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NMDA antagonists block expression of sensitization of amphetamine- and apomorphine-induced stereotypy

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Abstract

We have been studying sensitization of psychostimulant-induced stereotyped behavior in mice using both single and multiple pretreatment paradigms. In the present study, we tested whether NMDA receptor antagonists and an inhibitor of nitric oxide synthesis inhibit expression of sensitization in either of these models. Male CF-1 mice were pretreated with a single dose or with three daily doses of amphetamine (14 mg/kg) or apomorphine (40 mg/kg). Two days following these pretreatments, mice were injected with ((±)3-(2-carboxypiperazine-4yl)-propyl-1-phosphonic acid (CPP, 20 mg/kg), dizocilpine maleate (MK-801, 0.1 mg/kg), 7-nitroindazole (25 mg/kg), or vehicle 30 min before receiving amphetamine (7 mg/kg) or apomorphine (3 mg/kg). The stereotyped behavioral response was enhanced in mice pretreated with amphetamine or apomorphine, indicating that sensitization had developed. CPP, MK-801, and 7-nitroindazole prevented the expression of the sensitized stereotyped response induced by either amphetamine or apomorphine in both paradigms. These drugs did not attenuate the stereotypy elicited by amphetamine and apomorphine in drug-naïve mice. The effect of 7-nitroindazole was reversed by pretreatment with 500 mg/kg of L-arginine but not by D-arginine. These results suggest that glutamatergic transmission and subsequent NMDA receptor activation and the production of nitric oxide play a critical role in the expression of the sensitized stereotyped behavioral response elicited by amphetamine or apomorphine.

Keywords: Amphetamine; Apomorphine; Environmental context; Sensitization; Stereotypy

The intensity of locomotor activity and stereotyped behavior elicited by psychostimulant drugs, such as amphetamine and cocaine, and their potency in producing these effects can be augmented by prior exposure to one high dose or repeated administration of these drugs. This enhanced responding, which is referred to as sensitization, appears to be related to changes in mesolimbic and mesostriatal dopamine pathways, which play critical roles in the behavioral effects of psychostimulant drugs.

Activation of the NMDA subclass of glutamatergic receptors may be required for the induction of changes in dopaminergic pathways leading to the development of sensitization [19]. In experiments evaluating this concept, an antagonist of NMDA receptors is given before each administration of psychostimulant drug during a pretreatment schedule, and the psychostimulant drug is given alone in a subsequent test session. In such paradigms, the development of sensitization of the locomotor stimulant effects and the stereotyped behavioral effects of amphetamine, apomorphine, and cocaine is usually suppressed [6,11–13,19].

Whether activation of the NMDA subtype of glutamatergic receptors is important for the expression of enhanced responses in sensitized animals is not clear. In experiments evaluating this concept, a psychostimulant drug is given alone during a pretreatment schedule, and an NMDA antagonist is given before the psychostimulant drug in the test session. In several studies measuring locomotor activity or stereotypy responses in such a paradigm, NMDA antagonists did not block the expression of the sensitized response to amphetamine or cocaine [12,13,19,20] or did so only at doses higher than those needed to inhibit development of sensitization [5,10]. Thus, it appears that there might be a preferential role for NMDA receptors in the development versus the expression of sensitization for psychostimulants [19].

It has also been postulated that sensitization is an evolving process dependent on a variety of drug and context

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variables [17]. This postulate suggests that the importance of pharmacological interventions and anatomical substrates involved in sensitization may differ as a function of the behavior tested, the sensitization paradigm, and the time at which observations are made in the study of sensitized behaviors. Relating to this concept, we have been studying sensitization of psychostimulant-induced stereotyped behavior in mice using paradigms that involve variations in the number of pretreatment injections and in the environment of the pretreatment and test injections [1–3]. In these experiments, we have found that NMDA antagonists prevent development of sensitization in one paradigm but not in another [4]. In the present study, we have used these paradigms to determine whether the expression of a sensitized behavior in the different experimental protocols may require the activation of NMDA receptors. Both a non-competitive antagonist, dizocilpine maleate (MK-801), and a competitive antagonist, ((±)3-(2-carboxypiperazine-4yl)-propyl-1-phosphonic acid (CPP), were evaluated. Both amphetamine, which acts presynaptically to increase dopamine transmission, and apomorphine, which directly activates dopamine receptors, were studied to determine whether presynaptic or postsynaptic mechanisms may be more important in mediating sensitization. In addition, since NMDA receptor activation may lead to nitric oxide production [8,18], we also determined whether the neuronal nitric oxide synthase inhibitor, 7-nitroindazole, would have the same effects as CPP and MK-801. The results show that in the paradigms employed in these experiments, NMDA antagonists prevent expression of sensitization of amphetamine- and apomorphine-induced stereotyped behavior.

