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Long-term blockade of the expression of cocaine sensitization by ondansetron, a 5-HT_3 receptor antagonist

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Abstract

Intermittent cocaine administration induces sensitization (reverse tolerance) to its behavioral effects. The mechanism(s) mediating sensitization is not clear, however, previous research has implicated 5-HT_3 receptors in the expression of sensitization. The present experiment evaluated the ability of the 5-HT_3 receptor antagonist, ondansetron, administered during withdrawal from chronic intermittent cocaine administration, to block the expression of sensitization. Rats were pretreated for 14 days by daily subcutaneous injections of either 40 mg/kg cocaine or 0.9% saline. During the first 5 days of withdrawal from this pretreatment regimen, all rats received a daily subcutaneous injection of 0–1.0 mg/kg ondansetron. On days 7, 14 or 28 of withdrawal from the cocaine pretreatment, the rats received a 15.0-mg/kg cocaine challenge. Ambulatory behavior was automatically recorded for 60 min. Ondansetron had no significant effect on the subsequent behavioral response to cocaine in the saline control subjects. In contrast, daily injections of ondansetron blocked the expression of sensitization at all withdrawal times. We thus report that it is possible to permanently block the expression of sensitization once it has developed by administering a 5-HT_3 receptor antagonist.

Keywords: 5-HT_3 receptor; Cocaine; Ondansetron; Sensitization; (Rat)

1. Introduction

Stimulant sensitization is characterized by an increasing behavioral or neurochemical response to the stimulant with repeated intermittent administration (Post and Contel, 1983; Kalivas and Duffy 1990; Kalivas and Stewart 1991; King et al., 1993, 1994a). Sensitization lasts months, and is thought to represent a permanent change in the neurobiology of the organism (Kalivas and Stewart 1991). The mechanism(s) mediating sensitization is not clear, but the process of establishing sensitization can be divided into three phases: Development, transfer, and expression. The literature suggests that different mechanisms probably mediate each phase.

The most common method for evaluating the receptor(s) mediating the development of sensitization is to co-administer an antagonist with cocaine during the treatment regimen. Using this strategy, several reports have implicated D_1 receptors in amphetamine (Vezina, 1996), but not cocaine (White et al., 1998) sensitization. This method has also implicated NMDA and AMPA receptors (e.g., Karler et al., 1989, 1990, 1991, 1994; Kalivas and Alesdatter, 1993; de Montis et al., 1995; Li et al., 1997; Wolf, 1998; Wolf and Jezlorski, 1993; Wolf and Khansa, 1991; Wolf et al., 1993) in the development of both amphetamine and cocaine sensitization. We have previously reported that ondansetron, a 5-HT_3 receptor antagonist, co-administered with intermittent cocaine, can block the development of sensitization (King et al., 1997). These results were recently confirmed by Steketee and Crissman (1997) using other 5-HT_3 receptor antagonists locally administered into the ventral tegmental area.

There is evidence that the development and expression of sensitization and tolerance are distinct events (e.g., Cador et al., 1995) with a transfer phase between the development and expression of sensitization (White et al., 1998). First, maximal sensitization does not occur immediately after the last injection, but rather approximately 7–10

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days after the final injection (see Post, 1980, for a review of this literature). Furthermore, Kalivas and Weber (1988) reported that local administration of amphetamine into the A10 region induced sensitization to systemic amphetamine and cocaine. In contrast, local application of amphetamine into the nucleus accumbens does not induce sensitization (Dougherty and Ellinwood, 1981).

The expression of sensitization is associated with enhanced dopamine release in the nucleus accumbens (Nicolaysen et al., 1988; Kalivas and Duffy 1990; Kalivas et al., 1988; Pettit et al., 1990; Ng et al., 1991; King et al., 1993, 1994a,b). A common approach to evaluate the receptor basis of the expression of sensitization is to administer a single injection of an antagonist before the stimulant challenge at some time after the stimulant pretreatment regimen. Using this procedure, the literature indicates that neither NMDA (Wolf et al., 1995) nor AMPA (Karler et al., 1994) receptor antagonists blocked the expression of cocaine sensitization in rats. In contrast, we have reported that the 5-HT_3 receptor antagonist, ondansetron, does block cocaine sensitization in rats (King et al., 1994a,b).

