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Morphine Tolerance and Dependence in Mice with History of Repeated Exposures to NMDA Receptor Channel Blockers

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DRAVOLINA, O. A., I. V. BELOZERTSEVA, I. A. SUKHOTINA AND A. Y. BESPALOV. *Morphine tolerance and dependence in mice with history of repeated exposures to NMDA receptor channel blockers.* PHARMACOL BIOCHEM BEHAV **63**(4) 613–619, 1999.—Mice were subjected to two successive treatment protocols: first with NMDA receptor channel blockers (14 days, once a day) and second with morphine (5 mg/kg, 8 days, once a day). Treatment with the higher doses of dizocilpine (1 mg/kg), memantine (30 mg/kg), and MRZ 2/576 (30 mg/kg) upon discontinuation revealed only minor behavioral abnormalities attributable to the state of withdrawal. Following repeated administration of low-dose morphine, tolerance to morphine analgesia developed in mice preexposed to dizocilpine (1 mg/kg but not 0.3 mg/kg) but not memantine (10 and 30 mg/kg), MRZ 2/579 (10 and 30 mg/kg), or saline. There were no signs of morphine dependence in any treatment group. Overall, the present study found only minor effects of the subchronic administration of high doses of NMDA receptor channel blockers, suggesting that clinical use of NMDA receptor channel blockers such as memantine will not be accompanied by increased propensity to induction of morphine tolerance and dependence. © 1999 Elsevier Science Inc.

Dizocilpine Memantine MRZ 2/579 Morphine Tolerance Dependence Analgesia Social interaction test Mice

IT is widely accepted that learning and memory involve *N*-methyl-D-aspartate (NMDA) receptor-dependent mechanisms. Beside the prominent role of NMDA receptor in synaptic plasticity (7), vast experimental evidence implicates NMDA receptors in the development of drug tolerance and dependence (13,33). On the other hand, there is little doubt that repeated administration of NMDA receptor ligands themselves may result in altered sensitivity to their pharmacological effects [e.g., sensitization to locomotor stimulant effects; (39,41)]. Considering current perspectives on clinical use of NMDA receptor antagonists (14), one should note that knowledge of the functional consequences of chronic NMDA receptor blockade is limited.

Review of molecular data indicates that studies with repeated exposures to NMDA receptor antagonists yield quite controversial results. The outcome of the experiments (i.e., receptor upregulation, downregulation, or no effect) significantly depends on the type of antagonist used (noncompetitive vs. competitive), duration of exposure to the antagonist (3–28 days), dosing, and schedule of administration (intermittent vs. continuous) (2,16,20,21,22,29,32,36,38). Analysis of the reports relevant to the experimental design used in the present study reveals that repeated dizocilpine (MK-801) exposures increased both NMDAR1 mRNA (21,32) and [^{3}H]dizocilpine binding [(23,38); for memantine, see (16)]. Expression of non-NMDA subtypes of glutamate receptors is either unaffected (38) or affected in more complex ways (21). In addition, there is some evidence that NMDA receptor channel blockers may produce selective changes in binding to NMDA receptors, but not to other glutamate binding sites [i.e., AMPA; (9)].

Similarly, behavioral data also point to facilitated NMDA receptor function following repeated administration of NMDA receptor blockers. For instance, treatment with PCP (6),

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CGP39551, CGP37849 (29) resulted in greater behavioral ratings of seizure activity after NMDA administration [see (4) for conflicting data with the competitive antagonist CGS 19755]. Moreover, following dizocilpine administration the excitotoxic potential of NMDA (23) and quinolinic acid (26) was found to be increased. Assuming that clinical application of NMDA receptor antagonists would imply repeated administration schedules, such data may appear very distressing. Development of drug tolerance and dependence may also be affected in subjects with a history of NMDA receptor antagonist administration because earlier studies have implicated NMDA receptors in the mechanisms of drug tolerance and dependence [e.g., (13)]. Indeed, repeated exposures to dizocilpine, ketamine, and dextromethorphan were shown to increase the intensity of opioid abstinence syndrome [(18,19); see, however, (34) for contrasting findings with competitive antagonist LY274614].

The present study sought to further evaluate the severity of morphine tolerance/dependence in mice repeatedly exposed to NMDA receptor channel blockers. In addition to dizocilpine (MK-801), selected as a representative high-affinity NMDA receptor channel blocker (27,40), we tested memantine and MRZ 2/579, which have a lower affinity for the channel site of the NMDA receptor and show faster blocking and unblocking kinetics (27,28). A recent study by Hesselink et al. (16) demonstrated that [^{3}H]dizocilpine binding may be increased after 14 days of daily memantine injections (20 mg/kg). It is noteworthy that it is yet unclear whether repeated administration of NMDA receptor channel blockers with varying degrees of affinity would differentially affect expression of NMDA receptor channel site or NMDA receptor-dependent behaviors. For instance, repeated administration of ADCI, an ultralow-affinity channel blocker, did not affect [^{3}H]dizocilpine binding (30).

