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Effect of the Dopaminergic Neurotoxin MPTP on Cocaine-Induced Locomotor Sensitization

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ITZHAK, Y., J. L. MARTIN, M. D. BLACK AND S. F. ALI. *Effect of the dopaminergic neurotoxin MPTP on cocaine-induced locomotor sensitization.* PHARMACOL BIOCHEM BEHAV **63**(1) 101–107, 1999.—The blockade of dopamine (DA) uptake via the dopamine transporter (DAT) in the nucleus accumbens (NAC) and striatum by cocaine has a major role in the reinforcing and psychomotor stimulating effects of the drug. Here we investigated the effect of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the expression and induction of sensitization to the locomotor stimulating effect of cocaine. MPTP (20 mg/kg × 4) caused 72 and 76% depletion of DAT sites in the NAC and striatum, respectively, in C57BL/6 mice. The magnitude of this depletion 3 and 19 days after MPTP administration was the same. To determine the effect of MPTP on the expression of the sensitized response to cocaine, cocaine-experienced mice (20 mg/kg for 5 days) received MPTP 3 days before a challenge cocaine injection was given on day 15. Cocaine/MPTP mice were significantly more sensitive to the challenge cocaine injection than the cocaine/saline-pretreated mice. To determine whether depletion of NAC and striatal DAT affects the induction of sensitization to cocaine, mice were pretreated with MPTP 3 days before the administration of cocaine (20 mg/kg for 5 days). The magnitude of the sensitized response of MPTP/cocaine-pretreated mice to cocaine challenge was the same as the sensitized response of mice treated with saline/cocaine, while the number of DAT binding sites in the MPTP/cocaine group was significantly lower than the saline/cocaine group. The present study indicates that MPTP exacerbates the expression of locomotor sensitization to cocaine, but it had no effect on the induction of sensitization. We conclude that the expression, but not the induction, of locomotor sensitization to cocaine may be dependent on the level of DAT binding sites. © 1999 Elsevier Science Inc.

Cocaine 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Sensitization Locomotoractivity
Dopamine transporter Nucleus accumbens Striatum

EVIDENCE suggests that the reinforcing and psychomotor stimulating effects of cocaine are due primarily to the inhibition of the dopamine transporter (DAT) in the nucleus accumbens (NAC) and striatum (22,23,33,42). As a result, a marked increase in presynaptic dopamine (DA) transmission occurs. Furthermore, mutant mice lacking the synaptic DAT gene are insensitive to the psychomotor stimulating effect of cocaine and amphetamine (11). Repeated exposure to cocaine and amphetamines, causes the development of "reverse-tolerance" known as sensitization. In animal models, behavioral sensitization to psychostimulants is manifested by a progressive increase in locomotor activity after repeated administration of the drug. This phenomenon is considered relevant to the psychopathology, drug addiction, and craving that develop in humans abusing psychostimulants (27,29,35). Enhanced dopamine transmission in the NAC and striatum is thought to underlie the behavioral sensitization to cocaine (20). Results from studies on the regulation of the DAT following repeated exposure to cocaine are somewhat inconsistent. Some have reported no change in the number of striatal DAT binding sites (1,2,18,32), while others reported a decrease in DAT in the NAC, prefrontal cortex, and dorsal stri-

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atum (6,28,41) . In contrast, postmortem studies on human cocaine-related death (37) and single photon emission computed tomography (SPECT) studies on abstinent cocaine-abusing subjects (25) revealed an increase in striatal DAT binding sites.

Recently, we investigated how the depletion in striatal DAT binding sites affects cocaine and methamphetamine (METH)-induced locomotor activity in Swiss Webster mice. We reported that exposure to a neurotoxic dose of METH, which caused >60% depletion of striatal DAT binding sites, resulted in a marked locomotor sensitization in response to a challenge METH or cocaine injection (17). However, >60% depletion of striatal DAT induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) had no effect on cocaine- and METH-induced locomotor activity (e.g., acute response) compared with the response of naive animals to these psychostimulants (17).

