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Behavior of knock-in mice with a cocaine-insensitive dopamine transporter after virogenetic restoration of cocaine sensitivity in the striatum

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ABSTRACT

Cocaine's main pharmacological actions are the inhibition of the dopamine, serotonin, and norepinephrine transporters. Its main behavioral effects are reward and locomotor stimulation, potentially leading to addiction. Using knock-in mice with a cocaine-insensitive dopamine transporter (DAT-CI mice) we have shown previously that inhibition of the dopamine transporter (DAT) is necessary for both of these behaviors. In this study, we sought to determine brain regions in which DAT inhibition by cocaine stimulates locomotor activity and/or produces reward. We used adeno-associated viral vectors to re-introduce the cocaine-sensitive wild-type DAT in specific brain regions of DAT-CI mice, which otherwise only express a cocaine-insensitive DAT globally.

Viral-mediated expression of wild-type DAT in the rostrolateral striatum restored cocaine-induced locomotor stimulation and sensitization in DAT-CI mice. In contrast, the expression of wild-type DAT in the dorsal striatum, or in the medial nucleus accumbens, did not restore cocaine-induced locomotor stimulation. These data help to determine cocaine's molecular actions and anatomical loci that cause hyperlocomotion. Interestingly, cocaine did not produce significant reward – as measured by conditioned place-preference – in any of the three cohorts of DAT-CI mice with the virus injections. Therefore, the locus or loci underlying cocaine-induced reward remain underdetermined. It is possible that multiple dopamine-related brain regions are involved in producing the robust rewarding effect of cocaine.

1. Introduction

Cocaine is an inhibitor of the dopamine (DA), norepinephrine (NE), and serotonin (5-HT) transporters (Han and Gu, 2006; Ritz et al., 1987). It is simultaneously an addictive drug with euphorigenic effects, and adverse cardiovascular and psychiatric effects (Rotheram-Fuller et al., 2007). While inhibition of each of the monoamine transporters is likely to contribute to each of cocaine's effects in some way, there has been much effort to determine the specific role of each target in producing a behavioral effect. Knock-out mice with each of the monoamine transporters deleted still self-administer cocaine, indicating that none of these targets are individually required for its rewarding effect (Hall et al., 2002). Double knock-out mice lacking the dopamine transporter (DAT) and the serotonin transporter (SERT) do not show cocaine reward, suggesting that the monoamines are mutually or redundantly involved in producing cocaine reward (Uhl et al., 2002). However, the knock-out mice may have substantial adaptive changes. In contrast, using knock-in mice with a functional yet cocaine-insensitive dopamine transporter (DAT-CI mice), we determined that DAT inhibition is necessary for cocaine's rewarding and hyperlocomotive effects (Chen et al., 2006).

Abbreviations: AAV, adeno-associated virus; CPP, conditioned place-preference; DAT, dopamine transporter; DAT-CI mice, cocaine-insensitive dopamine transporter knock-in mice; dCPu, dorsal striatum/dorsal caudate-putamen; IHC, immunohistochemistry; lCPu, lateral striatum/lateral caudate-putamen; mNAc, medial nucleus accumbens; RTI-113, 2β-Carbophenoxy-3β-(4-chlorophenyl)tropane.

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It is well established that the mesolimbic dopamine system, containing dopaminergic projections from ventral tegmental area (VTA) to the nucleus accumbens (NAc) and other forebrain structures, plays a critical role in reward/reinforcement and that most addictive drugs elevate extracellular dopamine in the NAc (Carboni et al., 1989; Cass et al., 1992; Di Chiara, 1995; Koob, 1998). It was shown that rats self-administer amphetamine directly into the NAc (Hoebel et al., 1983), however, cocaine infusion to the NAc did not produce place-conditioning (Hemby et al., 1992). Additionally, cocaine was found to be readily self-administered into the prefrontal cortex (PFC) (Goeders and Smith, 1983). Interestingly, cocaine self-administration to the dorsal, and also to the ventral striatum (nucleus accumbens), is sufficient to induce hyperlocomotion (Delfs et al., 1990; Mao and Wang, 2000). It is therefore important to directly test how cocaine inhibition of DAT in various brain regions contributes to different behavioral responses induced by cocaine.