Lower doses of dizocilpine and memantine selected for the present study were aimed to be within clinically relevant range, as discussed earlier (15,16). In the case of MRZ 2/579, the dose range was the same as for memantine, because these compounds essentially exhibit similar parameters of interaction with the binding site with IC_{50} values of 1.4 and 2.3 M, respectively (1,3,24,27,28). Because of the variability of molecular data, selected compounds were to be administered over a period of 2 weeks, suggested by behavioral studies where significant tolerance and dependence developed to the effects of dizocilpine over this period of time (10,15,37).

METHOD

Subjects

Three hundred and eight adult male drug- and experimentally naive Swiss mice (22–29 g) bred at the State Breeding Farm "Rappolovo" (St. Petersburg, Russia) were used. Animals were housed in groups (n = 10), with food and water available ad lib. All experiments were conducted during the light period of a 12 L:12 D cycle (lights on at 0900 h). Experiments were approved by the Institutional Ethics Committee of Pavlov Medical University, and were performed in accordance with the recommendations and policies of the U.S. National Institutes of Health Guidelines for the Use of Animals.

Procedure

Immediately upon the arrival from the breeding center mice were housed in groups of 10. Preliminary social interaction tests and behavioral screening were conducted to characterize baseline social hierarchy in the groups, and to remove animals that exhibited aggressive behaviors (bites, attacks, threats) toward their cage mates. Experiments started at least 7 days after the arrival from the breeders.

On days 1 through 14 repeated injections of either NMDA receptor channel blocker or saline were administered once a day. Treatment groups were as follows: 1, dizocilpine, 0.3 mg/kg; 2, dizocilpine, 1 mg/kg; 3, memantine, 10 mg/kg; 4, memantine, 30 mg/kg; 5, MRZ 2/579, 10 mg/kg; 6, MRZ 2/579, 30 mg/kg; and 7, saline.

On day 15, 24 h after the last antagonist/saline injection, mice were tested using the social interaction (resident–intruder) test, and then pain thresholds were detected by means of the tail-flick test (see below for details). Immediately after the tail-flick test and on each day through day 22, mice were administered either morphine (5 mg/kg, SC) or saline. There was a total of 11 treatment groups (see Table 1 for treatment assignments and group sizes).

Twenty-four hours after the last morphine/saline injection (day 23) pain thresholds were detected by means of the tail-flick test. Saline was injected immediately after the initial measurement of baseline tail-flick latency. Thirty minutes after the saline administration tail-flick latencies were reassessed, and immediately thereafter mice were treated with the test dose of morphine (5 mg/kg). The third tail-flick test was held 30 min after morphine injection.

On day 24 (24 h after morphine injection during the tail-flick test), mice were injected with 5 mg/kg of morphine and 30 min later with 0.1 mg/kg of naloxone. Fifteen minutes after naloxone injection mice were retested using the social interaction test.

To achieve the reliability of the behavioral measurements, three independent observers, blinded to treatment conditions, recorded behaviors.

All injections and tests were conducted between 1300 and 1700 h. Immediately after the injections animals were placed

TABLE 1

TREATMENT CONDITIONS AND GROUP SIZES

Drug	Dose	MOR/SAL	Initial	Day 15	Day 23/24
Saline	—	SAL	21	20	20
Saline	—	MOR	20	20	20
Dizocilpine	0.3	SAL	20	20	20
Dizocilpine	0.3	MOR	20	19	19
Dizocilpine	1	SAL	21	19	19
Dizocilpine	1	MOR	21	20	20
Memantine	10	SAL	20	20	20
Memantine	10	MOR	20	20	19
Memantine	30	SAL	20	20	20
Memantine	30	MOR	22	22	21
MRZ 2/579	10	SAL	20	20	20
MRZ 2/579	10	MOR	20	17	17
MRZ 2/579	30	SAL	21	19	19
MRZ 2/579	30	MOR	22	21	21

Mice received injections of dizocilpine, memantine, MRZ 2/579, or saline once a day for 14 days followed by administration of either morphine (MOR) or saline (SAL) for 8 days. Tests were conducted 24 h after the last NMDA receptor channel blocker injection (day 15) and 24/48 h after the last morphine/saline injection (days 23 and 24). Group sizes are indicated as they were before drug administration and test days.

back into the home cages. Injections were given in the animal facility while tests were carried out in the experimental rooms.

An additional experiment was designed to assess morphine tolerance and dependence in groups of mice exposed to different regimens of repeated morphine administration (no injections of NMDA receptor channel blockers). Each of the four groups of mice ($n = 20$; not shown in Table 1) was subjected to treatment and testing protocol identical to the one described above, with the following exceptions. On days 15–22, mice were administered saline, 5, 10, or 20 mg/kg of morphine. On day 23, morphine doses of 10 and 20 mg/kg were administered in two injections: 5 mg/kg was administered prior to the tail-flick test, and the rest was supplemented after the tail-flick test.