However, this method may not adequately address the issue of the receptor bases of the expression of stimulant sensitization. As stated above, the transfer of necessary neurobiological changes from the ventral tegmental area to the nucleus accumbens is a process that requires a period of time (most likely several days). Thus, the changes in receptor mechanisms necessary for the consolidation occur over an extended period of time. As a result, a single injection of an antagonist at a specific time point may not be sufficient to probe the receptor bases of the transfer of sensitization from the soma to the terminal areas (i.e., ventral tegmental area to the nucleus accumbens).

Given these considerations, we have evaluated the receptor bases of this transfer process by administering antagonists for the first 5 days after the cessation of the cocaine pretreatment regimen. Using these procedures, we have reported that ondansetron can block the expression of sensitization on day 7 of withdrawal from the cocaine pretreatment regimen (King et al., 1998). However, the question arises as to whether this result represents a long-term/permanent blockade of sensitization, or whether ondansetron administration simply delayed the onset of sensitization. In other words, would sensitization appear at a later withdrawal time, following ondansetron administration, or was the expression of sensitization permanently blocked?

A long-term or permanent blockade of sensitization would suggest a critical role for 5-HT_3 receptors in the mediation of sensitization, while a delay in the expression of sensitization would suggest a more tangential role for 5-HT_3 receptors. The present experiment replicated our previous report (King et al., 1998), except that the subjects were withdrawn from the cocaine pretreatment regimen for 7, 14, or 28 days to determine whether the previous blockade of the expression of sensitization persists beyond 7 days. We predict that the cocaine plus vehicle subjects will exhibit sensitization at all withdrawal periods. In contrast, the cocaine plus ondansetron subjects will exhibit significantly less behavior at all withdrawal periods.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats, initially weighing 200 to 225 g (Charles River Laboratories), were acclimated to the vivarium on a 12 h light/dark cycle (light between 0700 to 1900 h) for 1 week prior to treatment. They were housed in pairs in plastic cages with continuous access to food and water. All subjects were treated according to the guidelines for the ethical treatment of subjects proposed by the American Psychological Association. The research was conducted under a protocol approved by the Duke University IACUC.

2.2. Drugs

Cocaine HCl (received from NIDA) was dissolved in 0.9 % sterile saline. Ondansetron hydrochloride dihydrate, (+)-1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*-carbazol-4-one, monohydrochloride dihydrate, (generously supplied by Glaxo Wellcome, Middlesex, UK) was dissolved in distilled water. All doses are calculated as the salt, and injection volume was based on the body weight.

2.3. Pretreatment regimen

The cocaine pretreatment regimen was for a 14-day period. On day 1 of treatment the animals received either daily s.c. injections of 40 mg/kg cocaine HCl (intermittent administration group), or daily s.c. injections of 0.9 % saline (saline control group). For the saline control and cocaine pretreatment subjects, each injection was done as two separate injections at separate sites (e.g., the cocaine subjects received two 20-mg/kg cocaine injections, one right after the other). This was done to minimize the development of tissue necrosis due to the vasoconstrictive properties of cocaine. As a result of this procedure, no significant necrosis developed in the cocaine-treated subjects. At the end of the 14-day pretreatment period, the subjects were exposed to a 7-, 14-, or 28-day withdrawal period. During the first 5 days of the withdrawal period, all subjects received a s.c. injection of ondansetron. The ondansetron doses were vehicle, 0.01, 0.1, and 1.0 mg/kg. These were the same doses that we used in our previous experiment (King et al., 1997) evaluating the ability of ondansetron to block the development and expression of sensitization and tolerance. Furthermore, previous research had indicated that this dose range spans the effective dose