1. Methods

1.1. Animals and drugs

Male CF-1 mice (Charles River Laboratories), weighing 28–32 g at the time of experimentation, were housed five per cage in a temperature (24 ± 1°C) and humidity (55–65%) controlled vivarium with a 12-h light/dark cycle. Food and water were provided ad libitum. All animal use procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Laboratory Animal Care and Use Committee. D-Amphetamine sulfate (Sigma, St. Louis, MO), CPP (Research Biochemicals International, Natick, MA), MK-801 (Merck Sharp & Dohme Chemical Laboratories, Rahway, NJ), D-arginine HCl (Sigma), and L-arginine HCl (Sigma) were dissolved in normal saline. *R*(−)-Apomorphine HCl (Research Biochemicals International) was dissolved in distilled water with 0.1% ascorbic acid. 7-Nitroindazole (Research Biochemicals International) was dissolved in dimethyl sulfoxide/propylene glycol/distilled water (1:3:6). All drug solutions were prepared immediately prior to administration. Dosages were calculated as milligrams of amphetamine sulfate, apomorphine HCl, CPP, MK-801, 7-nitroindazole, D-arginine HCl, or L-arginine HCl per kilogram of body weight. Drugs were administered in a volume of 0.05 ml/g body weight except for 7-nitroindazole, for which the volume was varied according to the dosage to be administered (drug concentration maintained at 2.5 mg/ml due to poor solubility). All drugs were administered intraperitoneally (i.p.) except for apomorphine that was administered subcutaneously (s.c.).

1.2. Evaluation of stereotyped behavior

All animals were evaluated for stereotyped behavior after amphetamine, apomorphine, or vehicle pretreatment and after amphetamine or apomorphine challenge during the test phase. The individual who evaluated the behavior of the mice was unaware of which mice received amphetamine, apomorphine, or vehicle. As described previously [1,2,11], the stereotyped behavioral response of the CF-1 mouse is well-defined with the mouse remaining stationary and exhibiting rapid, repetitive head and/or forelimb movements. This behavior corresponds to a score of 8 on a graded score of 9 in the rating scale described by Ellinwood and Balster [7]. After drug injection, mice were placed one per cage (amphetamine) or three per cage (apomorphine) and were observed for 1 min at 10-min intervals. Mice were scored positive for stereotyped behavior when this behavior was exhibited for greater than 30 s in a 1-min observation period. Group data are expressed as the percentage of mice rated as positive for stereotyped behavior. The value of the peak effect was used as the measure of the stereotyped behavioral response elicited by a drug. All studies were conducted between 10.00 and 16.00 h in a temperature (24 ± 1°C) and humidity (55–65%) controlled room. Animals were used in only one experiment.

1.3. Design and procedures

1.3.1. The effect of CPP, MK-801, or 7-nitroindazole on the expression of sensitization induced by amphetamine or apomorphine

Two paradigms of pre-exposure to amphetamine and apomorphine were utilized. For the single pre-exposure paradigm, mice were transported from the vivarium to the laboratory, pretreated with vehicle, amphetamine (14 mg/kg × 1), or apomorphine (40 mg/kg × 1), and placed in the "test" cages (28 × 17 × 11 cm with tan corncob bedding; Harlan Teklad, Madison, WI) for 120 min (amphetamine) or 90 min (apomorphine). At the end of this period, they were returned to their home cages in the vivarium. In the multiple pre-exposure paradigm, three amphetamine (14 mg/kg daily × 3 days), apomorphine (40 mg/kg daily × 3 days), or vehicle pretreatment injections were administered during the pretreatment

