotor activity during the 60 min after cocaine injection. D1-Gi-DREADD mice (groups 1-4), Kruskal-Wallis test value = 4.6, NS. WT mice (groups 5-8, independent experiment), Kruskal-Wallis test value = 6.25, NS. **C**) Pairwise comparison for each mouse of locomotor responses to cocaine on day 10 without CLZ pretreatment (data from Fig. 3E) and on day 11 with CLZ (data from Fig. 4B). Paired t-test, group 1, p = 0.001, group 3, p = 0.001, group 4, p = 0.038. Wilcoxon matched-pairs test, group 2, p = 0.001. **D-F**) On day 12, the same mice as in A were all injected with vehicle, placed in the locomotor activity box, and 30 min later, injected with cocaine (20 mg/kg). The time-course of locomotor activity was measured in 5-min bins. **D**) D1-Gi-DREADD mice, repeated measures 2-way ANOVA, interaction, $F_{(33, 397)}$ = 1.41, NS, time, $F_{(11, 396)}$ = 36.87, $p<10^{-4}$, groups, $F_{(3, 36)}$ = 0.63, NS. **E**) Total locomotor activity during the 60 min after cocaine injection. D1-Gi-DREADD mice (groups 1-4), Kruskal-Wallis test value = 2.39, NS. Wild type mice (groups 5-8, independent experiment) Kruskal-Wallis test value = 0.76, NS. **F**) Pairwise comparison for each mouse of locomotor responses to cocaine on day 10 without CLZ pretreatment (data from Fig. 3B) and on day 12 (data from Fig. 4E). Paired t-test, group 1, p = 0.009, group 2, p = 0.235, group 3, p = 0.001. Wilcoxon matched-pairs test, group 4, p = 0.014. In A and D, each point correspond to a group mean ± SEM. In B, C, E, and F, each point correspond to a mouse. In B and E, bars indicate means + SEM. NS, not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Fig 1

A

Drd1 promoter — Cre

X

CAG promoter — LoxP — STOP — LoxP — HA — Gi-DREADD — PTA — mCitrine

'D1-Gi-DREADD mice'

CAG promoter — HA — Gi-DREADD — PTA — mCitrine

B

Group	Mouse line	Day 0	Day 10	Day 11	Day 12
Group 1 (n = 8, M/F = 5/3)	D1-Gi-DREADD	Vehicle + Saline	Vehicle + Cocaine	CLZ + Cocaine	Vehicle + Cocaine
Group 2 (n = 11, M/F = 6/5)		Vehicle + Cocaine			
Group 3 (n = 11, M/F = 6/5)		CLZ + Saline			
Group 4 (n = 11, M/F = 6/5)		CLZ + Cocaine			
Group 5 (n = 20, M/F = 10/10)	Wild type	Vehicle + Saline			
Group 6 (n = 20, M/F = 11/9)		Vehicle + Cocaine			
Group 7 (n = 19, M/F = 10/9		CLZ + Saline			
Group 8 (n = 20, M/F = 10/10)		CLZ + Cocaine			

CLZ 0.1 mg kg^{-1} i.p. Cocaine 20 mg^{-1} kg i.p.

C

-3 -2 -1 0 10 11 12 Days

Habituation

Vehicle or CLZ + Saline or Cocaine

Vehicle + Cocaine

CLZ + Cocaine

Vehicle + Cocaine

Fig 2

A

D1-Gi-DREADD mice

Group A (n = 5, M/F = 4/1)
Group B (n = 5, M/F = 3/2)

Locomotor activity box

30 min — Vehicle / CLZ 0.1 mg kg^{-1}

Cocaine 20 mg kg^{-1}

90 min

Brain removal

B

Locomotor activity (1/4 turns per 60 min)

**

Vehicle CLZ

C

Merge   HA   Fos

Pretreatment Vehicle

CLZ

NAc shell

D

DM
DL
S C

DM, dorso-medial striatum
DL, dorso-lateral striatum
C, NAc core
S, NAc shell

E

NAc core — Fos+ / HA+ cells, % p = 0.075 Vehicle CLZ

NAc shell — Fos+ / HA+ cells, % * Vehicle CLZ

DM — Fos+ / HA+ cells, % *** Vehicle CLZ

DL — Fos+ / HA+ cells, % ** Vehicle CLZ

Fig 3

A Day 0

D1-Gi-DREADD mice
- Group 1 (Veh + Sal)
- Group 2 (Veh + Coc)
- Group 3 (CLZ + Sal)
- Group 4 (CLZ + Coc)

