Published in final edited form as:

Neuroreport. 2009 January 7; 20(1): 9–12. doi:10.1097/WNR.0b013e32831b9ce4.

Cocaine does not produce reward in absence of dopamine transporter inhibition

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Abstract

Previously we reported that knock-in mice with a cocaine-insensitive dopamine transporter (DAT-CI mice) do not experience cocaine reward, as measured by conditioned place-preference (CPP). This conclusion has come under scrutiny because some genetically modified mice show cocaine-induced CPP in a narrow dose range, i.e. responding at doses around 10 mg/kg, but not at 5 and 20 mg/kg, the doses we tested in DAT-CI mice. These results raise the possibility that we have missed the optimal dose for cocaine response. Here we report that cocaine does not produce reward in DAT-CI mice at low, moderate, and high doses, including 10 mg/kg. This study strengthens our conclusion that DAT inhibition is required for cocaine reward in mice with a functional dopaminergic system.

Keywords

Cocaine; dopamine transporter; knock-in mice; conditioned place preference

Introduction

Cocaine is a powerful psychostimulant with euphoric and stimulating properties that are thought to be a consequence of its ability to inhibit the reuptake transporters for the neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-HT) - DAT, NET and SERT, respectively. Of these transporters, DAT has often been viewed as the critical target for cocaine's rewarding and stimulating effects [1] [2] [3]. This view was challenged when it was shown that mice lacking DAT (DAT-KO mice) still self-administer cocaine [4] and still exhibit conditioned place-preference (CPP) in response to cocaine [5]. In fact, removal of any one of the three monoamine reuptake transporters does not eliminate cocaine's rewarding effects [5] [6] [7]. The DAT and SERT double knock-out mouse line does not show cocaine-induced CPP, raising the possibility that none of the targets are

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critical and that cocaine reward may be mediated by redundant pathways [8]. However, knockout mice may have profound adaptive changes to compensate for the total lack of an important gene product during development [9].

Previously we have reported the generation of a knock-in mouse line whose DAT has been engineered to be 80-fold less sensitive to cocaine inhibition (DAT-CI mice) [10]. In these mice, cocaine does not produce conditioned place-preference at doses of 5 mg/kg and 20 mg/kg, which are doses that produced robust CPP in wild-type mice [10]. These results argued strongly that inhibition of DAT was critical for cocaine's rewarding effects in mice with a functional dopaminergic system.

However, some studies indicate that in certain genetically modified mouse lines, there is a very narrow range of doses of cocaine that produce reward. In congenic female mice lacking DAT only the moderate dose of 10 mg/kg cocaine produces CPP, while the low and high doses of 5 mg/kg and 20 mg/kg do not [11]. In addition, a recent study has shown a similar results in a dopamine deficient (DD) mouse model in which tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis, is inactivated in dopamine neurons but not other catecholaminergic neurons [12]. The mice were given a diet supplemented with a dopamine precursor to allow dopamine production during development. The precursor was withheld during experiments that tested the response of the mice to cocaine. It was found that 5 mg/kg and 20 mg/kg cocaine did not produce CPP in these mice, while doses of 7.5 mg/kg, 10 mg/kg and 15 mg/kg cocaine did, with the maximum score at 10 mg/kg cocaine [12].

The dose-response curve of cocaine-induced CPP is shaped like an inverted "U" and altering the dopaminergic system seems to narrow the range of cocaine doses that produce CPP. Thus it is possible that instead of eliminating cocaine's rewarding effects, the removal of cocaine's ability to inhibit the dopamine transporter in our DAT-CI mice narrows the range of cocaine doses that produce reward and we might have missed the optimal dose by testing only the relatively low and high doses. In this study we investigate the ability of cocaine to induce CPP over a range of doses, including the moderate dose of 10 mg/kg.