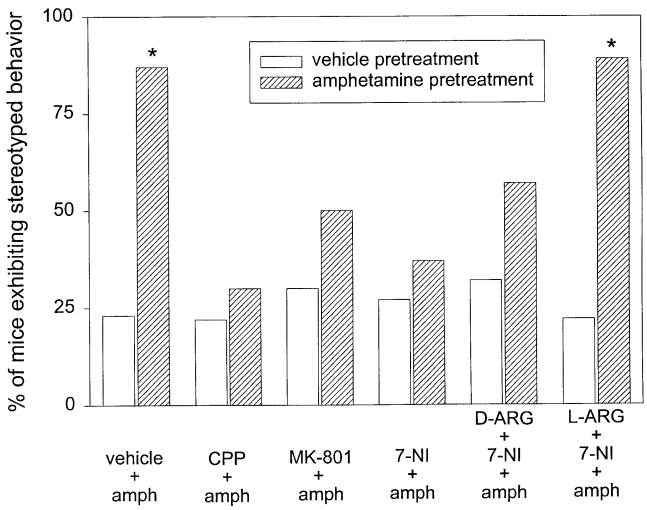

Fig. 1. The effect of CPP, MK-801, 7-nitroindazole (7-NI), and arginine (ARG) on the expression of sensitization induced by amphetamine (amph). Mice were pretreated with vehicle or 14 mg/kg of amphetamine for either 1 or 3 days and then tested for sensitization 3 days later. In the test, mice were injected with various agents — vehicle, 20 mg/kg CPP, 0.1 mg/kg MK-801, or 25 mg/kg 7-nitroindazole, ± 500 mg/kg D- or L-arginine — 30 min prior to administration of 7-mg/kg amphetamine challenge. Data shown for control, CPP, MK-801, and 7-nitroindazole studies are composites of four experiments: two pretreatment paradigms and two sequences of testing (see Methods). Data shown for arginine studies are composites of two experiments: two pretreatment paradigms. * Significantly different from vehicle control, as determined by χ^2 analysis ($p < 0.05$).

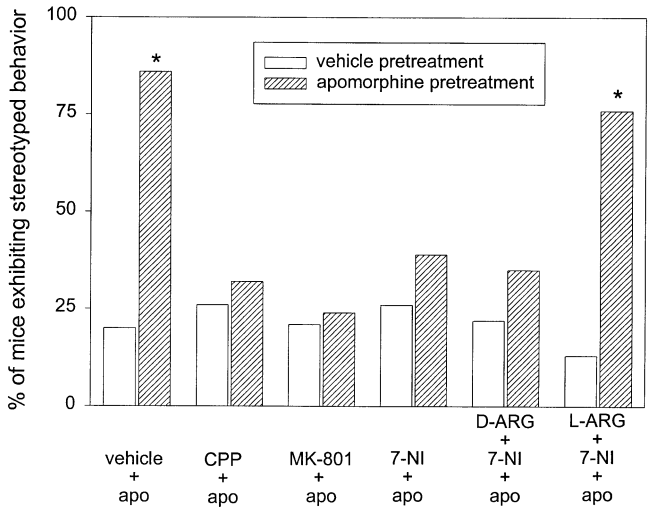

Fig. 2. The effect of CPP, MK-801, 7-nitroindazole (7-NI), and arginine (ARG) on the expression of sensitization induced by apomorphine (apo). Mice were pretreated with vehicle or 40 mg/kg of apomorphine for either 1 or 3 days and then tested for sensitization 3 days later. In the test, mice were injected with various agents — vehicle, 20 mg/kg CPP, 0.25 mg/kg MK-801, or 25 mg/kg 7-nitroindazole ± 500 mg/kg D- or L-arginine — 30 min prior to administration of 7-mg/kg amphetamine challenge. Data shown for control, CPP, MK-801, and 7-nitroindazole studies are composites of four experiments: two pretreatment paradigms and two sequences of testing (see Methods). Data shown for arginine studies are composites of two experiments: two pretreatment paradigms. * Significantly different from vehicle control, as determined by χ^2 analysis ($p < 0.05$).

phase, and the mice were placed in "diff" cages that were larger in size ($50 \times 25 \times 30$ cm) than the test cages and contained black colored bedding (Cellu-Dri-Shepherd Specialty Papers, Kalamazoo, MI) with a different texture than the tan corncob bedding of the test cages. In both paradigms, a test for sensitization was performed 3 days after end of the pretreatment. Mice were injected with CPP, MK-801, 7-nitroindazole, or respective vehicle in their home cage. Thirty minutes later, the animals were transported to the laboratory, administered the challenge dose of amphetamine (7 mg/kg) or apomorphine (3 mg/kg), placed in the test cages, and evaluated for stereotyped behavior. To determine that the block of sensitization by these drugs was reversible, a second test was performed 2 days later (5 days after pretreatment). Animals were transported to the laboratory, administered the challenge dose of amphetamine (7 mg/kg) or apomorphine (3 mg/kg), placed in the test cages and evaluated for stereotyped behavior. All experiments were repeated with the order of testing reversed — verification of sensitization first followed by effect of NMDA receptor antagonist or 7-nitroindazole second.