Here, we set out to determine specifically where DAT inhibition in the brain is involved in producing cocaine reward and hyperlocomotion. We tested the function of DAT inhibition in a specific region by restoring expression of the wild-type dopamine transporter (DAT_{wt}) in DAT-CI mice using adeno-associated viruses (AAV). We injected DAT-CI mice with AAV-DAT_{wt} in the dorsal striatum (dCPu), medial nucleus accumbens (mNAc), and in both the dorsal and ventral portions of the lateral striatum (lCPu). After expression of DAT_{wt} in these regions, we exposed the DAT-CI mice to cocaine, and tested for restoration of the reward and locomotor behaviors.

2. Materials and methods

2.1. Animal subjects

In this study, knock-in mice with a cocaine-insensitive dopamine transporter (DAT-CI mice) were used, which were generated as described previously (Chen et al., 2006). These mice contain a triply mutated dopamine transporter (DAT) which is composed of the following substitutions: L104V/F105C/A109V (termed DAT_{vcv}). C57-congenic DAT-CI and wild-type littermates were generated from sibling pairings of heterozygous mice.

The animal subjects then underwent AAV injections, followed by behavioral testing, and finally by immunohistochemical analysis. A timeline of this paradigm is in Fig. 1, and the details of each method are described below.

During the course of these experiments, all mice were kept in standard housing conditions, which include ad libitum access to food/water and 12 h each of dark/light. Only male mice were used, and all mice were between 2 and 6 months of age at the time of behavioral testing. Experimental groups compared to one another were age matched. All animal procedures were approved by The Ohio State University Internal Laboratory Animal Care and Use Committee (ILACUC).

2.2. Packaging and purification of viral vectors

Recombinant adeno-associated viral vectors containing a hemagglutinin (HA)-tagged wild-type mouse dopamine transporter (AAV-DAT_{wt}) were used in this study. The vectors were prepared by the OSU viral vector core, where viruses were packaged and purified similar to procedures described elsewhere (Clark et al., 1999). Briefly, HEK293 cells were triple co-transfected via the calcium phosphate method with the following plasmids: a capsidation plasmid (AAV1 serotype), a helper plasmid, and the recombinant genome plasmid. The viruses containing wild-type mouse DAT were then isolated from the cell and media fractions of the culture by a series of ultracentrifugation and chromatography purifications. The final preparation was titered by real-time PCR and determined to be at a concentration of 2.6×10^{12} vg/mL. The virus was diluted to 2.6×10^{11} vg/mL for microinjections.

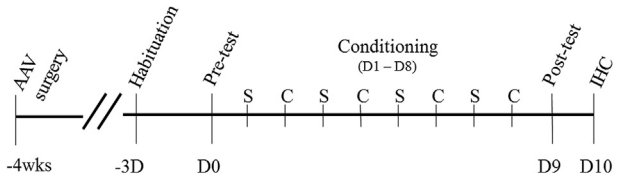

Fig. 1. Timeline and design of in-vivo experiments. The stereotaxic AAV injection surgery was performed and stable expression is reached during a four week recovery. The mice were habituated to being handled for 3 days. Then the mice went through the CPP paradigm with the pre-test on day 0 (D0), followed by four treatment/environment pairings of saline (S) and cocaine (C), and the post-test on D9. After the last behavioral test, the mice were sacrificed and processed for immunohistochemical (IHC) localization of the AAV injection site. Although the data in the Fig. 3 are presented chronologically as in this timeline, the mouse cohorts were counterbalanced for treatment order – with half receiving cocaine on day 1, and half receiving saline.