Methods

Animals

The knock-in mice were generated by gene targeting in SV129 ES cells [10] and backcrossed to C57BL/6J for at least 10 generations. The homozygous DAT-CI mice and wild-type control mice were drug naïve male littermates and were treated in accordance with The Ohio State University's ILACUC. They were group housed and kept on a 12-hour day and 12-hour night cycle with the lights on at 6 am and off at 6 pm and provided food and water *ad libitum*. CPP was performed during the daylight hours between 8 a.m. and 12 p.m.

Drugs

Cocaine was kindly provided by NIDA drug supply program. Cocaine was dissolved in 0.9% sterile saline and injected interperitoneally.

Conditioned Place Preference

Conditioned place preference was performed as previously described [10]. CPP was performed using unbiased chambers and a video monitoring system (Limelight, Coulbourn Instruments). CPP was performed in acrylic boxes 12.5 X 25 cm with three chambers per box. The side chambers had different sensory cues while the middle chamber was neutral. One side had a thick-texture floor mat and a grid pattern on the walls while the other side had a fine-textured floor mat and a wavy wall pattern. Briefly, on day one animals were placed in the box for 30 minutes and given free access to all three chambers. Their time spent in each chamber was recorded. Based on the results of the pretest, the drug-paired chambers (mixed of both cues) were assigned in a way that the saline and cocaine groups were both counterbalanced and unbiased toward the environmental cues at pre-conditioning.

The mice were conditioned for 30 minutes each day by confining them to the appropriate chamber and cue-set immediately after cocaine or saline injection. Mice in the test group received saline on days 2, 4, 6, and 8 while exposed to one chamber and received cocaine on the alternate days while being confined to the opposite chamber. Mice in the control group received saline on all conditioning days in both chambers. On day 10, the mice were placed in the box and given full access to all of the chambers. The time they spent in each chamber was recorded. CPP was scored by subtracting the time spent in the drug-paired chamber post-conditioning from the time spent in the chamber preconditioning.

Statistics

Statistics were performed using SPSS. Two-way ANOVA was used to assess overall effects of the drug on the mice and to look for drug X genotype interactions. One-way ANOVA was used to determine if there was a significant difference within each genotype. Post hoc Bonferroni tests were performed after one-way ANOVA to look for differences between saline and drug within each genotype.

Results

Figure 1 shows the results of CPP for both wild-type and DAT-CI mice over a range of doses, including 10 mg/kg cocaine. Two-way ANOVA revealed significant effects for genotype ($F_{1,60} = 40.496$, $p < 0.001$), drug dose ($F_{3,60} = 11.047$, $p < 0.001$), and genotype X drug dose interaction ($F_{3,60} = 2.996$, $p < 0.05$). One-way ANOVA was performed to analyze the within genotype effects of cocaine. Cocaine produced robust CPP in wild-type animals ($F_{3,27} = 13.98$, $p < 0.001$), and post hoc Bonferroni test revealed significant increases in CPP at doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg ($p < 0.001$, for each). In contrast, one-way ANOVA revealed that there were no significant differences between the CPP scores of the saline group and the mouse groups receiving each of the cocaine doses, including the 10 mg/kg dose in DAT-CI animals ($F_{3,32} = 2.002$, $p > 0.05$). Post-hoc Scheffé's tests have also been performed to analyze within-group effects and the results are in agreement with those from post-hoc Bonferroni tests.

Discussion

Here we show that doses of 5 mg/kg, 10 mg/kg and 20 mg/kg cocaine produce strong CPP in wild-type mice. In contrast, none of these doses produce CPP in DAT-CI mice. These doses of cocaine do not inhibit the cocaine-resistant DAT mutant.

Previously, we tested only the 5 mg/kg and 20 mg/kg cocaine doses for their ability to produce CPP [10]. We thought that by testing a low and a high dose of cocaine we would capture the effects of cocaine over the normal range of doses. However, several studies have shown that only a narrow range of cocaine doses are able to consistently produce CPP in some mouse lines with genetic alterations in the dopaminergic system [11,12], and this range centered on the 10 mg/kg dose. Since other reports indicate that 10 mg/kg may be a “sweet spot” for cocaine’s rewarding effects, particularly in some transgenic animals, the tests were performed again and included the 10 mg/kg dose.