1.3.2. The effect of D- or L-arginine plus 7-nitroindazole on the expression of sensitization induced by amphetamine or apomorphine

The pretreatment procedures were the same as described above. Three days after animals received their last pre-treatment injection, mice were administered either vehicle or 7-nitroindazole (25 mg/kg) and were placed in their home cage for 30 min. All mice were then transported to the laboratory, given amphetamine (7 mg/kg) or apomorphine (3 mg/kg), placed in the test cage, and evaluated for stereotyped behavior to verify that sensitization had developed. A second test was performed 2 days later (5 days after pretreatment). In this test, mice were treated with D-arginine (500 mg/kg) along with the 7-nitroindazole (25 mg/kg) as a positive control to verify that 7-nitroindazole inhibited expression of sensitization in the presence of the inactive arginine stereoisomer. Two days later (7 days after

Table 1
The effect of CPP, MK-801, or 7-nitroindazole on the acute stereotyped behavioral response elicited by 14 mg/kg of amphetamine

Drug	Percent of mice exhibiting stereotypy
Vehicle	83
CPP, 20 mg/kg	73
Vehicle	95
MK-801, 0.1 mg/kg	100
MK-801, 0.25 mg/kg	40*
Vehicle	86
7-Nitroindazole, 25 mg/kg	80

* $p < 0.05$ compared to vehicle control as determined by χ^2 analysis.

pretreatment), mice were treated with L-arginine (500 mg/kg) along with the 7-nitroindazole (25 mg/kg).

1.4. Statistics

The percentage of mice in different experimental groups that exhibited stereotyped behavior was compared by χ^2 analysis. The Fisher exact test was used whenever 20% of the expected values in a contingency table were less than 5. The value of $p<0.05$ was considered significant.

2. Results

The NMDA receptor antagonists, CPP and MK-801, and the neuronal nitric oxide synthase inhibitor, 7-nitroindazole, blocked the expression of sensitization induced by either amphetamine (Fig. 1) or apomorphine (Fig. 2). Mice that exhibited a sensitized behavioral response after amphetamine or apomorphine administration at 3 days after the pretreatment phase did not exhibit sensitization when CPP, MK-801, or 7-nitroindazole was given prior to amphetamine or apomorphine 2 days later. The effect of CPP, MK-801, or 7-nitroindazole was reversible. Mice, in which the sensitized response to amphetamine or apomorphine was inhibited by CPP, MK-801, or 7-nitroindazole, showed an enhanced response to amphetamine or apomorphine given 2 days later in the absence of the antagonists. Results were identical for both the single pre-exposure and the multiple pre-exposure paradigms. It is important to note that the antagonists prevented the expression of sensitization at doses that did not affect the acute stereotyped behavioral response to either amphetamine (Table 1) or apomorphine (Table 2).

In order to determine whether 7-nitroindazole exerted its effects through a decrease in available nitric oxide, we attempted to counteract the inhibition of the expression of sensitization, produced by 7-nitroindazole, with the active isomer of the nitric oxide synthase substrate, arginine. The data presented in Figs. 1 and 2 show that L-arginine, the isomer that is a substrate for nitric oxide synthase, prevented the effects of 7-nitroindazole while D-arginine, the inactive isomer, did not. Neither D- nor L-arginine significantly affected the stereotyped behavioral response to amphetamine or apomorphine in non-sensitized animals (data not shown).

Table 2
The effect of CPP, MK-801, or 7-nitroindazole on the acute stereotyped behavioral response elicited by 40 mg/kg of apomorphine

Drug	Percent of mice exhibiting stereotypy
Vehicle	93
CPP, 20 mg/kg	87
Vehicle	87
MK-801, 0.25 mg/kg	80
Vehicle	90
7-Nitroindazole, 25 mg/kg	80

