Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice

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Antagonists of the $N$-methyl-$\text{D}$-aspartate (NMDA) receptor complex have been shown to inhibit the expression of place preferences conditioned with several drugs that are abused by humans, which suggests that this class of compounds may be beneficial in the treatment of substance dependence. Therefore it is important to assess the specificity of this effect, of whether inhibitory effects of NMDA receptor antagonists on conditioned drug stimuli generalize to behaviors produced by nondrug reinforcers. The present study was designed to compare the effects of the NMDA receptor channel blocker, memantine, on the expression of place preferences conditioned with: (1) consumption of regular laboratory food; (2) sexual encounters with females; and (3) injection of morphine (10 mg/kg) in adult male Swiss mice. For all three experiments reported here, unconditioned stimuli (food, receptive female or morphine) were presented before the exposures to the ‘to-be-conditioned’ environments. Significant place preferences developed as a result of explicit pairings of the environmental context and food consumption, sexual encounter and morphine administration. Memantine (7.5 mg/kg, given prior to the post-conditioning test) inhibited the expression of place preferences conditioned with morphine and sexual encounter, but had no effects in food-conditioned mice. These findings suggest that the effects of NMDA receptor blockade may not be limited to drug-reinforced behaviors. 

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Keywords: conditioned place preference, NMDA receptor antagonist, memantine, morphine, sexual interaction, food reward, mouse

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Introduction

The behaviors that are involved in drug abuse are known to be highly sensitive to environmental control. Environmental cues that were previously associated with drug intake can later elicit drug craving and trigger relapse to drug taking in abstinent individuals (Childress et al., 1988; O’Brien et al., 1998). Therefore, agents that effectively reduce responding to such drug-associated cues may be considered beneficial for medication development. For example, preliminary clinical evidence suggests that a clinically used $N$-methyl-$\text{D}$-aspartate (NMDA) receptor channel blocker, memantine (Parsons et al., 1999), may be capable of attenuating craving and relapse rate in heroin addicts (Bespalov et al., 2001).

Antagonists of the NMDA subtype of glutamate receptors have been shown repeatedly to attenuate both development and expression of place preferences conditioned with various drugs of abuse (reviewed by Bisaga and Popik, 2000). In this paradigm, drug-conditioned responses are expressed as increased time spent in the drug-associated environment and are thought to represent an indirect measure of a drug’s rewarding effects (Carr et al., 1989). It has been reported that memantine blocked the expression of morphine- (Popik and Danysz, 1997) and cocaine- (Kotlinska and Biala, 2000) induced conditioned place preferences in rats.

The important feature of drug abuse pharmacotherapy is that it should selectively interfere with the drug-related responses while leaving other biologically significant appetitive behaviors intact. In experimental studies, food-maintained behavior is often taken as a baseline against which the drug-maintained behavior is assessed. For instance, experimental medications are required to alter drug self-administration while having little or no effects on food-seeking and eating (e.g., Mello et al., 1993). Similarly, in place conditioning studies, effects of the potential medications are assessed in both drug- and food-conditioned animals (Popik and Danysz, 1997; Papp et al., 2002).
There are at least two reasons to investigate the effects of NMDA receptor antagonists on behaviors maintained by other types of reinforcing stimuli than drugs of abuse. First, one of the commonly held views on the mechanism of compulsive drug seeking and taking expects both ‘drug’ (e.g. opiate-induced) and ‘natural’ (food, sex) rewards to be mediated by a common neuroanatomical pathway, most likely by the mesocorticolimbic systems (Di Chiara et al., 1998). If NMDA receptor antagonists have an effect on behaviors maintained by both drug and nondrug reinforcers, this could suggest a common mechanism or pathway involved in the processing of these stimuli. Secondly, NMDA receptor antagonists were shown to reduce the intensity of a variety of behaviors related to drug addiction and dependence (reviewed by Herman et al., 1995; Bisaga and Popik, 2000). As NMDA (and other glutamate) receptor antagonists are probed as potential candidates for developing an effective drug abuse medication, the specificity of their effects becomes of critical significance.