The dopaminergic neurotoxicity produced by MPTP is thought to be initiated after the conversion of MPTP to 1-methyl-4-phenylpyridinium (MPP+) by monoamine oxidase B. MPP+ is taken up by the DAT and then accumulated in the mitochondria of dopaminergic neurons (14,24). The damage caused to nigrostriatal dopaminergic neurons by MPP+ is similar to that detected in Parkinson's disease [(7), for review]. The aim of the present study was twofold. First, to investigate whether the degree of MPTP-induced dopaminergic neurotoxicity in the NAC and striatum is similar; second, to determine how depletion of the DAT in NAC and striatum affects the induction and expression of sensitization to the locomotor stimulating effect of cocaine in C57BL/6 mice (this mouse strain is particularly sensitive to MPTP-induced dopaminergic neurotoxicity). We report that >70% depletion of NAC and striatal DAT binding sites by MPTP had no effect on the induction of sensitization to cocaine, but it produced an augmentation in the expression of the sensitized response to cocaine.

METHOD

Drugs and Chemicals

MPTP-HCl was purchased from Research Biochemical International (Natick, MA), and cocaine-HCl, was purchased from Sigma (St. Louis, MO). All drugs were dissolved in saline (0.9% NaCl). [^{3}H]Mazindol (24.0 Ci/mmol) was purchased from New England Nuclear (Wilmington, DE).

Animals and Schedule of Drug Administration

Male C57BL/6 mice (8–10 weeks old; Jackson Laboratories, Bar Harbor, ME) were maintained on a 12-h light/dark schedule and housed in groups of five with free access to food and water. The principals of laboratory animal care (NIH publication No. 85-23, revised, 1985) were followed. Drug solutions were administered by intraperitoneal (IP) injection in a volume of 0.1 ml per 10 g of body weight.

Experiment 1. Effect of MPTP on striatal and NAC dopaminergic markers. In the first experiment the dopaminergic neurotoxicity caused by the administration of four injections of 10 or 20 mg/kg MPTP was investigated. Mice (n = 10 per group) received either four injections of saline, 10 mg/kg MPTP or 20 mg/kg MPTP in 2-h intervals. After 72 h animals were sacrificed and the striatum and NAC were dissected and frozen at −80°C. Tissue from one hemisphere was used for determination of the levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the tissue from the other hemisphere was used for labeling of the DAT by [^{3}H]mazindol. Because the high dose of MPTP (20 mg/kg × 4) resulted in a marked depletion of DAT (in both NAC and striatum), all subsequent experiments were conducted with the high dose of MPTP.

Experiment 2. Effect of MPTP on the expression of sensitization to the locomotor stimulating effect of cocaine. One group of mice (n = 10) received saline from day 1 through 5. A second group (n = 20) received cocaine (20 mg/kg) from day 1 through 5. Cocaine-induced locomotor activity was recorded on day 1 to determine the acute effect of the drug, and later on day 15 after a 10-day drug-free period to determine the sensitized response to a challenge cocaine injection. Based on our previous studies, cocaine-induced sensitization is manifested on the fifth day of cocaine administration, but after a 10-day drug-free period the sensitized response to cocaine was even greater (15). Because MPTP exerts its optimal neurotoxic effect 72 h after its administration, mice were treated with MPTP 72 h before day 15, which we chose as the test day for sensitization. Accordingly, on day 12, one group of the cocaine-experienced mice (n = 10) received MPTP (20 mg/kg × 4), and the second group of mice (n = 10) received saline. On day 15, the mice were challenged with cocaine (20 mg/kg) and locomotor activity was recorded. The three groups of mice, saline/control, cocaine/saline, and cocaine/MPTP (which correspond to groups a, b, and c, respectively, in Fig. 4) were sacrificed on day 16, and the striata were processed to determine the number of DAT binding sites using [^{3}H]mazindol.

Experiment 3. Effect of MPTP on the induction of sensitization to the locomotor stimulating effect of cocaine. Mice (n = 20) received four injections of MPTP (20 mg/kg) in 2-h intervals 72 h before the administration of cocaine began. In Figs. 3 and 4 the time point of MPTP administration is designated as "day −3" (e.g., 3 days before animals began receiving cocaine). Then, from days 1 through 5, one group of mice (n = 10) received daily cocaine injections (20 mg/kg), and the second MPTP-treated group (n = 10) received daily saline injections. In parallel, another group of saline-treated mice (n = 10) received cocaine injections (20 mg/kg) from days 1 through 5. Cocaine-induced locomotor activity was measured on day 1 (e.g., acute effect) and then again on day 15, after a 10-day drug-free period, to determine the sensitized response to cocaine. The control group received saline injections throughout the same time. The four groups of mice, saline/control, saline/cocaine, MPTP/cocaine, and MPTP/saline, (which correspond to groups a, b, d, and e, respectively, in Fig. 4) were sacrificed on day 19 from the starting date of MPTP treatment (which corresponds to day 16 from the starting date of cocaine administration). The striatum was processed to determine the number of DAT binding sites using [^{3}H]mazindol.

