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Restoration of cocaine stimulation and reward by reintroducing wild type dopamine transporter in adult knock-in mice with a cocaine-insensitive dopamine transporter

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ABSTRACT

In previous studies, we generated knock-in mice with a cocaine-insensitive dopamine transporter (DAT-CI mice) and found cocaine does not stimulate locomotion or produce reward in these mice, indicating DAT inhibition is necessary for cocaine stimulation and reward. However, DAT uptake is reduced in DAT-CI mice and thus the lack of cocaine responses could be due to adaptive changes. To test this, we used adeno-associated virus (AAV) to reintroduce the cocaine-sensitive wild type DAT (AAV-DATwt) back into adult DAT-CI mice, which restores cocaine inhibition of DAT in affected brain regions but does not reverse the adaptive changes. In an earlier study we showed that AAV-DATwt injections in regions covering the lateral nucleus accumbens (NAc) and lateral caudate-putamen (CPu) restored cocaine stimulation but not cocaine reward. In the current study, we expanded the AAV-DATwt infected areas to cover the olfactory tubercle (Tu) and the ventral midbrain (vMB) containing the ventral tegmental area (VTA) and substantia nigra (SN) in addition to CPu and NAc with multiple injections. These mice displayed the restoration of both locomotor stimulation and cocaine reward. We further found that AAV-DATwt injection in the vMB alone was sufficient to restore both cocaine stimulation and reward in DAT-CI mice. AAV injected in the VTA and SN resulted in DATwt expression and distribution to the DA terminal regions. In summary, cocaine induced locomotion and reward can be restored in fully developed DAT-CI mice, and cocaine inhibition of DAT expressed in dopaminergic neurons originated from the ventral midbrain mediates cocaine reward and stimulation.

1. Introduction

Cocaine is a powerfully addictive stimulant and a popular drug of abuse. Cocaine inhibits the transporters for dopamine (DA), norepinephrine (NE), and serotonin (5-HT) at roughly similar concentrations in both brain preparations and in cultured cells expressing the cloned transporters (Ritz et al., 1987; Amara and Kuhar, 1993; Surratt et al., 1993; Gu et al., 1994; Han and Gu, 2006). Knockout mice with each of the transporters disrupted individually still exhibit the rewarding effect of cocaine in conditioned place preference (CPP) and/or cocaine self-administration experiments (Rocha et al., 1998; Sora et al., 1998; Xu et al., 2000), suggesting that none of the these transporters are required for cocaine reward. However, the knock-out mice showed very significant adaptive changes to compensate for the lack of an important transporter (Giros et al., 1996; Bengel et al., 1998; Rocha et al., 1998; Sora et al., 1998; Li et al., 1999; Rioux et al., 1999; Xu et al., 2000). We have made a knock-in mouse line carrying a functional but cocaine-insensitive dopamine transporter (DAT-CI mice) (Wu and Gu, 2003; Chen et al., 2006). In these mice, cocaine does not block DAT and does not produce reward or stimulate locomotion, indicating that cocaine blockade of DAT is required for the cocaine

Abbreviations: 5-HT, serotonin; AAV, adeno-associated virus; AAV-DATwt, adeno-asssociated virus carrying cocaine-sensitive wild type DAT; CPP, conditioned place-preference; CPu, Caudate Putamen; DA, dopamine; DAT, dopamine transporter; DAT-CI mice, cocaine-insensitive dopamine transporter knock-in mice; HA-DAT, hemagglutinin-tagged DAt; IHC, immunohistochemistry; NAc, nucleus accumbens; NE, norepinephrine; SERT, serotonin transporter; SN, substantia nigra; Tu, olfactory tubercle; VTA, ventral tegmental area; vMB, ventral midbrain.

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reward and stimulation in "normal" mice. In addition, cocaine actually produces conditioned place aversion (CPA) and locomotor suppression in these mice (Chen et al., 2006; O'Neill et al., 2013). However, DAT function in DAT-CI mice is also significantly reduced which might also cause some adaptive changes. It is possible that the lack of cocaine responses in these mice is due to adaptive changes. To test this possibility, we use viral vectors to reintroduce the wild type DAT (DATwt) back into the brains of fully developed adult DAT-CI mice, which partially restore cocaine inhibition of DAT but will not reverse any adaptive changes occurred during development.