The present study aimed to establish place preferences conditioned with food, sexual encounter and morphine administration in male mice and to compare the effects of memantine on the expression of place preference responses. Mice have already been used in place conditioning experiments. However, with some exceptions (Hutcheson et al., 1998; Martin et al., 2000; Valjent and Maldonado, 2000; Manzanedo et al., 2001), most of the experiments were carried out using the ‘biased’ version of this procedure. In the ‘biased’ version, the rewarding treatment is paired with the initially nonpreferred environment, while its control condition is paired with the initially preferred environment. Because the use of the ‘biased’ procedure has many limitations (Bozarth, 1987; Spyraki, 1988), the present study involved an ‘unbiased’ procedure. In a conventional place conditioning study design, there is a potentially critical difference between drug versus nondrug conditioning protocols. For drug conditioning, animals are administered a drug before being placed into the to-be-conditioned environment. For nondrug conditioning, the unconditioned stimuli (e.g. food or receptive female) are typically presented after the animals were placed into the conditioning apparatus. This difference may bias the interpretation of the effect of memantine on the expression of drug- versus nondrug-conditioned place preferences (see Discussion). Thus, to avoid this potentially confounding difference in conditioning protocols, in the present study mice, were exposed to the nondrug unconditioned stimuli before being placed into the conditioning apparatus. This allowed us to eliminate the potential effects of memantine on the behavioral performance and/or consummatory phase of behaviors that were to be conditioned.

Method

Subjects

Male and female albino Swiss mice (Breeding Facility of the Institute of Pharmacology, Polish Academy of Sciences) weighing 25-30 g were housed separately in groups of 3-9 in standard plastic cages (43 × 27 × 15 cm), in a colony room maintained at a temperature of 21 ± 1°C and 50–70% humidity. Artificial light was provided daily from 07.00 hours to 19.00 hours, for all mice, except for those used in the sexual conditioning experiment, which were maintained under a reversed light/dark cycle (lights off at 07.00 hours, on at 19.00 hours). Unless indicated otherwise, food and water were freely available. Experimental procedures were approved by the Institutional Animal Care and Use Committee and were performed in accordance with the recommendations and policies of the US National Institute of Health Principles of Laboratory Animal Care (1996).

Apparatus

The conditioning apparatus consisted of three rectangular arms (30 × 15 × 20 cm) placed at 120° to each other, all of which were accessible from a triangular (central) platform (Popik et al., 2003). The apparatus was made of Metaplex and the three arms differed in distinctive visual, tactile and olfactory cues. One arm was white, had a black floor with small holes, and was marked with peppermint odor; one arm was black, had a rough white floor and was marked with anise odor; the final arm was black with a plain black floor, and had no odor. These distinct cues served as conditioned stimuli. The use of visual, olfactory and tactile cues ensured direct contact with the conditioned stimuli during the preference testing (Mucha et al., 1982). The guillotine doors, colored according to the respective wall colors, were inserted during the conditioning sessions and were removed during the pre- and post-conditioning tests. The ceiling of the three arms was made of transparent Plexiglas. During testing, the location of the mouse was monitored through a closed-circuit TV camera positioned directly above the apparatus. The testing room had dim indirect lighting, comprised of two 15W bulbs positioned about 1 m above the apparatus. A loudspeaker positioned above the apparatus delivered white noise. The apparatus was repeatedly washed and dried.