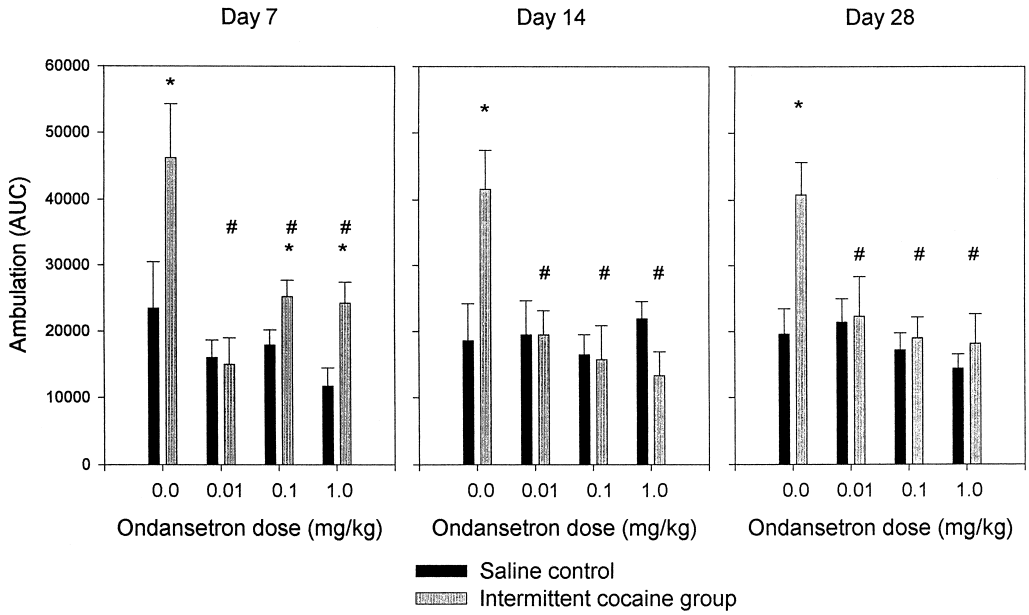

Fig. 1. The mean AUCs for the behavioral response to a 15.0-mg/kg cocaine challenge, as a function of withdrawal day, separately for each pretreatment group are presented. The top panel presents the mean AUCs for the saline control subjects, and the bottom panel presents the data for the intermittent cocaine subjects. The bars represent one standard error of the mean. The asterisks (*) indicate a significant difference between the cocaine pretreated and saline control subjects. The pound sign (#) indicates a significant difference between an ondansetron dose and the vehicle.

range for ondansetron (see Costall, 1993, Costall et al., 1990c for reviews).

2.4. Behavioral testing

On day 7, 14, or 28 following the cocaine pretreatment regimen (or 2, 9, or 23 days after the final ondansetron injection), the animals were acclimated to the test room in their home cage for 30 min under normal light conditions. The animals were then transferred to the center of plexiglas boxes ($43.2 \times 43.2 \times 21$ cm) inside Opto-Varimex ''minor'' activity monitors, and allowed to acclimate to the test cages for an additional 30 min. The activity monitors had 15 photobeams, spaced 2.5 cm apart, along each side of the monitor. All subjects received a 15.0 mg/kg i.p. cocaine injection. Activity counts (horizontal movements and ambulation) were taken in 5 min bins for 60 min.

2.5. Data analysis

The current experiment is a mixed model design. Specifically, there were three group factors (cocaine pretreatment, ondansetron dose, and withdrawal period) that produces 24 separate groups (two pretreatment groups $\times$ four ondansetron doses $\times$ three withdrawal periods), and one repeated measures factor (time). There were 10 rats per group, for a total of 240 subjects. In the current experiment, the subjects were randomized according to a Latin Square design. The data were analyzed by standard analyses of variance (ANOVA). Differences between specific groups were determined by a-priori *t*-tests. The significance level is set at $p < 0.05$ for all comparisons.