Measurement of Tissue Content of DA, DOPAC, and HVA

Concentrations of DA and its metabolites DOPAC and HVA were quantitated by a modified method of high-performance liquid chromatography (HPLC) combined with electrochemical detection as we described previously (16). Each striatum was weighed in a measured volume (20% w/v) of 0.2 N perchloric acid containing 100 ng/ml of the internal standard 3,4-dihydroxybenzylamine. The tissue was then disrupted by ultrasonication, centrifuged at 4°C (15,000 × g; 7 min), and 150 μl of the supernatant was removed and filtered through a 0.2-μm Nylon-66 microfilter (MF-1 centrifugal filter, Bioanalytical System, W. Lafayette, IN). Aliquots of 25 μl representing 2.5 mg of brain tissue were injected directly onto the

HPLC/EC system for separation of the analytes. The concentration of DA, DOPAC, and HVA were calculated using standard curves generated by determining in triplicate the ratio between three different known amounts of the amine or its metabolites and a constant amount of internal standard.

Binding of [^{3}H]Mazindol to the Dopamine Transporter

NAC or striatum from three to four mice was pooled together, homogenized in 20 vol of Tris-HCl buffer (50 mM; pH 7.7) containing 300 mM NaCl and 5 mM KCl, and centrifuged (40,000 × *g*; 15 min; 4°C). The pellet was resuspended in 6 vol of sucrose (0.32 M), and aliquots were stored at −80°C. For binding assays, the tissue was resuspended in 15 vol of the buffer (0.3–0.4 mg protein/ml). Saturation binding assays were carried out in a final volume of 0.5 ml containing various concentrations of [^{3}H]mazindol (1.0–20.0 nM) and desipramine (300 nM; to occlude binding to the norepinephrine transporter). Nonspecific binding was determined in the presence of 20 μM benztropine. Following a 60-min incubation at 4°C, the reaction was stopped by a rapid vacuum filtration (Brandel M-12) through Whatman GF/B filters. Radioactivity remaining on filters was determined by scintillation counting. The binding parameters of [^{3}H]mazindol, for example, the maximal number of binding sites (B_{max}) and the dissociation constant (K_d) were determined by the LIGAND program, version 2.3.10.

Measurement of Animals' Locomotor Activity

Routinely, mice locomotor activity was measured between 1000 and 1500 h. Initially, spontaneous locomotor activity was measured for a 60-min period, and then cocaine-induced locomotor activity was measured for a 30-min period. The following groups were investigated: (a) saline/control: this group consisted of 20 mice. One group (n = 10) from Experiment 2 received daily saline injection for 5 days, and locomotor activity was recorded on day 15. The second group (n = 10) from Experiment 3 received four saline injections in 1 day and then after 3 days daily saline injections for 5 days. Locomotor activity was recorded on day 18. Because the various schedules of saline injections had no effect on mice locomotor activity, the results were combined and represent n = 20 for the control group in Fig. 2. (b) MPTP group from Experiment 3. One group consisted mice (n = 10) that received MPTP 3 days before spontaneous locomotor activity was determined, and a second group (n = 10) that received MPTP 18 days before spontaneous locomotor activity was measured (marked as "MPTP day 3" and "MPTP day 18" in Fig. 2. (c) Cocaine group from Experiment 2 (n = 10) and Experiment 3 (n = 10). The spontaneous locomotor activity of the two groups on day 15 did not differ from each other, and the results were combined and represent n = 20 (marked as "cocaine day 15" in Fig. 2). (d) Cocaine group (n = 10) that received MPTP on day 12 from the time that cocaine administration commenced (Experiment 2; marked as "cocaine/MPTP day 15" in Fig. 2). (e) Cocaine group (n = 10) that received MPTP 3 days before cocaine administration commenced (Experiment 3; marked as "MPTP/cocaine day 18" in Fig. 2). Following the 60-min sessions of measuring spontaneous locomotor activity, selected groups received cocaine (20 mg/kg) injection and locomotor activity was recorded for a 30-min period. Animals' activity was monitored by activity meter, Opto-Varimex Mini (Columbus Instruments, Columbus, OH), which consists of an array of 15 infrared emitter/detector pairs, spaced at 2.65-cm intervals, measuring activity along a single axis of motion. Each emitter and detector were mounted along side the length of a standard transparent rectangular rodent cage (42 × 42 × 20 cm high). Ambulatory counts, which correspond to horizontal activity, were recorded and transferred by a computer interface to an IBM computer.