General procedure

The conditioned place preference experiments were conducted using procedure described previously (Popik et al., 2003). Each experiment consisted of five separate test days. During adaptation (Day 1), male mice were brought into the testing room, where the experimenter weighed and handled animals by moving them from one standard home cage to another, which was near the apparatus. The aim of this phase was to reduce the novelty and stress associated with handling, injections and exposure to the apparatus. During the
pre-conditioning test (Day 2), male mice were placed individually on to the central triangular platform of the apparatus, with free access to all three arms for 20 min. Time spent in each arm, as well as the number of arm entrances (a raw measure of locomotor activity), were recorded. Based on the results of this pre-conditioning test, two arms with the most similar preferences were identified individually for each mouse. One of these arms was later paired with the effects of ‘rewarding conditions’ and the other arm was paired with the respective ‘control conditions’ (see below). During the first conditioning session (Day 3), animals were confined to the first of the pre-assigned arms of the apparatus for 45 min. Before the placement, one group of the male mice was fed with standard laboratory food, another group was exposed to a receptive female, and the third group received an injection of morphine (active conditions). During the second conditioning session (Day 4), animals were confined to the second of the pre-assigned arms for 45 min. Before the placement, the first group of animals was not fed, the second group was exposed to a non-receptive female, and the third group was given saline injections (control conditions). Assignment to the treatment–arm pairings was counterbalanced, with half of the animals receiving treatment in the reverse order. The post-conditioning test (Day 5) was carried out in the manner identical to the pre-conditioning test. Twenty minutes prior to the post-conditioning test, mice were injected with either saline or memantine (7.5 mg/kg).

**Conditioning with food**

For the duration of this experiment, mice were deprived of food for 20 h/day. During the first 8 days, mice were adapted to the testing room, ‘feeding cages’ and the feeding procedure. Subjects were transferred into the testing room, adjacent to the animal facility room, and placed individually for 4 h (15.00–19.00 h) into the ‘feeding cages’ (standard plastic laboratory cages measuring 27.5 × 21.5 × 13 cm and equipped with standard lab chow, a bottle with tap water and sawdust bedding). The amount of laboratory food consumed was monitored by weighing it before and after feeding. At the end of this 8-day period, mice achieved ~80% of their original body weight and consumed an average of 1.9 ± 0.1 g of chow per 4-hour period. There were no differences among various treatment groups with regard to these measures. Preliminary data (not shown) indicated that mice deprived of food for 20 h consumed the majority of food during the first hour of the 4-hour feeding period. Nine days after the beginning of the food-deprivation procedure, the pre-conditioning test took place, followed by the conditioning sessions as described above. Mice were placed in the ‘feeding cages’, with or without food, for 45 min. Then subjects were transferred into the designated arm of the conditioning apparatus for 45 min. The control groups were not fed prior to either of the two conditioning sessions.

**Conditioning with sexual encounter**

Female mice were bilaterally ovariectomized under pentobarbital anesthesia 3–4 weeks prior to testing. All male mice used in this experiment were sexually naïve before they were given four screening tests for masculine copulatory behavior. Screening tests were conducted at 7-day intervals during the dark phase of the light–dark cycle. Each male mouse was placed into the testing chamber (50 × 50 × 50 cm) with sawdust bedding, for 20 min of habituation, followed by the introduction of a sexually receptive female for 60 min. Females were rendered sexually receptive by the administration of estradiol benzoate (20 μg), 48 h before the test, and progesterone (500 μg), 6 h before the test. During the screening tests, sexual behavior of male mice was recorded by a trained observer and only mice demonstrating at least three ejaculations in four screening tests were used in the subsequent conditioning experiments (approximately 95% of mice tested). The conditioning procedure was essentially the same as the one used in the food experiment, except that mice were transferred to the apparatus after the interaction with the female mice that lasted until the first ejaculation (receptive female conditioning) or for 15 min (nonreceptive female conditioning). Male mice that needed more than 15 min to achieve ejaculation (~10%) were not used. Male mice did not interact with the same female mouse more than once.

**Conditioning with morphine**

The design of experiments with morphine was essentially the same as for the food and sexual encounter conditioning procedures, with the exception that there were no pre-exposures to morphine. Saline and morphine (10 mg/kg) injections were given immediately before placement into designated arms of the place conditioning apparatus. Control groups received saline injections prior to both conditioning sessions. In order to reduce the number of mice, this experiment did not include a control group that would receive a memantine injection prior to the post-conditioning test in the nonmorphine condition. Such treatment conditions were already represented in the food or sexual conditioning experiments, which included control treatment groups that were not fed or were exposed to nonreceptive females, respectively, prior to each conditioning session. Moreover, our previous data indicate that memantine does not affect place preference of saline-conditioned mice (Popik et al., 2003).