3. Results

Fig. 1 presents the mean area under the curve (AUC) for each pretreatment group as a function of the ondansetron dose administered during withdrawal, separately for each withdrawal period. A three-way ANOVA was conducted on the AUCs presented in Fig. 1. The factors were pretreatment group (saline vs. cocaine), ondansetron dose (vehicle, 0.01, 0.1, 1.0 mg/kg), and withdrawal day (day 7, 14, or 28). The results of the ANOVA indicated that the main effects of the pretreatment group [$F(1,216) = 15.38$] and the ondansetron dose [$F(3,216) = 14.69$] were significant, but that the main effect of withdrawal day [$F(2,216) = 0.28$] was not. Furthermore, the results indicated that the pretreatment group $\times$ ondansetron dose [$F(3,216) = 8.62$] interaction was significant, but no other interaction was significant [pretreatment group $\times$ withdrawal day: $F(2,216) = 1.32$; withdrawal day $\times$ ondansetron dose: $F(6,215) = 0.84$; pretreatment group $\times$ withdrawal day $\times$ ondansetron dose: $F(6,216) = 0.74$].

4. Discussion

The present results support and extend previous findings, which suggest a role for 5-HT_3 receptors in cocaine sensitization. The current results demonstrate that daily

injections of the 5-HT_3 antagonist, ondansetron, during the first 5 days of withdrawal from chronic cocaine can permanently block the expression of behavioral sensitization.

The present results indicate that chronic ondansetron had no consistent, systematic effect on the subsequent behavioral response to cocaine in the saline control subjects. This result is consistent with previous research indicating that these compounds have no effect on locomotor behavior when administered alone in drug naive rats (see Costall and Naylor 1974, Costall et al., 1987, 1989, 1990a,b,c for examples). This result is also consistent with our previous reports (King et al., 1997). The absence of an effect of ondansetron on the behavioral response to cocaine in the saline control subjects indicates that the effects of ondansetron in the cocaine-pretreated subjects is not the result of some non-specific effect of ondansetron on behavior.

The results presented in Fig. 1 highlight several findings. First, the intermittent cocaine subjects that received vehicle injections on days 1–5 of withdrawal all exhibited sensitization to the 15.0-mg/kg cocaine challenge at all withdrawal periods. Further, the magnitude of sensitization did not significantly change over the withdrawal periods. This pattern of results is consistent with previous literature indicating that sensitization is a long-term response to intermittent cocaine administration (Kalivas and Stewart 1991).

Second, the results in Fig. 1 also indicate that ondansetron blocked the expression of sensitization induced by intermittent cocaine administration at all withdrawal periods. The results in Fig. 1 indicate that there were no significant differences in the behavioral response to the cocaine challenge between the saline control subjects and the cocaine subjects that received different doses of ondansetron. Further, the magnitude of the blockade of sensitization did not change over the withdrawal periods. In other words, there was no increase, at any ondansetron dose, in the behavioral response to the cocaine challenge over the three withdrawal periods. The elimination of sensitization was not due to the subjects exhibiting stereotypies following the cocaine challenges. An analysis of behavior ratings using the Ellinwood and Balster (1974) rating scale (data not presented) did not indicate that any ondansetron-treated subject exhibited stereotypies. This result demonstrates that the blockade of sensitization was long-term (if not permanent), and questions the assumption that sensitization, once it has developed, represents a permanent change on the neurobiology of the organism.

The current results are also consistent with our previous research (King et al., 1997) and the report of de la Garza and Cunningham (1993) who found that the co-administration of zacopride, another 5-HT_3 receptor antagonist, and cocaine also blocked the development of sensitization. The results are also consistent with the findings of Steketee and Crissman (1997) who reported that the intraventral tegmental area administration of a variety of 5-HT_3 receptor antagonists blocked the development of sensitization. Overall, these reports, as well as the current results, strongly suggest a role for 5-HT_3 receptors in both the development and expression of cocaine sensitization.