In our prior study, we injected recombinant adeno-associated viral (AAV) vectors containing the wild-type mouse DAT (AAV-DATwt) into various brain regions of DAT-CI mice. We found that restoring DAT inhibition in the lateral striatum including the dorsal and ventral areas is sufficient for restoring cocaine's stimulating effect but not for the rewarding effect (O'Neill et al., 2014). In the current study, we expanded the tested areas to identify the brain regions where cocaine inhibition of DAT leads to stimulation and reward.

2. Materials and methods

2.1. Animal subjects

Homozygous C57-congenic DAT-CI mice (Chen et al., 2006; O'Neill and Gu, 2013) and wild-type littermates were produced from sibling pairings of heterozygous DAT-CI mice. Mice were kept in a 12-h light/dark cycle and group housed up to five per cage. Water and standard rodent chow were provided ad libitum. Nesting material and hiding/nesting devices were provided for enrichment purposes. Only male mice were used for experiments, and all mice were between 2 and 6 months of age at the time of testing. All animal related procedures were performed in accordance with the Ohio State University Institutional Animal Care and Use Committee (IACUC). The animals went through surgeries of AAV injections 4 weeks before behavioral tests followed by immunohistochemical analysis. Fig. 1 illustrate the timeline of these procedures.

2.2. Viral vectors

Recombinant adeno-associated viral vectors (AAV) carrying the wild-type mouse dopamine transporter (AAV-DATwt) were used in this study. The virus carried DAT has a hemagglutinin (HA) tag at the N-terminal, allowing the detection of its expression. The vectors were prepared by the OSU viral vector core, where viruses were packaged and purified using a procedure similar to that described (Clark et al., 1999). Briefly, HEK293 cells were co-transfected with a capsidation plasmid (AAV1 serotype), a helper plasmid, and the recombinant genome plasmid containing HA tagged wild type mouse DAT. The AAV-DATwt viruses were then isolated from the cell culture by ultracentrifugation and chromatography purification. The final preparation was tittered by real-time PCR and the concentration was 2.6×10^{12} vg/ml. The virus stock was diluted to 2.6×10^{11} vg/ml for microinjections.

2.3. Surgeries and microinjection of viral vectors

The surgery and microinjection procedures are similar to those described in our earlier publication (O'Neill et al., 2014). Briefly, mice were anesthetized with a mixture of 100 mg/kg ketamine and 20 mg/kg xylazine (Sigma–Aldrich, St. Louis, MO). Mice were mounted on a stereotaxic frame and a small straight incision was made along the midline of the head to expose the underlying skull. The bregma coordinates were recorded and used as the reference point.

The stereotaxic injection setup consisted of a Hamilton syringe connected to a 33 gauge injector cannula (Plastics one, Roanoke VA). A volume of 2–10 μl of virus was injected per mouse, at a rate of 0.1–0.25 μl/min controlled by a syringe pump.

Microinjections of viral vectors were carried out to cover multiple regions. Injection 1 covered olfactory tubercle (Tu), nucleus accumbens shell (Acbsh) and core (AcbC), and the Caudate Putamen (CPu). Injection 2 covered the ventral midbrain (vMB) including the ventral tegmental area (VTA) and the substantia nigra (SN). In injection 1, four boli of 1 μL virus were infused along the injector's path at 4 different dorsal/ventral coordinates (Table 1). The coordinates for each of the targeted regions are listed in Table 1.

After infusion was finished, the injector was left in place for 2 min before being raised. The mice were sutured after the surgery and administered post-operative care for one week. After four weeks recovery, the wild-type DAT would be stably expressed in the injected region.