3. Discussion

The results of this work demonstrate that antagonists of the NMDA glutamate receptor blocked the expression of sensitization of amphetamine- and apomorphine-induced stereotyped behavior in the paradigms employed. Both the competitive NMDA receptor antagonist, CPP, and the non-competitive NMDA receptor antagonist, MK-801, were effective inhibitors of sensitization. This suggests that, in the paradigms used in our studies, glutamatergic transmission and subsequent NMDA receptor activation play a critical role in the expression of the sensitized stereotyped behavioral response elicited by amphetamine or apomorphine. The paradigms employed in the present work involve single and multiple pretreatment protocols, which differ in the role that environmental context plays in the expression of sensitization [3]. The observation that NMDA receptor antagonists were equally effective in inhibiting expression of sensitization in both paradigms suggests that the activation of this receptor does not account for the varying importance of environment in the expression of sensitization. Furthermore, neuronal nitric oxide synthase inhibition similarly blocked the expression of sensitization. Thus, it is hypothesized that the effects of NMDA receptor activation in the expression of the sensitized stereotyped behavior could be mediated through nitric oxide production.

Previous studies of the effects of NMDA receptor antagonists on expression of sensitization of stereotyped behavior elicited in mice by amphetamine reported either an inhibition of the sensitized response [5] or no effect [12,13]. A comparison of the methods used in these studies suggests that the role of activation of NMDA receptors in the expression of sensitized behavior may depend on the paradigm employed. In studies in which NMDA receptor antagonists inhibited the sensitized response (this study and Ref. [5]), a high dose of amphetamine sufficient to induce the stereotyped response in more than 80% of the mice was given in the pretreatment. In contrast, in studies in which NMDA receptor antagonists did not inhibit the expression of the sensitized response [12,13], the initial dose of amphetamine given in the pretreatment did not elicit stereotyped behavior. In the latter experiments, the pretreatment phase consisted of 10–12 amphetamine injections administered once daily, a schedule that produced a progressive augmentation of the behavioral response. Similarly, in a study in rats in which the first exposure to amphetamine did not elicit stereotypy, neither MK-801 nor CGS 19755 inhibited the expression of sensitization of stereotyped behaviors [20]. These observations suggest that the sensitization induced

by pretreatment with a high dose of amphetamine and that produced by the repeated intermittent administration of several low doses of amphetamine may be mediated by different mechanisms. This conclusion is consistent with the concept that the importance of pharmacological interventions and anatomical substrates involved in sensitization may differ as a function of the behavior tested, the sensitization paradigm, and the time at which observations are made in the course of the study of sensitized behaviors [17].

In studies of locomotor activity elicited by amphetamine, NMDA receptor antagonists at doses that prevented the development of sensitization did not prevent the expression of sensitization [14,20]. In fact, the amount of locomotor activity elicited by the co-administration of some NMDA receptor antagonists and amphetamine in the test for sensitization was higher than that elicited by amphetamine alone [14]. This is attributed to the additive effects of amphetamine and those NMDA receptor antagonists (e.g., MK-801) that stimulate locomotor activity when used alone. Thus, studies of locomotor activity may not be a valid model for evaluating the role of these NMDA antagonists on the expression of sensitization.

A comparison of effects of NMDA antagonists on the development versus the expression of sensitization shows interesting differences. Whereas NMDA antagonists were equally effective in inhibiting expression of sensitization in our single and multiple pretreatment paradigms, NMDA antagonists inhibited development of sensitization in the single pretreatment paradigm but not in the multiple pretreatment paradigm [4]. Furthermore, the doses of NMDA antagonists effective at inhibiting development of sensitization in the single pretreatment paradigm were similar to those inhibiting the expression or sensitization. This differs from other studies where the general observation has been that NMDA antagonists are more effective at inhibiting development than expression of sensitization [19]. As mentioned above, there are substantial differences in the protocols used in these experiments, and the varying results of pharmacological interventions support the idea that sensitization is a multifactorial phenomenon involving many brain circuits.

In addition to NMDA receptor activation, nitric oxide production appears to play an essential role in the expression of sensitization of stereotyped behavior. This is supported by the fact that the relatively selective competitive inhibitor of neuronal nitric oxide synthase, 7-nitroindazole [15,16], blocked the expression of sensitization induced by amphetamine and apomorphine. 7-Nitroindazole did not appear to elicit its effects non-specifically since the attenuation of the expression of sensitization produced by 7-nitroindazole was reversed by the administration of L-arginine but not D-arginine. A previous study demonstrated that the non-selective inhibitor of nitric oxide synthase, L-NAME (*N*ω-nitro-L-arginine methyl ester) attenuated the expression of sensitization of methamphetamine-induced locomotor activity [9]. Thus, nitric oxide production is important in the expression of sensitization in several experimental paradigms.

