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Dopamine transporter inhibition is required for cocaine-induced stereotypy

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Abstract

The primary mechanism by which cocaine induces stereotypy has been difficult to discern because cocaine has three high affinity targets, the reuptake transporters for dopamine (DAT), norepinephrine, and serotonin. To dissect out the role of DAT in cocaine effects, we generated a knock-in mouse line with a cocaine insensitive DAT (DAT-CI mice). DAT-CI mice provide a powerful tool that can directly test whether DAT inhibition is important for cocaine-induced stereotypy. We found that acute cocaine failed to produce stereotypy in DAT-CI mice. In fact, 40 mg/kg cocaine suppressed stereotypy in DAT-CI mice but produced profound stereotypy in wild-type mice. These findings suggest that DAT inhibition is necessary for cocaine-induced stereotypy. Furthermore, mechanisms independent of DAT inhibition appear to inhibit stereotypy.

Keywords

Cocaine; dopamine transporter; knock-in mice; stereotypy

Introduction

Stereotypy is characterized by repeated, purposeless movements. Stereotypy can be produced by high doses of psychostimulants, such as cocaine and it is considered a direct measure of cocaine's psychoactive properties [1] [2]. The primary mechanism by which cocaine produces stereotypy is unclear because cocaine has three high affinity targets in the central nervous system, the reuptake transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT) [3][4]; and cocaine inhibits these transporters with similar affinities [4–6].

There is considerable evidence showing that elevated dopaminergic neurotransmission is very important for stereotypy. For example, restoration of dopamine to dopamine deficient mice induces stereotyped movements and dopamine receptor 1 agonists induce stereotypy in these mice, while antagonists reduce stereotypy [7–9]. The ability of amphetamine to produce stereotypy is attenuated in rats treated with the dopaminergic selective neurotoxin 6-OHDA [10]. The selective dopamine transporter inhibitor GBR 12909 produces stereotypy in rats [11]. However, studies have suggested that norepinephrine and serotonin systems also play a role in cocaine-induced stereotypy. Mice lacking norepinephrine due to a knock-out of the beta-hydroxylase gene are more sensitive to the stimulating effects of amphetamine [12]. Serotonergic agents produce a more complex picture with some studies

reporting serotonin signaling reducing stereotyped movements and others reporting a positive effect [13–17].

In this study, we used a knock-in mouse line that expresses a cocaine-insensitive dopamine transporter (DAT-CI mice). Importantly, in DAT-CI mice cocaine still inhibits NET and SERT. We have previously found that cocaine does not elevate extracellular dopamine in DAT-CI mice, it suppresses locomotor activity and does not produce conditioned place preference [18]. Here we investigate the importance of DAT inhibition for cocaine-induced stereotypy, using DAT-CI mice which have a cocaine resistant dopamine transporter.

Methods

Animals

Mice were group housed with 2 to 4 animals per cage, in accordance with the University Lab Animal Resources at The Ohio State University. They were provided food and water *ad libitum*. The animals were kept on a 12-hour day/night cycle with lights on at 6 a.m. and off at 6 p.m. All experiments were performed between 10 a.m. and 2 p.m. The DAT-CI mice were on a mixed C57Bl-6/SV129J background that had been backcrossed to C57BL/6 for more than 10 generations.

Drugs

Cocaine HCl was kindly provided by NIDA's drug supply program. Animals were injected with either 0.9% saline or cocaine (5 mg/kg or 40 mg/kg, i.p.) dissolved in 0.9% saline.

Behavior Experiments

All experiments were performed using the Versamax system (Columbus, OH). This system has two layers of infrared light beams traversing the animal cage from front to back and from left to right. A test animal in the cage will break some of the beams revealing its location and movements. A software analyzes the movement and provides scores for locomotor activity, repeated movements, rearing, circling, etc. Animals were habituated to the chambers for 30 minutes prior to testing. Animals were then injected with drug or saline and behavior was monitored for 30 minutes. Mice with saline or cocaine injection were carefully observed after they were placed into the behavior test chambers. We rarely observed chewing or gnawing stereotypies, thus these behaviors were not scored and analyzed.

Statistics

SPSS was used for statistical calculations. We used two-way analysis of variance (ANOVA) to ascertain if there was difference in the baseline behaviors in wild-type mice versus DAT-CI mice. One-way ANOVA was used to look for differences between saline and drug treatment within genotypes. Normal distributions are assumed.

Results

We have previously studied cocaine's effect on locomotor activity in DAT-CI mice using a system with video based tracking and shown that cocaine reduces locomotor activity in DAT-CI mice [18]. We measured the effects of cocaine on locomotor activity and horizontal activity in wild type and DAT-CI mice using the Versamax system. The results of these tests were similar to previous results, with cocaine reducing locomotor and horizontal activity in DAT-CI mice (data not shown).