Statistical Analysis

Analyses of the effects of a particular drug (MPTP or cocaine) on dopaminergic markers or locomotor activity on a specific day were done by one-way ANOVA followed by post hoc Newman–Keuls test. The effects of cocaine on animals' locomotor activity across time (e.g., day 1 vs. day 15) were analyzed by a two-way ANOVA (drug treatment × time) with time as the repeated measure. Bonferroni multiple comparison adjustment was performed to determine differences between specific groups.

RESULTS

Experiment 1. Effect of MPTP on Striatal and NAC Dopaminergic Markers

The effects of two different dose regimens of MPTP on striatal and NAC dopaminergic markers are illustrated in Fig. 1. The low dose of MPTP (10 mg/kg × 4) resulted in a 22–25% decrease in DA and HVA levels and a 35% decrease in DAT binding sites in the striatum. Similarly, in the NAC, the low dose of MPTP resulted in a 25–45% decrease in the level of DA and its metabolites and a 32% decrease in DAT binding sites. However, the higher dose of MPTP (20 mg/kg × 4) produced greater neurotoxicity. In the striatum, a 90% depletion of DA and a 76% depletion of DAT were observed, and in the NAC, a 71% depletion of DA and 72% depletion in DAT sites were detected. The actual values of control DA, DOPAC, HVA, and DAT binding sites are given in the legend to Fig. 1. These results indicate that the magnitude of MPTP-induced neurotoxicity in the striatum and NAC was similar. For all subsequent experiments the high dose regimen of MPTP was used.

Experiment 2. Effect of MPTP on the Expression of Sensitization to the Locomotor Stimulating Effect of Cocaine

In this experiment we investigated whether MPTP-induced depletion of DAT binding sites affects the expression of sensitization to the locomotor stimulating effect of cocaine. Before the investigation of cocaine-induced locomotor activity, we sought to examine whether the various drug treatments caused any changes in animals' spontaneous locomotor activity. Results in Fig. 2 illustrate a 60-min period of spontaneous locomotor activity of the mice. Analysis of the data indicated that none of the drug treatments caused any changes in animals' spontaneous locomotor activity compared with the saline/control group. Thus, it appears that >70% deficit in dopaminergic markers caused by MPTP had no effect on mice spontaneous locomotor activity. Similarly, the spontaneous locomotor activity of cocaine-experienced mice that received either saline or MPTP was not different from saline/control (Fig. 2).

Although no significant changes in animals' spontaneous locomotor activities were observed between the various groups, cocaine-induced locomotor activities were different. As expected, mice treated with cocaine for 5 days developed a sensitized response to a challenge cocaine injection given on day 15 (Fig. 3). Mice treated with cocaine for 5 days, which subsequently received MPTP (20 mg/kg × 4) 72 h before they were challenged to cocaine, showed a greater sensitized response to