**Drugs**

Both morphine hydrochloride (Polfa, Kraków, Poland) and memantine (Sigma-Aldrich, Poznan, Poland) were dissolved in sterile physiological saline and administered intraperitoneally (i.p.). The dose of morphine was calculated for the base form of the drug. Estradiol benzoate and progesterone (Sigma-Aldrich, Poznan, Poland) were prepared in sunflower oil and administered subcutaneously (s.c.). Injection volume was 10 ml/kg.
Data analysis
The times spent in all three arms of the conditioning apparatus before and after conditioning were subjected to statistical analyses. Additionally, the post-conditioning data were summarized as the difference in time spent in the designated arm relative to the pre-conditioning values (called ‘delta’). Data were analyzed using two- and three-way analyses of variance (ANOVA) with repeated measures whenever appropriate. Because of the unbalanced design with unequal cell sizes, the General Linear Model (GLM) procedure was applied (SAS-STAT software, SAS Institute Inc., Cary, North Carolina). The impacts of the following main factors were analyzed: (1) pre- versus post-conditioning test; (2) conditioning (food versus no food, receptive versus nonreceptive female); (3) memantine treatment (memantine versus saline). Because of the unbalanced design (see above), one- and two-way ANOVAs were used for the analysis of the morphine conditioning results (treatment groups: saline + saline, morphine + saline, morphine + memantine). One- and two-way ANOVAs were used to analyze the effect of treatments on the number of arm entrances recorded during the post-conditioning tests (due to technical reasons, such data were available only for food- and morphine-conditioned place preference experiments). The post-hoc Newman–Keuls test was applied to test between-group differences whenever indicated by ANOVA results.

Results
During the pre-conditioning test, the mean time spent by mice in the ‘to-be-conditioned’ arm ranged from 268 s to 343 s and did not differ significantly among the experiments (Table 1). The sham conditioning procedure did not affect the arm preference scores, as no change in time spent in the test arm was observed in mice that were pretreated with either saline or memantine prior to the test (Fig. 1, open and black bars). Conditioning with food, sexual encounter and morphine produced a significant increase in time spent in the designated arms (hatched bars) and successful conditioning was confirmed by ANOVA for all three experiments \[F(1,40) = 16.9, \ P < 0.001; \quad F(1,28) = 5.2, \quad P < 0.05; \quad F(2,31) = 5.3, \quad P < 0.01, \] respectively. Pretreatment with memantine (cross-hatched bars) blocked the expression of place preference conditioned with sexual encounter.

Table 1 Time spent in each arm of the conditioning apparatus before and after conditioning with food, sexual encounter or morphine