Sal or Coc

Vehicle or CLZ

B Day 0 (0-30 min)
Basal locomotor activity

NS

****

Group: 1 2 3 4 5 6 7 8
Pretreatment: Veh CLZ Veh CLZ
Genotype: D1-DREADD WT

C Day 0 (30-90 min)
Cocaine-induced activity

Saline
Cocaine

**** ****
* NS

Group: 1 2 3 4 5 6 7 8
Pretreatment: Veh CLZ Veh CLZ
Genotype: D1-DREADD WT

D Day 0 (30-90 min)

D1-Gi-DREADD mice
1 2 3 4
M F M F M F M F

WT mice
5 6 7 8
M F M F M F M F

E Day 10

D1-Gi-DREADD mice
- Group 1
- Group 2
- Group 3
- Group 4

Coc

Vehicle

F Day 10 (30-90 min)
Veh + Coc

* **
*** NS

Group: 1 2 3 4 5 6 7 8
Genotype: D1-DREADD WT

Fig 4

A Day 11

D1-Gi-DREADD mice

Previous treatment on Day 0
- Group 1 (Day 0 Veh + Sal)
- Group 2 (Day 0 Veh + Coc)
- Group 3 (Day 0 CLZ + Sal)
- Group 4 (Day 0 CLZ + Coc)

B Day 11 (30-90 min)
CLZ + Coc

C Day 10 vs Day11 (30-90 min)
D1-Gi-DREADD mice

D Day 12

D1-Gi-DREADD mice
- Group 1
- Group 2
- Group 3
- Group 4
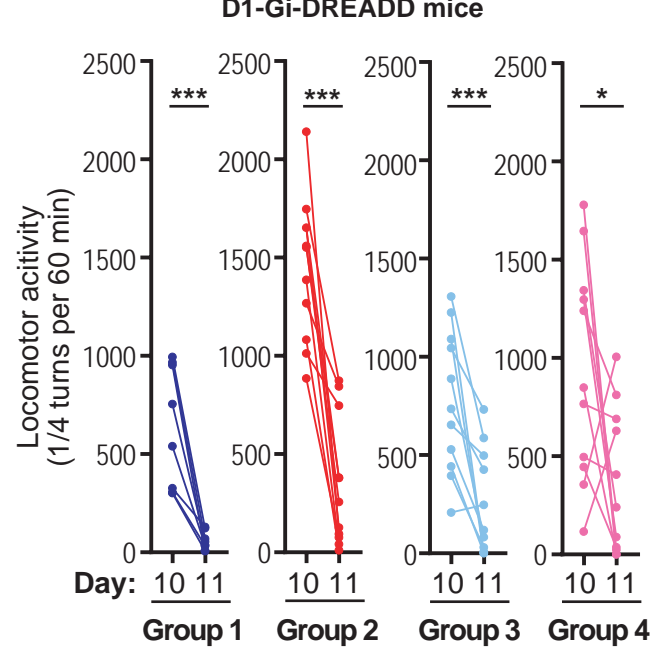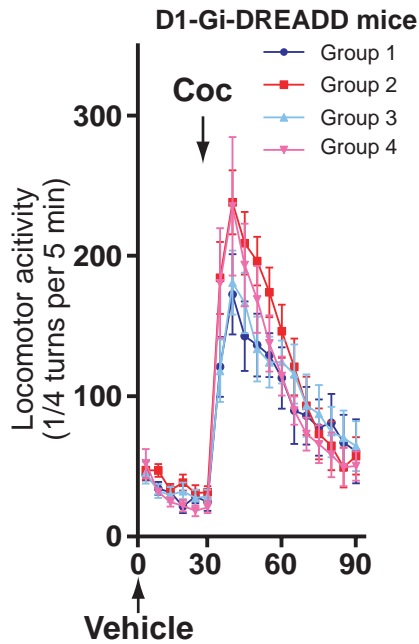
E Day 12 (30-90 min)
Veh + Coc
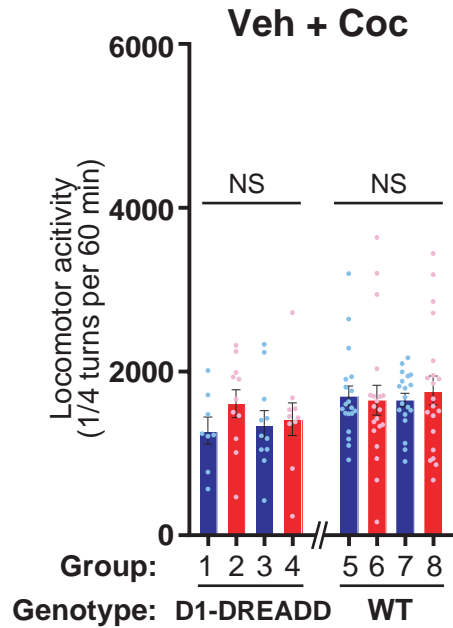
F Day 10 vs Day12 (30-90 min)
D1-Gi-DREADD mice