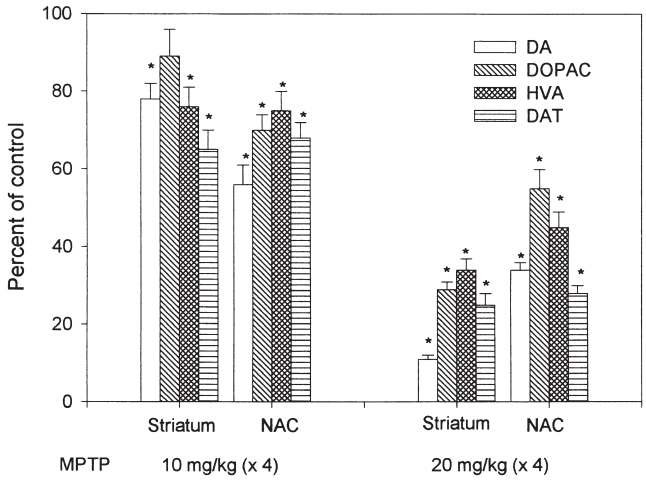

FIG. 1. Effect of MPTP on DA, DOPAC, HVA, and DAT binding sites in the striatum and NAC of C57BL/6 mice. Mice ($n = 10$ per group) were administered four injections of either saline, 10 mg/kg or 20 mg/kg MPTP in 2-h intervals. Seventy-two hours after the last MPTP injection, mice were sacrificed, and the striatum and NAC were processed for the determinations of monoamines and DAT binding sites as described in Material and Methods. Results represent the mean ± SEM ($n = 10$ for monoamines levels and $n = 3$ for DAT levels) as percent of control values (*$p < 0.05$ compared to control). The values obtained for control striatal tissue are: 947 ± 47, 141 ± 10, and 113 ± 8 ng/100 mg tissue, which correspond to the levels of DA, DOPAC, and HVA, respectively; and 1453 ± 102 fmol/mg protein corresponds to the number of DAT binding sites. The values obtained for control NAC tissue are: 605 ± 33, 61 ± 3, and 72 ± 4 ng/100 mg tissue, which correspond to DA, DOPAC, and HVA, respectively; and 1015 ± 83 fmol/mg protein corresponds to the number of DAT sites.

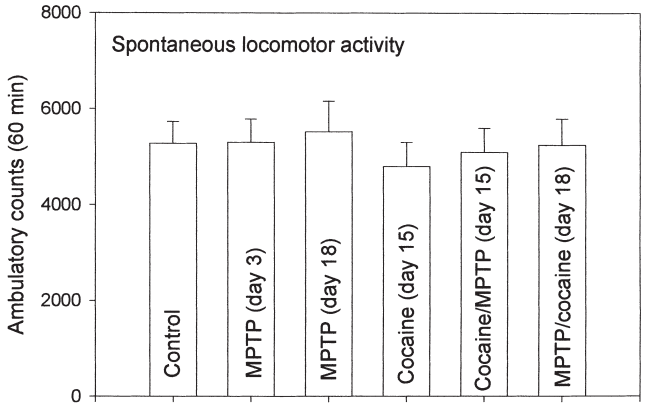

FIG. 2. Spontaneous locomotor activity of C57BL/6 mice following various drug treatments. Mice were administered either saline (control), MPTP (20 mg/kg × 4), cocaine (20 mg/kg; 5 days), MPTP after cocaine (Experiment 2) or MPTP before cocaine (Experiment 3). The spontaneous locomotor activity of the MPTP treated mice was measured 3 and 18 days after the administration of the neurotoxin alone. The spontaneous locomotor activity of mice that were treated with cocaine was measured 10 days after cocaine administration was stopped, and the spontaneous locomotor activity of control mice was determined following saline injections as described in the Method section. A two-way ANOVA (drug treatment × time), with time as the repeated measure, was employed to analyze MPTP effects, and a one-way ANOVA followed by the post hoc Neuman–Keuls test for the comparison between the spontaneous locomotor activity of the other groups tested. Results indicated no significant differences between any of the groups tested.

cocaine compared with mice that received cocaine/saline (Fig. 3; $p < 0.05$). These results suggest that MPTP caused augmentation in the expression of the sensitized response to cocaine.

We also compared the number of the striatal DAT binding sites in the cocaine/MPTP group vs. the cocaine/saline group. Results in Fig. 4 show the number of DAT sites on day 16 (24 h after the challenge cocaine injection was given). While cocaine treatment per se resulted in a slight (22%) but a significant increase in the number of DAT sites compared to control, MPTP administration to the cocaine-experienced mice caused a 75% decrease in striatal DAT sites (Fig. 4). It appears, therefore, that the marked decrease in DAT binding sites in cocaine/MPTP-experienced mice is associated with augmentation in the sensitized response to cocaine compared with mice that received cocaine/saline.