<table>
<thead>
<tr>
<th>Unconditioned stimulus</th>
<th>Saline/memantine</th>
<th>N</th>
<th>Test</th>
<th>Time spent per arm (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conditioned</td>
</tr>
<tr>
<td>Food</td>
<td>Saline</td>
<td>19</td>
<td>Pre</td>
<td>284.4 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>Memantine</td>
<td>8</td>
<td>Pre</td>
<td>304.8 ± 17.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>371.8 ± 17.6</td>
</tr>
<tr>
<td>No food</td>
<td>Saline</td>
<td>8</td>
<td>Pre</td>
<td>332.5 ± 13.4</td>
</tr>
<tr>
<td></td>
<td>Memantine</td>
<td>9</td>
<td>Pre</td>
<td>356.8 ± 13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>335.1 ± 21.0</td>
</tr>
<tr>
<td>Pre/post × conditioning</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,40) = 16.9)</td>
</tr>
<tr>
<td>Pre/post × memantine</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,40) = 0.6)</td>
</tr>
<tr>
<td>Pre/post × conditioning × memantine</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,40) = 0.8)</td>
</tr>
<tr>
<td>Receptive female</td>
<td>Saline</td>
<td>8</td>
<td>Pre</td>
<td>291.9 ± 19.4</td>
</tr>
<tr>
<td></td>
<td>Memantine</td>
<td>8</td>
<td>Pre</td>
<td>343.3 ± 16.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>328.3 ± 16.3</td>
</tr>
<tr>
<td>Nonreceptive female</td>
<td>Saline</td>
<td>8</td>
<td>Pre</td>
<td>307.4 ± 19.4</td>
</tr>
<tr>
<td></td>
<td>Memantine</td>
<td>8</td>
<td>Pre</td>
<td>343.5 ± 13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>329.6 ± 16.3</td>
</tr>
<tr>
<td>Pre/post × conditioning</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,28) = 5.2)</td>
</tr>
<tr>
<td>Pre/post × memantine</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,28) = 14.9)</td>
</tr>
<tr>
<td>Pre/post × conditioning × memantine</td>
<td></td>
<td></td>
<td></td>
<td>(F(1,28) = 5.5)</td>
</tr>
<tr>
<td>Morphine</td>
<td>Saline</td>
<td>10</td>
<td>Pre</td>
<td>268.2 ± 21.0</td>
</tr>
<tr>
<td></td>
<td>Memantine</td>
<td>13</td>
<td>Pre</td>
<td>311.9 ± 15.2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>11</td>
<td>Pre</td>
<td>315.6 ± 14.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>310.6 ± 18.2</td>
</tr>
</tbody>
</table>

*Saline or memantine (7.5 mg/kg) was injected once prior to the post-conditioning session. Values are means ± SEM.

\(^1P<0.05 \text{ versus pre-test}; \quad ^2P<0.01.\)
Previously paired with food consumption, successful significant preferences were observed in mice for places conditioned with food, sexual encounter and morphine in male mice. There were two major findings in this study. First, the NMDA receptor channel blocker, memantine, blocked the expression of place preference conditioned with morphine and sexual encounter, without affecting place preference conditioned with food. Morphine-conditioned place preference is a well-established experimental phenomenon (Carr et al., 1989). The present study confirmed that it can be observed readily in mice after a single conditioning trial in an unbiased conditioning procedure. However, to our knowledge, this paradigm has never been applied to study food- and sexual interaction-induced place preferences in mice, the species most commonly used in behavioral genetics research (e.g. Crawley et al., 1997). Moreover, the present design (three-arm apparatus) allowed us to demonstrate that place preference responses observed in these studies cannot be explained by the ‘novelty’ account (Bevins and Bardo, 1999), because we found no increase of the time spent in the ‘novel’ arms that could be related to the conditioning procedure and/or drug treatment.

Behaviors maintained by nondrug reinforcers are often used as baseline controls for analyzing the drug effects in a variety of experimental procedures, such as intravenous drug self-administration (Mello et al., 1993) or drug-conditioned place preference (Popik and Danysz, 1997; Papp et al., 2002). For example, Papp et al. (2002) demonstrated recently that the partial agonist at the glycine/NMDA site, ACPC (1-aminocyclopropane carboxylic acid), inhibited the acquisition and expression of morphine-induced conditioned place preference in rats. ACPC (200 mg/kg) affected neither the acquisition nor expression of place preferences conditioned with natural reinforcers (food, sucrose, social interaction and novelty). Similarly, Popik and Danysz (1997) evaluated the effects of memantine (7.5 mg/kg) on the expression of morphine- and food-conditioned place preferences in rats, and found that morphine, but not the food-conditioned response, was inhibited by memantine, indicating different mechanisms of food- and drug-induced conditioned place preference or, alternatively, a different sensitivity of these responses to NMDA receptor blockade.