In summary, NMDA receptor activation and nitric oxide production appear to be critical for the expression of sensitization of amphetamine- and apomorphine-induced stereotyped behavior in two paradigms that differ in the importance of environmental context. Since the effects of NMDA receptor antagonists and 7-nitroindazole on the expression of sensitization were the same for amphetamine, which increases synaptic dopamine, and apomorphine, which directly activates dopamine receptors, the results also suggest that the expression of sensitization requires the activation of NMDA receptors and production of nitric oxide at sites downstream from the dopamine synapse.

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References

[1] Battisti JJ, Chang C-H, Uretsky NJ, Wallace LJ. Sensitization of stereotyped behavior to amphetamine is context and response dependent. Pharmacol Biochem Behav 1999;63:263–9.
[2] Battisti JJ, Uretsky NJ, Wallace LJ. Sensitization of apomorphine-induced stereotyped behavior in mice is context dependent. Psychopharmacology 1999;146:42–8.
[3] Battisti JJ, Uretsky NJ, Wallace LJ. Importance of environmental context in the development of amphetamine- or apomorphine-induced stereotyped behavior after single and multiple doses. Pharmacol Biochem Behav 2000;66:435–41.
[4] Battisti JJ, Uretsky NJ, Wallace LJ. NMDA glutamate receptor role in the development of context-dependent and independent sensitization of the induction of stereotypy by amphetamine or apomorphine. Behav Brain Res (in press).
[5] Bedingfield JB, Calder LD, Thai DK, Karler R. The role of the striatum in the mouse in behavioral sensitization to amphetamine. Pharmacol Biochem Behav 1997;56:305–10.
[6] Druhan JP, Jakob A, Stewart J. The development of behavioral sensitization to apomorphine is blocked by MK-801. Eur J Pharmacol 1993;243:73–7.
[7] Ellinwood EH, Balster RL. Rating the behavioral effects of amphetamine. Eur J Pharmacol 1974;28:35–41.
[8] Garthwaite J. Glutamate, nitric oxide and cell–cell signalling in the nervous system. Trends Neurosci 1991;14:60–7.
[9] Inoue H, Arai I, Shibata S, Watanabe S. *N*-G-Nitro-L-arginine methyl ester attenuates the maintenance and expression of methamphetamine-induced behavioral sensitization and enhancement of striatal dopamine release. J Pharmacol Exp Ther 1996;277:1424–30.
[10] Karler R, Calder LD, Bedingfield JB. Cocaine behavioral sensitization and the excitatory amino-acids. Psychopharmacology 1994;115:305–10.
[11] Karler R, Calder LD, Chaudhry IA, Turkanis SA. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 1989;45:599–606.

[12] Karler R, Calder LD, Turkanis SA. DNQX blockade of amphetamine behavioral sensitization. Brain Res 1991;552:295–300.
[13] Karler R, Chaudhry IA, Calder LD, Turkanis SA. Amphetamine behavioral sensitization and the excitatory amino acids. Brain Res 1990;537:76–82.
[14] Li Y, Wolf ME. Can the "state-dependency" hypothesis explain prevention of amphetamine sensitization in rats by NMDA receptor antagonists? Psychopharmacology 1999;141:351–61.
[15] Moore PK, Handy RLC. Selective inhibitors of neuronal nitric oxide synthase — is no NOS really good NOS for the nervous system? Trends Pharmacol Sci 1997;18:204–11.
[16] Moore PK, Wallace P, Gaffen Z, Hart SL, Babbedge RC. Characterization of the novel nitric-oxide synthase inhibitor 7-nitro indazole and related indazoles — antinociceptive and cardiovascular effects. Br J Pharmacol 1993;110:219–24.
[17] Post RM, Weiss SRB, Fontana D, Pert A. Conditoned sensitization to the psychomotor stimulant cocaine. In: Kalivas PW, Samson HS, editors. The Neurobiology of Drug Addiction, vol. 654. New York: New York Academy of Sciences, 1992. pp. 386–99.
[18] Snyder SH. Nitric oxide: first in a new class of neurotransmitter? Science 1992;257:494–6.
[19] Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog Neurobiol 1998;54: 679–720.
[20] Wolf ME, Dahlin SL, Hu X-T, Xue C-J, White K. Effects of lesions of prefrontal cortex, amygdala, or fornix on behavioral sensitization to amphetamine: comparison with *N*-methyl-D-aspartate antagonists. Neuroscience 1995;69:417–39.