Experiment 3. Effect of MPTP on the Induction of Sensitization to the Locomotor Stimulating Effect of Cocaine

To investigate the effect of MPTP on the induction of sensitization to cocaine, mice were treated with MPTP (20 mg/kg × 4) 72 h before cocaine administration was started. The spontaneous locomotor activity of the mice measured 72 h after the administration of the neurotoxin was not different from control (Fig. 2). The initial response of the MPTP-treated mice to the acute effect of cocaine on day 1 was similar to the response of saline-treated mice to the acute effect of cocaine (Fig. 3). Thus, despite the marked depletion of NAC and striatal DAT sites, 72 h after MPTP administration (Fig. 1), the response to cocaine was not affected. Following the 5-day cocaine administration, animals remained drug free for 10 days. On day 15 from the first cocaine injection (e.g., day 18 from the MPTP injection), animals' spontaneous locomotor activity was similar to the saline/control group (Fig. 2). On day 15, the effect of a challenge cocaine injection (20 mg/kg) was investigated. Results in Fig. 3 indicate that mice that received MPTP before cocaine administration commenced developed the same degree of sensitization to cocaine as the saline/cocaine-treated mice. This finding suggests that MPTP pretreatment had no effect on the induction of sensitization to cocaine.

At the time when MPTP/cocaine-pretreated mice showed the sensitized response to cocaine, the level of DAT sites was only 30% of the control value (Fig. 4). Results in Fig. 4 also indicate that the depletion in striatal DAT sites observed 18 days after MPTP administration persisted in animals that were treated either with saline or cocaine. This finding suggests that cocaine administration had no further effect on DAT sites.

DISCUSSION

The major finding of the present study is that more than 70% depletion of NAC and striatal DAT binding sites had no effect on the induction of locomotor sensitization to cocaine, but this depletion caused an enhancement in the expression of the sensitized response to cocaine. The NAC and striatum are considered as the major neural substrates for the psychomotor stimulating effect of cocaine. Recently, we have shown that MPTP-induced depletion of DA and DAT in the stria-

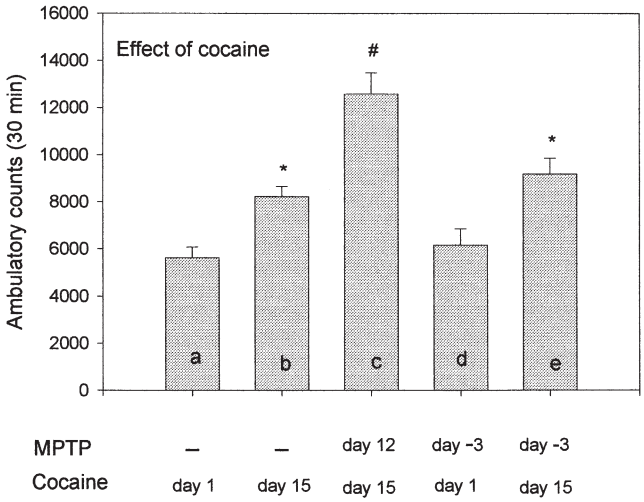

FIG. 3. Cocaine-induced locomotor activity on days 1 and 15 and the effect of MPTP. C57BL/6 mice received cocaine (20 mg/kg) injections on days 1 through 5 and after a 10-day drug-free period they were challenged to cocaine (day 15). To determine the effect of MPTP (20 mg/kg × 4) on the expression of locomotor sensitization, mice received the neurotoxin on day 12. To determine the effect of MPTP on the induction of sensitization, mice received the neurotoxin 3 days before the cocaine administration started (designated as day −3). The comparison between cocaine effects on day 1 and 15 by a two-way ANOVA (drug treatment × time) with time as the repeated measure yielded a significant interaction ($p = 0.003$). Bonferroni multiple comparison adjustments showed that on day 15, ambulatory counts in the cocaine group (bar b) and the MPTP (day −3) group (bar e) were significantly higher than the corresponding counts on day 1 ($^*p = 0.01$; bars a and d, respectively). The responses of the three different groups, saline/cocaine (bar b), cocaine/MPTP (bar c), and MPTP/cocaine (bar e) to cocaine challenge on day 15 were analyzed by one-way ANOVA and yielded a significant drug pretreatment effect: $F(2, 27) = 12.5$, $p < 0.001$. The MPTP (day 12)-treated group (bar c) was significantly more sensitive to challenge cocaine than the other two groups ($^{\#}p < 0.05$; Newman–Keuls test).