In contrast to previous studies with food place conditioning (Popik and Danysz, 1997; Papp et al., 2002), in the present study mice had no opportunity to consume food while in the place-conditioning apparatus. Instead, they were first allowed to consume food for a specified period of time (as in drug-conditioning sessions when morphine was given before mice were exposed to the conditioning environment) and then were placed into the conditioning apparatus. Similarly, unlike the previous place-conditioning studies with sexual behavior (Miller and Baum, 1987; Hughes et al., 1990; Mehrara and Baum, 1990), in the present study conditioning sessions did not start until the interaction with the females (receptive or nonreceptive) was complete. Thus, the design of our experiments was

\[ F(1,28) = 14.9, P < 0.001 \] and morphine \[ F(2,31) = 5.5, P < 0.01 \], but not with food \[ F(1,40) = 0.6, \text{NS} \]. A similar analysis conducted for the time spent in the sham-conditioned or novel arms did not reveal any significant increases in the time spent in these compartments as a result of conditioning or memantine treatment (Table 1). However, as indicated in the Table 1, there was a significant interaction between pre-/post-conditioning test and food-conditioning factors for a neutral arm. This interaction was not due to the increased time spent in the neutral arm as a result of the conditioning procedure. Instead, for both food-conditioned groups (treated with saline or memantine prior to the post-conditioning test), the time spent in the neutral arm was slightly decreased. The mean numbers ± SEM of entrances into the ‘rewarded’ arm recorded during the post-conditioning test for mice fed/treated with placebo, fed/treated with memantine, nonfed/treated with placebo and nonfed/treated with memantine were: 41.9 ± 2.53, 35.5 ± 1.86, 42.6 ± 5.08 and 35.3 ± 5.63, respectively. There were no significant differences among treatments \[ F(3,40) = 1.0, \text{NS} \]; the effects of memantine were also not significant \[ F(1,40) = 2.96, \text{NS} \]. For mice treated with morphine/placebo, morphine/memantine and placebo/placebo, these values were 31.0 ± 1.96, 38.7 ± 3.76 and 30.5 ± 1.91, respectively, and did not differ from each other \[ F(2,31) = 2.68, \text{NS} \].

**Discussion**

There were two major findings in this study. First, significant preferences were observed in mice for places previously paired with food consumption, successful sexual encounter and morphine administration. Secondly, the NMDA receptor channel blocker, memantine, blocked the expression of place preference conditioned with morphine and sexual encounter, without affecting place preference conditioned with food. Morphine-conditioned place preference is a well-established experimental phenomenon (Carr et al., 1989). The present study confirmed that it can be observed readily in mice after a single conditioning trial in an unbiased conditioning procedure. However, to our knowledge, this paradigm has never been applied to study food- and sexual interaction-induced place preferences in mice, the species most commonly used in behavioral genetics research (e.g. Crawley et al., 1997). Moreover, the present design (three-arm apparatus) allowed us to demonstrate that place preference responses observed in these studies cannot be explained by the ‘novelty’ account (Bevins and Bardo, 1999), because we found no increase of the time spent in the ‘novel’ arms that could be related to the conditioning procedure and/or drug treatment.
similar to those used by Agmo and co-workers (Agmo and Berenfeld, 1990; Agmo and Marroquin, 1997) and Lett et al. (2000), in that the unconditioned stimuli were presented before rather than during the exposure to the conditioned environment. Although this approach may resemble the backward conditioning paradigm, most evidence suggests that backwardly conditioned cues become inhibitory (Hall, 1984). In contrast, in the present study significant conditioned place preferences were observed in experiments with both food consumption and sexual encounters [see, however, Maes and Vossen (1993), who reported that such a conditioning procedure is less likely to produce the place preference response compared with the conventional protocols, in which the animals consume food while in the to-be-conditioned environment].

Memantine inhibited the expression of place preferences conditioned with morphine and sexual encounter, while having little or no effect on the expression of food-conditioned responses. These findings are somewhat surprising, since most, if not all, previous studies reported the lack of effects of NMDA receptor antagonists on place preferences conditioned with nondrug reinforcers (e.g. Popik and Danysz, 1997; Papp et al., 2002). Inhibitory effects on morphine-induced place preference confirm the previously reported inhibition of morphine- and cocaine-conditioned place preference by memantine in rats (Popik and Danysz, 1997; Kotlinska and Biala, 2000) and are also consistent with earlier reports on the decreased expression of drug-conditioned place preference by other NMDA receptor antagonists (Bespalov, 1996; Delpozo et al., 1996; Mead and Stephens, 1999; Papp et al., 2002). Thus, the effects of memantine are most likely attributable to the NMDA receptor blockade.