The effects of ondansetron on the subsequent behavioral response to the cocaine challenge were not dose dependent. In other words, ondansetron exhibited a flat dose–response curve. This result is not surprising, as the effects of 5-HT_3 receptor antagonists often show either an inverted U-shaped or flat dose–response curve (Costall et al., 1990a,b). Furthermore, the current results are consistent with the results of our previous report (King et al., 1998), where the effects of ondansetron were also not dose-dependent. The results presented in Fig. 1 suggest that the two highest doses of ondansetron did not completely eliminate sensitization on day 7 of withdrawal. This result is consistent with our previous report (King et al., 1998), which examined the ability of ondansetron to block the development of cocaine tolerance and sensitization. In that report, similar to the current results, the lowest and highest ondansetron doses attenuated, but did not completely eliminate, the development of sensitization. These two results suggest that multiple mechanisms may be involved in the development and expression of sensitization.

In contrast to the current results, White et al. (1998) reported that SCH 23390, a dopamine D_1 receptor antagonist, administered during the first 4 days of withdrawal from an intermittent cocaine administration regimen, failed to block the expression of sensitization on day 7. Thus, the current results suggest that 5-HT_3 receptors, but not D_1 receptors, are critical to the subsequent expression of sensitization.

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References

Cador, M., Bijou, Y., Stinus, L., 1995. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience 65, 385–395.

Costall, B., 1993. The breadth of action of the 5-HT_3 receptor antagonists. Int. Clin. Psychopharmacol. 8 (Suppl. 2), 3–9.

Costall, B., Domeney, A.M., Naylor, R.J., Tyers, M.B., 1987. Effects of the 5-HT_3 receptor antagonist GR 38302F on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br. J. Pharmacol. 92, 881–890.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1990a. Ondansetron inhibits a behavioural consequence of withdrawing from drugs of abuse. Pharmacol. Biochem. Behav. 36, 339–344.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1990b. Sites of action of ondansetron to inhibit withdrawal from drugs of abuse. Pharmacol. Biochem. Behav. 36, 339–344.

Costall, B., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1989. Neuroanatomical sites of action of 5-HT_3 receptor agonists and antagonists for alteration of aversive behaviour in mice. Br. J. Pharmacol. 96, 325–332.

Costall, B., Naylor, R.J., 1974. Extrapyramidal and mesolimbic involvement with the stereotypic activity of D- and L-amphetamine. Eur. J. Pharmacol. 25, 121–129.

Costall, B., Naylor, R.J., Tyers, M.B., 1990c. The psychopharmacology of 5-HT_3 receptors. Pharmacol. Ther. 47, 181–202.

de la Garza, R., Cunningham, K.A., 1993. Effects of the 5-HT_3 antagonist zacopride on the development and expression of cocaine sensitization. Soc. Neurosci. Abstr. 19, 1850, (part 3).

De Montis, M.B., Gambarana, C., Ghliglieri, O., Tagliamonte, A., 1995. Reversal of stable behavioral modifications through NMDA receptor inhibition in rats. Behav. Pharmacol. 6, 562–567.

Dougherty, G.G., Ellinwood, E.H., 1981. Chronic D-amphetaminein nucleus accumbens: lack of tolerance or reverse tolerance to locomotor activity. Life Sci. 28, 2295–2298.

Ellinwood, E.H., Balster, R.I., 1974. Rating the behavioral effects of amphetamine. Eur. J. Pharmacol. 28, 35–41.

Kalivas, P.W., Alesdatter, J.E., 1993. Involvement of *N*-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. J. Pharmacol. Exp. Ther. 267, 486–495.

Kalivas, P.W., Duffy, P., 1990. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5, 48–58.

Kalivas, P.W., Duffy, P., Dumars, L.A., Skinner, C., 1988. Behavioral and neurochemical effects of acute and daily cocaine administration in rats. J. Pharmacol. Exp. Ther. 245, 485–492.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.

Kalivas, P.W., Weber, R., 1988. Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J. Pharmacol. Exp. Ther. 245, 1095–1102.

Karler, R., Caldor, L.D., Bedingfield, J.B., 1994. Cocaine behavioral sensitization and the excitatory amino acids. Psychopharmacology 115, 305–310.

Karler, R., Caldor, L.D., Chaudhry, I.A., Turkanis, S.A., 1989. Blockade of ''reverse tolerance'' to cocaine and amphetamine by MK-801. Life Sci. 45, 599–606.