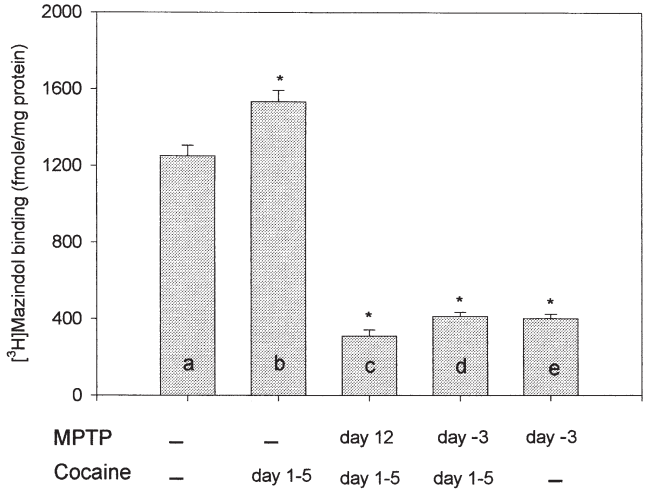

FIG. 4. The number of striatal DAT binding sites labeled with [^{3}H]mazindol. C57BL/6 mice received one of the following treatments: (a) saline injections (first bar from left), (b) five daily cocaine injections (20 mg/kg each) and saline on day 12, (c) five daily cocaine injections and MPTP (20 mg/kg × 4) on day 12, (d) MPTP 3 days before cocaine administration was started (designated as day −3), and (e) MPTP only. Groups b, c, and d received challenge cocaine injections on day 15. On day 16 (e.g., day 19 from the time MPTP was administered to groups d and e), mice were sacrificed and the striatum was processed for [^{3}H]mazindol binding. Saturation binding assays were carried out as described in the Method section. The dissociation constants of mazindol (K_d) in the various tissues investigated were in the range of 3.8 to 4.3 nM. However, there were significant differences in the maximal number of binding sites (Bmax in units of fmol/mg protein). One-way ANOVA yielded a significant drug pretreatment effect: $F(4, 25) = 183$, $p < 0.001$. The Bmaxs of groups b, c, d, and e were significantly different from control (a) ($^*p < 0.05$; Newman–Keuls test).

tum of Swiss Webster mice had no effect on animals' acute response to cocaine and METH (17). Moreover, METH-induced dopaminergic neurotoxicity was associated with a sensitized response to cocaine and METH (17). Previously, we suggested that the lack of effect of MPTP on cocaine- and METH-induced locomotor activity may be due to the resistance of the NAC to dopaminergic neurotoxicity compared with the striatum. It has been reported that the striatum is more sensitive than the NAC to METH-induced neurotoxicity (5,36). Studies on 6-hydroxydopamine (6-OHDA) lesions of the NAC revealed reduction in cocaine-induced locomotor activity (19,21), amphetamine-induced conditioned locomotion (12), and cocaine self-administration (3,10,34). However, a sensitized response to the direct dopamine receptor agonist, apomorphine, in NAC 6-OHDA–lesioned rats was attributed to supersensitivity of DA receptor following the lesions (40).

In the present study we found that the striatum and NAC are equally sensitive to MPTP-induced dopaminergic neurotoxicity. The high dose of MPTP (20 mg/kg × 4) resulted in 72 and 76% depletion of DAT sites in the NAC and striatum, respectively. Thus, the purpose of the present study was to investigate how the depletion of DAT sites in the NAC and striatum affects the expression and induction of sensitization to cocaine. In the first set of experiments, the effect of MPTP on the expression of the sensitized response to cocaine was investigated. As expected, cocaine-experienced mice that received a challenge cocaine injection after a 10-day drug-free period developed a sensitized response to cocaine (Fig. 3). However, cocaine-experienced mice that received MPTP 3 days before the challenge cocaine injection showed significantly greater response to cocaine compared with the cocaine/saline group that did not receive the neurotoxin (Fig. 3). This finding by itself could suggest that ablation of the DAT binding sites by MPTP impairs the mechanism of DA uptake, which leads subsequently to a sensitized response to cocaine. However, other results of this study do not support this hypothesis. First, we found that cocaine administration alone for 5 days—which induced locomotor sensitization—resulted in a small (22%) but a significant increase in striatal DAT binding sites compared to control (Fig. 4). Second, results from the experiment on the effect of MPTP on the induction of locomotor sensitization to cocaine also suggest that the decrease in DAT sites per se is not associated with augmentation in the effect of cocaine. Results in Fig. 3 indicate that pretreatment with MPTP that resulted in >70% decrease in NAC and striatal DAT affected neither the acute nor the sensitized response to cocaine compared with mice that did not receive the neurotoxin.