As in our previous study, none of the doses of cocaine produced CPP in DAT-CI mice while all the doses produced significant CPP in wild type mice (Fig. 1). Specifically, 10 mg/kg cocaine did not produce significant CPP in DAT-CI mice. Therefore, low, moderate and high doses of cocaine do not produce reward in mice bearing a cocaine-resistant DAT – DAT-CI mice.

It should be noted that the mutant DAT in DAT-CI mice have reduced DA uptake activity, resulting in higher basal dopaminergic tone [10]. It is possible that the lack of cocaine reward in DAT-CI mice is a result of adaptive changes. We think this is unlikely due to the following reasons: 1) Heterozygous DAT-KO mice with 50% DAT expression level and DAT knockdown mice with 10% DAT expression level both retain the rewarding effects of cocaine despite a markedly reduced DA uptake activity and elevated basal dopaminergic tone [4] [5] [13] [14] [15] [16]; 2) DAT-KO mice with highly elevated DA tone and severe adaptive changes still retain the rewarding effect of cocaine [4] [5]; 3) Amphetamine produces CPP in DAT-CI mice, suggesting that the reward pathway in DAT-CI mice is functional and the mice are capable of sensing the rewarding effects of the psychostimulants [10].

Our results were potentially in conflict with those found in DAT-KO mice. The fact that cocaine still produces reward in the mice that do not have DAT clearly indicates that DAT inhibition is not absolutely required for cocaine’s rewarding effects. Since DAT-CI mice carry a DAT mutant with relatively normal DA uptake function while DAT-KO mice have no DA uptake and more severe adaptive changes, we believe that DAT inhibition is critical for cocaine reward in normal mice but not required under certain circumstances, such as after adaptive changes during development in the total absence of DAT expression.

In addition it was found that the selective SERT or NET inhibitors also elevated extracellular DA levels and produced reward in DAT-KO mice but not in wild-type mice [13] [7] [14], suggesting alterations in the reward pathway. Intriguingly, inhibition of SERT with fluoxetine also produces CPP in the DD mice with deficient dopamine synthesis but not in the wild type control mice [12]. These studies suggest that cocaine can produce reward by

inhibiting SERT or NET under certain conditions such as when the dopaminergic system is damaged or severely altered.

Conclusion

Cocaine does not produce CPP in DAT-CI at all tested doses, including 10 mg/kg. This result supports the hypothesis that DAT inhibition is required for cocaine's rewarding effects in normal mice with a functional dopaminergic system. However, cocaine can produce reward through SERT, NET and/or other possible targets of cocaine under certain circumstances, such as in DAT knockout mice or possibly in animals with a severely altered dopaminergic system.

Acknowledgments

We would like to thank Pauline Chen for her assistance with this study and the NIDA Drug Supply Program for drugs used in this study. This research was funded by NIH grant DA 014610.

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Figure 1.

Cocaine-induced conditioned place preferences (CPP) in wild-type and DAT-CI mice. The scores of CPP are presented as the postconditioning time (in seconds) minus preconditioning time in the paired chamber in a 30 min session (mean $\pm$ standard error). For each drug dose and saline control groups, 8 – 12 mice were examined. A range of cocaine doses were tested, including 5, 10, and 20 mg/kg. Two-way ANOVA revealed significant effects for genotype, drug dose, and genotype X drug dose interaction. One-way ANOVA within each genotype revealed that cocaine produced robust CPP in wild-type animals ($F_{3, 27} = 13.98$, $p < 0.001$), and post hoc Bonferroni test revealed significant increases in CPP at doses of 5 mg/kg, 10 mg/kg, and 20 mg/kg ($p < 0.001$, for each). However, no significant differences were found in CPP scores among groups of the DAT-CI animals treated with saline or different doses of cocaine.