Karler, R., Caldor, L.D., Turkanis, S.A., 1991. DNQX blockade of amphetamine behavioral sensitization. Brain Res. 532, 295–300.

Karler, R., Chaudhry, I.A., Caldor, L.D., Turkanis, S.A., 1990. Amphetamine behavioral sensitization and the excitatory amino acids. Brain Res. 537, 76–82.

King, G.R., Joyner, C., Ellinwood, E.H., 1994a. Continuous or intermittent cocaine administration: effects of flupenthixol treatment during withdrawal. Pharmacol. Biochem. Behav. 49, 883–889.

King, G.R., Joyner, C., Ellinwood, E.H., 1994b. 5-HT_3 receptor modulation of behavior during withdrawal from continuous or intermittent cocaine. Pharmacol. Biochem. Behav. 47, 339–407.

King, G.R., Kuhn, C., Ellinwood, E.H., 1993. Dopamine efflux during withdrawal from continuous or intermittent cocaine. Psychopharmacology 111, 179–184.

King, G.R., Xiong, Z., Ellinwood, E.H., 1997. Blockade of cocaine sensitization and tolerance by the co-administration of ondansetron, a 5-HT_3 receptor antagonist, and cocaine. Psychopharmacology 130, 159–165.

King, G.R., Xiong, Z., Ellinwood, E.H., 1998. Blockade of the expression of sensitization and tolerance by ondansetron, a 5-HT_3 receptor antagonist, administered during withdrawal from intermittent and continuous cocaine. Psychopharmacology 135, 263–269.

Li, Y., Wolf, M.E., White, F.J., 1997. Co-administration of the NMDA receptor antagonist MK-801 with the dopamine D2 receptor agonist quinpirole reverses behavioral sensitization to cocaine. Soc. Neuorsci. Abstr. 23, 261.

Ng, J.P., Hubert, G.W., Justice, J.B. Jr., 1991. Increased stimulated release and uptake of dopamine in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. J. Neurochem. 56, 1485–1492.

Nicolaysen, L.C., Hwai-Tzong, P., Justice, J.B. Jr., 1988. Extracellular cocaine and dopamine concentrations are linearly related in rat striatum. Brain Res. 456, 317–323.

Pettit, H.O., Hwai-Tzong, P., Parsons, L.H., Justice, J.B. Jr., 1990. Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J. Neurochem. 55, 798–804.

Post, R.M., 1980. Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life Sci. 26, 1275–1282.

Post, R.M., Contel, N.R., 1983. Human and animal studies of cocaine: implications for development of behavioral pathology. In: Creese, I. (Ed.), Stimulants: Neurochemical, Behavioral, and Clinical Perspectives. Raven Press, New York, pp. 169–203.

Steketee, J.D., Crissman, A., 1997. The effects of intra-ventral tegmental area injection of 5-HT_3 receptor antagonists on cocaine-induced sensitization. Poster presented at the 37th Annual Meeting of the American College of Neuropharmacology, Waikoloa, HI.

Vezina, P., 1996. D_1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. J. Neurosci. 16, 2411–2420.

White, F.J., Joshi, A., Koeltzow, T.W., Hu, X.-T., 1998. Dopamine receptor antagonists fail to prevent induction of cocaine sensitization. Neuropsychopharmacology 18, 26–40.

Wolf, M.E., 1998. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog. Neurobiol. 54, 1–42.

Wolf, M.E., Dahlin, S.L., Ju, X.-T., Xue, C.-J., White, F.K., 1995. Effects of lesions of prefrontal cortex, amygdala, or fornix on behavioral sensitization to amphetamine: comparison with *N*-methyl-D-aspartate antagonists. Neuroscience 69, 417–439.

Wolf, M.E., Jezlorski, M., 1993. Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res. 613, 291–294.

Wolf, M.E., Khansa, M.R., 1991. Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. Brain Res. 562, 164–168.

Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J., Khansa, M.R., 1993. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. J. Pharmacol. Exp. Ther. 264, 249–255.