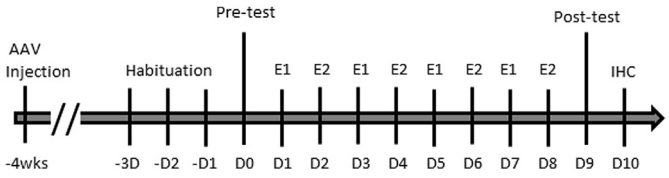

Fig. 1. Timeline of AAV injection and behavior tests. The stereotaxic surgery was performed to inject AAV vectors into selected brain regions 4 weeks before the behavior tests. The 4 weeks of recovery after the surgery is necessary for stable expression of virus carried genes. The mice were first habituated to being handled each day for 3 days (−D3 to −D1). Then the mice went through the CPP paradigm with the pre-test on day 0 (D0), followed by four treatment/environment pairings of two alternating environments (E1 and E2) which are saline or cocaine injection. Half of the mice had cocaine treatment first and half of the mice had saline treatment first. The post-conditioning test was on day 9 (D9). After the last behavioral test, the mice were sacrificed and processed for immunohistochemical (IHC) localization of the AAV injection site (D10) and HA-DAT expression.

2.4. Drugs administered

All drugs were dissolved in 0.9% saline and given in a volume of 10 μl/g body weight. Cocaine HCL was provided by the NIDA drug supply program, and administered at 20 mg/kg doses intraperitoneally (i.p.). Ketamine and xylazine were administered for anesthesia before the virus injection surgeries at doses of 100 mg/kg and 20 mg/kg respectively (i.p.).

2.5. Conditioned place-preference and locomotion test

The conditioned place-preference (CPP) scores and the locomotor activities were recorded during the same CPP procedure (O'Neill et al., 2014). The apparatus was an acrylic box divided into three interconnected compartments: two side compartments and a center compartment. The CPP procedure is outlined in Fig. 1. Mice were first habituated to being handled for three days. On the pre-conditioning test day (D0), the three compartments had distinct visual and tactile cues, creating three different "environments". Mice were placed into the center compartment and allowed to explore all three compartments for 30 min. Mouse locations and movements were recorded by the AnyMaze video tracking system (Stoelting Co.). Time mice spent in each of the three compartments and the total distance mice traveled were calculated. Their preference was defined as the difference in time spent in one side compartment versus the other side. The pre-test (preexisting) preference was counterbalanced in each group of mice by designating individual mice to receive cocaine in either their initially preferred or initially non-preferred environment—such that the group bias was minimized.

During the conditioning phase (D1 to D8), all three compartments had the same environmental cue set. The mice were then administered the treatment (either cocaine or saline) corresponding with one of the two environments and immediately placed in the environment for 30 min. The mice were monitored and the distance traveled was recorded. On the following day, the opposite agent was administered and mice were placed in the alternate environment for 30 min. These alternations proceeded for 4 cycles (four pairings, D1–D8). The distances traveled after the first saline injection and after the first cocaine injection were used to assess the basal locomotion and cocaine induced locomotor stimulation in these mice.

On the post-conditioning test day (D9), the apparatus was configured to have three-environments and the mice were tested exactly the same way as on the pre-conditioning test day. There were no treatments during the pre or post-conditioning tests. The "CPP score" is defined as the time spent in the cocaine-paired environment (conditioned stimulus, CS+) minus the time spent in the saline-paired environment (unconditioned stimulus, CS−) during the pre- and post-conditioning tests. Differences in CPP score during the pre-conditioning test versus the post-conditioning test indicate an effect of the drug.

Table 1
Coordinates used for stereotaxic AAV-DATwt injection.

Brain region	Injection site relative to bregma (in mm)		
	Anterior/Posterior	Medial/Lateral	Dorsal/Ventral
CPu	+1.5	±1.3	−3.5
AcbC			−4.6
AcbSh			−5.0
Tu			−5.5
vMB	−2.9	±0.9	−4.5

The stereotaxic coordinates for targeted brain regions in mm relative to bregma. CPu: caudate putamen (dorsal striatum); AcbC and AcbSh: nucleus accumbens core and shell; Tu: olfactory tubercle; vMB: ventral MidBrain including ventral tegmental area (VTA) and substantia nigra (SN).