To explain these differential effects of memantine, one should analyze the differences of drug, sexual interaction and food reinforcers. Satiety thresholds exist for various types of drug and nondrug reinforcing stimuli (e.g. Hilliard and Domjan, 1995; Tsibulsky and Norman, 1999) and the deprivation state may facilitate responding to both drug- and nondrug-related stimuli, although it may not be a necessary condition. In contrast to the fact that the pre-existing drug dependence is not a prerequisite for acquisition and maintenance of drug self-administration behavior (Katz, 1989), mice consume regular types of food only when in the deprived state and it is commonly assumed that food intake in food-restricted subjects is ‘driven mainly by energy needs, with taste or palatability being relatively less important’ (Zhang et al., 1998, p. 909). Since both humans and animals will consume highly palatable food even in the nondeprived (satiated) state, one could argue that the place preference established with palatable food in nondeprived subjects would be a more adequate comparison condition (e.g. for evidence on the differential effects of naloxone on the intake of palatable and regular types of food, see Segall and Margules, 1989; Levine et al., 1995). Thus, future studies should probably focus on sucrose as the potential candidate for analyzing conditioned place preference responding in nondeprived subjects (e.g. as in Agmo and Marroquin, 1997; Papp et al., 2002). However, one should note that in a study by Papp and colleagues (2002), place preferences conditioned with sucrose were not affected by ACPC, which is a glycine/NMDA receptor partial agonist (Popik et al., 1995), rather than a full antagonist of NMDA receptor complex. For example, it was observed in electrophysiological studies that ACPC demonstrated intrinsic activity of 92% at the glycine/NMDA receptor (Karczubicha et al., 1997) while acting as the low-affinity competitive antagonist at the glutamate site (Nahum-Levy et al., 1999). Thus, it is likely that the effects of ACPC are not necessarily identical to those seen for NMDA receptor antagonist with little or no intrinsic activity. Moreover, Papp et al. (2002) reported that ACPC did not affect drug-induced place aversions and had no effect on the expression of amphetamine-conditioned place preference. This also contrasts with some previous studies suggesting that NMDA receptor antagonists may be capable of inhibiting both conditioned place aversions as well as amphetamine-conditioned place preference (Higgins et al., 1992; Bespalov, 1996; Popik and Danysz, 1997; Mead and Stephens, 1999). On the other hand, it has been reported that while memantine blocked the expression of cocaine-induced place preference (Kotlinska and Biala, 2000), neither the glycine/NMDA site antagonist L-701,324 (Kotlinska and Biala, 1999), the partial agonist at the glycine/NMDA site, ACPC (Papp et al., 2002), nor the uncompetitive NMDA receptor antagonist, dizocilpine (Cervo and Samanin, 1995), were able to affect cocaine-induced place preferences. Other data indicate that while ACPC did not block expression of amphetamine-induced place preference (Papp et al., 2002), the competitive NMDA receptor antagonist (±)-CPP (conditioned place preference) in rats (Bespalov, 1996) and the glycine/NMDA antagonist 7-chloro-4-hydroxy-3-(2-phenoxy) phenyl-2(1H)-quinolone in mice, did block expression of amphetamine-induced place preferences (Mead and Stephens, 1999). These data indicate that various NMDA receptor antagonists do not affect the expression of psychostimulant-induced place preferences uniformly, with the cocaine-induced response being most difficult to inhibit. Further data on the effects of memantine on expression of drug-induced place preferences are necessary. With regard to the natural rewards, while the present study demonstrates an initial positive effect, it remains to be evaluated whether NMDA receptor antagonists inhibit the expression of place preferences conditioned with non-drug reinforcers in the nondeprived state(s).
One of the behavioral models of motivation proposes that two separate systems contribute to reward processes. The first system is associated with the state of nondeprivation (e.g., satiety) and the second system is associated with the state of deprivation (e.g., hunger in the case of food reward). This distinction is substantiated by the differences between behaviors acquired in the nondeprivation and deprivation state. A number of studies have suggested that these systems (states) are readily discriminated by the neurobiological and pharmacological interventions that selectively alter one of the systems/states (e.g., Nader et al., 1997). We thus hypothesize that the observed pattern of the effects of memantine is due to the selective interaction with motivated behaviors acquired in the nondeprived state. In addition, we further suggest that in certain cases, food-reinforced behaviors may not serve as appropriate controls for drug-maintained behaviors.