Stereotypy counts are the number of times the mouse breaks the same beam in succession without breaking an adjacent beam. In figure 1A the effects of increasing doses of cocaine on stereotypy counts is shown. Two-way ANOVA found a main effect of drug X genotype interaction (see details in figure legends). One-way ANOVA was used to assess the effects of cocaine on stereotyped activities within each genotype. A dose of 5 mg/kg cocaine did not have a significant effect on the stereotypy counts of either genotype. However, 40 mg/kg greatly increased the stereotypy count of wild-type mice and decreased the stereotypy count of DAT-CI mice in comparison to saline injected animals. There was also a large difference in the stereotypy counts of saline injected animals, with DAT-CI mice showing significantly higher counts of stereotyped movements than wild type. Another measure is stereotypy number, which is the number of sessions the mouse performs a series of stereotyped movements. Cocaine did not significantly affect the stereotypy number and the drug X genotype interaction was also not significant, as seen in Figure 1B. In addition there was no difference between saline treated animals of each genotype.

High doses of psychostimulants can induce circling behaviors in wild-type mice. Figure 2A shows the effect of cocaine on circling. Two-way ANOVA revealed a main effect for the drug X genotype interaction. A dose of 40 mg/kg greatly enhanced circling behavior in wild-type animals, when compared to saline treated animals, but cocaine did not significantly affect the circling behavior of DAT-CI mice.

Psychostimulants can also induce rearing behaviors in animals. Figure 2B shows the effects of cocaine on the rearing activities of wild-type and DAT-CI mice. Two-way ANOVA revealed a drug X genotype interaction as the main effect. Unlike the behavioral measures above, the cocaine effect on rearing activity is not statistically significant in the wild-type animals. It is possible that increasing the number of mice tested may result in a significant but slight increase. In DAT-CI mice, 5 mg/kg cocaine also did not affect rearing activity; however, 40 mg/kg cocaine reduced rearing activity when compared to saline treated animals.

Discussion

In this study, we compared cocaine-induced stereotypy, rearing, and circling behaviors in wild-type and DAT-CI mice. We have found that in DAT-CI mice with a cocaine-resistant DAT, cocaine reduces stereotypy counts, which is opposite to its effect in wild-type mice. Interestingly, cocaine does not change rearing activity in wild-type mice in our test conditions, but it decreases rearing in DAT-CI mice. Furthermore, cocaine increases circling in wild-type mice, but has no effect on circling in DAT-CI mice. These results indicate the molecular basis for these effects are not identical.

We have generated a mouse line with a cocaine resistant dopamine transporter, DAT-CI mice. Previously we have reported that cocaine does not produce CPP, and it suppresses locomotor activity in these mice. To further understand the role of DAT-inhibition in cocaine's effects we tested the ability of cocaine to produce stereotypy in these mice. Stereotypy is considered a direct measure of cocaine's psychoactive properties. Cocaine-induced stereotypy and locomotor stimulation may share neural pathways [19]. In DAT-CI mice, the mutated DAT has reduced DA uptake which is not expected to fundamentally change the response of DAT-CI mice to cocaine because DAT knockdown mice with only 10% DA uptake respond normally to cocaine [20].

We found that a high dose of cocaine increased stereotypy in wild-type mice and decreased stereotypy in DAT-CI mice, highlighting the important role of the dopaminergic system in cocaine-induced stereotypy. An analysis of stereotypy counts, which is how many times

Versamax scored a movement as stereotypy, is shown in Figure 1A. DAT-CI mice had higher control stereotypy count scores (saline injection) presumably due to the higher basal extracellular DA levels in DAT-CI mice. A dose of 5 mg/kg cocaine did not affect the stereotypy count of either genotype. However, 40 mg/kg cocaine had opposite effects on the two genotypes, a large increase in stereotypy count scores in wild-type mice and a significant decrease in DAT-CI mice. Thus, cocaine's effect on one or more of its other targets, for example SERT and/or NET, reduces stereotypy.

Cocaine did not change stereotypy number, the number of sessions of stereotyped behavior as shown in Figure 1B shows. In addition, there was no difference between genotypes. These data indicate that a change in dopaminergic neurotransmission does not play a key role in the initiation of a session of stereotypy (as defined by the Versamax system) however, it does greatly increase the number of times stereotypy is performed once a session is started. High doses of potent psychostimulants can reduce locomotor activity by increasing the amount of time the animals spend doing other types of movement, such as sniffing, circling or other forms of stereotypy [2]. Therefore, it is possible that the decreased locomotor activity observed in DAT-CI mice in response to cocaine was due to increased stereotypy. However, cocaine markedly decreased both locomotor activity and stereotypy in DAT-CI mice, which suggest that the cocaine-induced locomotion suppression in DAT-CI mice is not due to increased stereotypy.