2.3. Surgeries and microinjection of viral vectors

Mice were anesthetized with a mixture of 100 mg/kg ketamine and 15 mg/kg xylazine (Sigma–Aldrich). Using aseptic surgical procedures, the mice were then fixed into a stereotaxic frame (Stoelting Co., IL.) and a small skin incision was made over the skull. The skull was made level, and the location of the bregma landmark was recorded.

The stereotaxic injection setup consisted of a Hamilton syringe and tubing system, primed with water and connected to a 33 gauge injector cannula (Plastics One, Roanoke VA). A volume of 2–4 μL of virus was injected per mouse, at a rate of 0.1–0.25 μL/min using a syringe pump.

For all behavioral studies, the AAV was injected bilaterally. Microinjections of viral vectors were carried out for three different brain regions: 1) the rostrolateral striatum/lateral caudate-putamen (lCPu), 2) the dorsal striatum/dorsal caudate-putamen (dCPu), and 3) the medial accumbens (mNAc). The coordinates targeted for each of the regions are listed in Table 1.

After infusion, the injector was left in place for 2 min, and then raised. For the lCPu region, three boli were infused along the injector's path during withdrawal, such that both ventral and dorsal striatum were targeted. The mice were sutured after the surgery and administered post-operative care for one week. Expression of the recombinant vector genome was allowed during a four week recovery. AAV does not replicate and the infected region does not spread over time. AAV-mediated expression increases gradually and then stabilizes. The four week recovery period is sufficient to reach high and stable levels of expression.

2.4. Drugs administered

All drugs administered to the mice were dissolved in a vehicle of 0.9% saline at a concentration such that 10 μL/g body weight would deliver the desired dose. Cocaine HCl was provided by the NIDA drug supply program, and administered at 10 and 20 mg/kg doses intraperitoneally (i.p.). The DAT selective inhibitor, 2β-carbophenoxy-3β-(4-chlorophenyl) tropane (Research Triangle Institute, North Carolina) was administered at 5 mg/kg i.p. This drug is hereafter referred to as RTI-113.

Ketamine and xylazine were administered for anesthesia during the stereotaxic surgeries preceding the behavioral experiments, at doses of 100 mg/kg and 15 mg/kg respectively (i.p.).

2.5. Conditioned place-preference and locomotion test

A conditioned place-preference (CPP) apparatus was used to measure cocaine-induced reward and hyperlocomotion simultaneously. The apparatus was a 12.5 cm × 42.5 cm acrylic box subdivided into the following three interconnected compartments: two side compartments (12.5 cm × 17.5 cm) and a center compartment (12.5 cm × 7.5 cm). The CPP procedure, outlined in Fig. 1, consists of a preconditioning test (D0), a cocaine/saline conditioning phase (D1–D8), and a postconditioning test (D9).

Mice were habituated to handling for three days, prior to the pre-conditioning test. On the pre-conditioning test day (D0), the three compartments were made distinct from one another by 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. Time spent in each of the three compartments, as well as the total distance traveled, were automatically recorded by the AnyMaze video tracking system (Stoelting Co.) Their preference was defined as the difference in time spent in one side compartment versus the other side. Their unconditioned (pre-existing) preference was counterbalanced in each group by designating

Table 1
Coordinates used for stereotaxic injection of AAV.

Brain region	Axis: Adjustment relative to bregma (in mm)			Approach angle
	Anterior/ posterior	Medial/ lateral	Dorsal/ ventral	
lCPu	+1.5	±1.2	−4.6	15° lateral
dCPu	0.0	±2.2	−3.3	0
mNAc	+1.5	±0.5	−4.8	18° cross midline

The stereotaxic coordinates targeted are listed in the table, in mm relative to bregma. In cases where an angle was used, trigonometric adjustments were made to the manipulanda displacements, in order to ensure that the targeted region occurred at the listed location.