There are alternative hypotheses that may explain the differential effect of memantine on the expression of conditioned place preferences induced by various stimuli. Experimental evidence suggests that separate neural circuits may be involved in the mediation of behaviors maintained by different reinforcers. For instance, nonoverlapping populations of neurons are known to encode information about abused drugs (cocaine) and food reinforcement (Carelli et al., 2000). Moreover, it is possible that cocaine activates populations of cells that normally process information about the reinforcing properties of sexual behavior (Childress et al., 1998). Although cocaine-responsive neural circuits may not be the same as those for opiate drugs (Chang et al., 1998), it is still worth noting that the present results support the view on common mechanisms encoding behaviors maintained by drug and sexual reinforcement. Furthermore, reinforcing stimuli may differ with regard to their novelty, i.e., the extent to which they were present in an individual subject’s history. These differences may have correlates in neural organization of behaviors maintained by these reinforcers. For instance, cocaine self-administration produced significant changes in striatal levels of dopamine transporter, and patterns of these changes depended on the duration of drug exposure (Letchworth et al., 2001). Finally, food consumption is not an ‘optional’ activity as is sexual interaction, or, especially, drug seeking and taking in nondependent subjects.

Attenuation of place preferences conditioned with both morphine and sexual encounter, taken together with above-mentioned data, could suggest that NMDA receptor antagonists may impair discrimination between the environments, produce general motivational deficits and/or nonspecific disruption of the test performance. However, such interpretation is not likely, for several reasons. First, memantine lacked any significant effects in the food-conditioning studies. Secondly, the numbers of arm entrances recorded after the post-conditioning test in food- and morphine-conditioned place preference experiments did not suggest any potentially important effects of memantine on locomotor activity. Also, it should be noted that, in previous studies, NMDA receptor antagonism was found to inhibit the expression of drug-conditioned place preference irrespective of its effect on locomotor activity (Popik and Kolasiewicz, 1999). Finally, although it is possible that memantine could make the retrieval of place preference state-dependent, the studies by Tschentke and Schmidt (1997) and Papp et al. (2002) provided arguments against a state-dependency explanation.

One of the limitations of the present study was that a single dose of memantine was used. However, this dose (7.5 mg/kg) is well within the dose range that is known to result in brain concentrations (~1 μmol/l) sufficient to block NMDA receptors (Parsons et al., 1999). Tests with higher doses of memantine are less desirable in behavioral experiments, since they may result in the loss of selectivity towards the NMDA receptors, as well as in a substantial change in the behavioral profile. At doses above 10 mg/kg, memantine is more likely to produce behavioral effects typical for phencyclidine-like NMDA receptor antagonists (Grant et al., 1996). Moreover, there is no experimental evidence that would suggest significant sensorimotor, attention and discrimination deficits produced by memantine in mice, at the dose level used in the present study. In addition, at the same dose, memantine itself produces neither conditioned place preference nor aversion (Popik and Danyts, 1997) that could explain its effects on drug- and nondrug-induced place preference. The results of this study demonstrate that memantine blocked the expression of place preferences conditioned with morphine, as well as those conditioned with successful sexual encounter in male mice. Altogether, contrary to some previous expectations, effects of NMDA receptor blockade may not be limited to drug-reinforced behaviors.

References
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