Circling is commonly observed behavior in mice in response to psychostimulants and it is believed to be dependent upon increased dopaminergic signaling in the striatum [21], [22]. As shown in Figure 2A, 40 mg/kg cocaine greatly increased circling behaviors in wild-type mice but had very little effect on the circling behavior in DAT-CI mice. This is in contrast to cocaine-induced decreases in other activities in DAT-CI mice. However, it is possible the lack of effect on DAT-CI mice was due to a floor effect since the circling activity was low under the control condition. Figure 2B shows the effect of 5 mg/kg and 40 mg/kg cocaine on the rearing activity of wild type and DAT-CI mice. The 40 mg/kg dose of cocaine did not seem to affect the rearing activity of wild-type mice. However, the rearing activity of DAT-CI was greatly reduced by 40 mg/kg cocaine.

The results suggest that the three behaviors do not respond to cocaine-induced neurochemical changes in the same way. When comparing wild type with DAT-CI mice the cocaine effects might be conceptually divided into two opposing components: the stimulating effect mediated by DAT inhibition and the suppressing effect of cocaine actions on targets other than DAT. For stereotypy, the stimulating effect is dominant in wild type mice and the suppressing effect only becomes apparent in DAT-CI mice when DAT is not blocked by cocaine (Fig. 1A). For circling behaviors, the stimulating effect is very robust (wild type mice) while the suppressing effect is not significant (DAT-CI mice, Fig. 2A). The situation for rearing activity is different. The stimulating and suppressing effects of cocaine seem to be in balance in wild type mice and the suppressing effect leads to decreased activity in the absence of the opposing effect in DAT-CI mice (Fig. 2B). It is important to note that these behaviors can be affected by both drug treatment and experimental conditions. This may complicate the comparison of this study with other studies using different apparatus and experimental setups. We used exactly the same condition when studying the two genotypes of mice.

A sedative is a compound that reduces anxiety or has a calming effect. In DAT-CI, cocaine does not act as a stimulant. In fact, it reduces activity by many measures, including locomotor activity, stereotypy and rearing. Thus in the absence of DAT-inhibition, cocaine appears to have sedative-like qualities, as opposed to it being a strong stimulant as it is for wild-type animals. The molecular mechanism for this is not known, though it is likely to be

a result of cocaine's ability to inhibit NET and SERT. It is known that selective NET inhibitor atomoxetine can reduce hyperactivity and increase the ability to focus of children with attention deficit hyperactive disorder [23]. In addition tricyclic antidepressant drugs are sometimes sedatives, presumably due to their impact on noradrenergic and/or serotonergic systems [24].

Conclusion

Cocaine does not induce stereotypic behaviors in mice with a cocaine resistant DAT, which suggest that cocaine must inhibit DAT in order to produce stereotypy. In addition, cocaine reduces locomotor activity and stereotypy in DAT-CI mice, which is likely due to the inhibition of NET and/or SERT, and the subsequent increase in signaling of their respective neurotransmitters.

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Figure 1.

The effects of cocaine on the stereotypy of wild-type and DAT-CI mice. (A) Effect of 5 mg/kg and 40 mg/kg cocaine, compared to saline, on stereotypy counts in 30 minutes, defined as the number of movements the mouse performed that was scored as stereotypy. Two-way ANOVA revealed the main effect to be a drug X genotype interaction ($F_{2, 38} = 31.017$, $p < 0.001$). One-way ANOVA was used to compare drug treatments to saline treatments within genotypes ($F_{2, 18} = 27.879$, $p < 0.001$ and $F_{2, 18} = 6.474$, $p < 0.01$ for wild-type and DAT-CI mice, respectively.) (B) Effect of 5 mg/kg and 40 mg/kg cocaine, compared to saline, on the number of sessions of a series of stereotypic movements in 30 minutes. Two-way ANOVA revealed no effect for drug ($F_{1, 38} = 1.383$, $p > 0.05$) and the drug X genotype interaction was also not significant ($F_{2, 38} = 0.947$, $p > 0.05$). In addition there was no significant difference between saline treated mice of each genotype (T-test, $n = 12$, $p > 0.05$).

Figure 2.

The effects of cocaine on circling and rearing movements of wild-type and DAT-CI mice. (A) Effect of 5 mg/kg and 40 mg/kg, compared to saline, on the revolution numbers of circling movements in 30 minutes. Two-way ANOVA revealed the main effect to be a drug X genotype interaction ($F_{2, 38} = 16.41$, $p < 0.001$). One-way ANOVA was used to compare drug treatments to saline treatments within genotypes ($F_{2, 18} = 28.04$, $p < 0.001$ and $F_{2, 18} = 1.92$, $p > 0.05$ for wild-type and DAT-CI mice, respectively). (B) Effects of cocaine on the rearing activity in 30 minutes. Two-way ANOVA revealed the main effect to be a drug X genotype interaction ($F_{2, 38} = 8.65$, $p < 0.01$). One-way ANOVA was used to compare drug treatments to saline treatments within genotypes ($F_{2, 18} = 1.736$, $p > 0.05$ and $F_{2, 18} = 11.306$, $p < 0.01$ for wild-type and DAT-CI mice, respectively). Bonferonni post-hoc analysis (** = $p < 0.01$).