The differential effect of MPTP on the expression and induction of sensitization to cocaine is not entirely clear. Postsynaptic regulation of D_1D_2 dopamine receptors following the administration of MPTP may be one reason, although there is

no consensus on the extent of DA receptors regulation by the neurotoxin. In monkeys rendered Parkinsonian with MPTP, an increase in D_2 and no change in D_1 receptors in the caudate nucleus and putamen was reported (13,30). Also, an increase in the D_2 receptor mRNA and a decease in the D_1 receptor mRNA in the caudate and putamen of MPTP-treated monkeys was reported (26). In contrast, no change in D_2 receptors and a decrease in D_1 receptors in the dorsal caudate of MPTP-treated cats (8), and no change in D_1 and D_2 receptors in striatum of MPTP-treated C57BL/6 mice were reported (4). In the present study, it is unlikely that the enhancement of the sensitized response to cocaine is associated with MPTP-induced upregulation of DA receptors, because MPTP affected neither the acute response to cocaine nor the induction of sensitization. Also, because in the present study mice were treated with cocaine and MPTP, any speculation on DA receptor regulation may be misleading.

A more plausible explanation for the differential effect of MPTP on the expression and induction of sensitization to cocaine may relate to neural adaptation and plasticity that develop in the course of sensitization to psychostimulants. When cocaine was given to mice preexposed to MPTP (e.g., "induction" experiment), the NAC and striatum had already only 24–28% of the normal level of DAT sites. This level of DAT sites is apparently sufficient to produce not only the "normal" acute response to cocaine, but also the sensitized response afterwards (Fig. 3). In this situation, the NAC/striatal system has already adjusted to the low level of DAT sites, and may function evidently as a normal network. This hypothesis is supported by the finding that spontaneous locomotor activity of MPTP-treated mice was not different from control (Fig. 2).

However, when cocaine was given to mice with intact NAC/striatal system, and they became sensitized to the drug, abrupt destruction of the DAT sites by MPTP (e.g., "expression" experiment) may have caused impairment in the mechanism of DA uptake compared with the pre-MPTP exposure phase. Such impairment in DA uptake may lead to an augmentation in the sensitized response to cocaine.

Although the DAT has a major role in the acute response to cocaine, the process of sensitization to the drug is more complex and probably involves multiple neurotransmitter systems. Evidence suggests that glutamatergic transmission has a major role in the development of sensitization to psychostimulants [(27), for review]. In addition, we have shown that brain nitric oxide (NO)—which is thought to be coupled to activation of glutamatergic transmission (9,39)—is involved in the sensitization to psychostimulants (15,16). NO is also involved in MPTP-induced dopaminergic neurotoxicity (31,38). It appears, therefore, that the neuronal nitric oxide synthase (nNOS) has a major role in both the effects of cocaine and MPTP. Consequently, it is possible that the increase in the expression of the sensitized response to cocaine after MPTP administration is due to an increase in nNOS activity, which then leads to exacerbation of the effect of cocaine. According to this hypothesis, if there is a synergism between the effects of cocaine and MPTP on NOS activity, this synergism may be apparent only after the neural system had already been sensitized to cocaine (e.g., "expression" experiment). However, further studies are necessary to determine if there are any mutual interactions between cocaine, MPTP, and nNOS.

In summary, the present study indicates that the depletion of NAC and striatal DAT sites has a differential effect on the induction and expression of sensitization to cocaine. The finding that depletion of DAT sites did not disrupt the induction of sensitization to cocaine supports the view that neural systems other than NAC/striatal DAT may contribute to the induction of sensitization to cocaine.

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