Tail-Flick Procedure

A mouse's tail (about 1 cm from the base) was exposed to a focused heat source (300-W white bulb). By withdrawing or removing the tail from the path of the stimulus and thereby exposing a photocell located in the apparatus ("Farmakolog," St. Petersburg, Russia) immediately below the tail, the mouse could terminate the noxious stimulation and the reaction time was then recorded. An animal that failed to respond before 10 s (cutoff time) was removed from the apparatus and assigned a latency of 10 s. Stimulation intensity was adjusted so that the baseline tail-flick latency for drug-naive mice was between 2.8 and 3.8 s. During each of the test days tail-flick latencies were measured three times for each subject. Mice were returned to their home cages after each injection and/or tail-flick test.

Social Interaction Test

Behavioral observations were done in the home cages ($300 \times 200 \times 200$ mm) covered with a transparent plastic lid and with both food hoppers and bottles with drinking water removed, and there were 10 g of standard food chow (d = 9–10 mm) spread all over the cage and mixed with sawdust bedding. After a 1-min adaptation period, a group-housed nonaggressive male intruder (randomly selected from the pool of 90 mice) was placed into the home cage of an experimental subject. During the next 4 min of observation, duration and sequences of 40 items of experimental mouse's behavior (acts and postures) were recorded by means of the customized PC-based data acquisition system. The behavioral inventory included agonistic behaviors, sociability items, various individual behaviors, as well as a number of signs commonly used in scoring opioid withdrawal syndrome (i.e., jumping, stretching, "wet-dog"–like shaking).

After the social interaction test was completed, intruders were returned to its home cage while experimental subjects were placed individually into the glass cylinders (h = 200 mm, d = 120 mm; covered with a transparent, perforated lid) for 15 min. The following parameters were recorded during the 15-min observation period: number of jumps, stretches, "wet-dog"–like shakes and the presence of ptosis, piloerection, diarrhea, chewing, salivation, genital grooming, and tremor of forelimbs. Cylinders had a single-use disposable paper floor, and were cleaned and deodorized after each test.

Drugs

Morphine hydrochloride ("Endocrinnyj Zavod," Moscow, Russia), memantine (1-amino-3,5-dimethyladamantane), and MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride; both from Merz+Co., Frankfurt am Main, Germany), dizocilpine maleate [(+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate; (+)MK-801] and naloxone hydrochloride (both from Research Biochemicals International, Natick, MA) were dissolved in physiological saline. Morphine, naloxone, and their vehicles were injected subcutaneously (SC), while dizocilpine, memantine, MRZ 2/576, and their vehicles were administered intraperitoneally (IP). Injection volume was 10 ml/kg. Doses are based upon the forms of the drugs listed above.

Data Analysis

For social interaction test data, relative durations [(cumulative time of the element expression)/(session duration) $\times$ 100%] and relative frequencies (amount of times the element was expressed)/(total number of behavioral counts per session) $\times$ 100%] of each of the 40 behavioral elements were compared across treatment groups. For the sake of clarity and brevity, results of the analysis are represented using only the relative durations of the selected clustered elements, for example, sociability (nose/body/genital sniffing, grooming of the partner, huddling, passive contact, self-exposing for grooming, approaching, crawling over/under the partner), static (static sitting, lying), and defense (sideway postures, upright postures, pushing, freezing, shriveling, retreats, postures on the back).

Tail-flick latencies obtained on day 23 were converted to percentage of maximal possible effect. The individual mouse values of the percent of analgesia were calculated according to the formula: $(T_{EXP} - T_{BL}) \times 100/(10 - T_{BL})$, where T_{EXP} is the test tail-flick latency (s), and T_{BL} is the baseline latency (s).

Data were analyzed using SAS-STAT software (ver. 6.11, SAS Institute, Cary, NC). Following rank transformation, data were subjected to the multivariate analysis of variance (ANOVA) adjusted for an unbalanced design with unequal group sizes. A repeated-measures design was applied wherever needed (e.g., tail-flick test on day 23). Tukey's test was used for post hoc pairwise group comparisons wherever indicated by ANOVA results.

RESULTS

Tail-Flick Tests

On day 15 (NMDA receptor channel blocker withdrawal), significant differences were observed in tail-flick latencies across treatment groups (Table 2). Mice repeatedly exposed to dizocilpine (1 mg/kg), memantine (30 mg/kg), and MRZ 2/579 (30 mg/kg) had shorter reaction latencies than mice treated with saline or lower doses of the drugs [dizocilpine: $F(2, 95) = 3.1$, $p < 0.05$; memantine: $F(2, 99) = 4.0$, $p < 0.05$; MRZ 2/579: $F(2, 94) = 8.3$, $p < 0.01$].

On day 23 (morphine tolerance test day), tail-flick latencies following saline challenge (3.3 $\pm$ 0.0 s) were not different from baseline levels (3.3 $\pm$ 0.0 s; $n = 276$). There were no differences between treatment groups in tail-flick performance following saline administration, $F(1, 204) = 0.8$. Tests conducted in drug-naive mice (repeatedly treated with saline instead of both NMDA receptor channel blocker and morphine, $n = 20$) indicated that the acute treatment with morphine (5 mg/kg) produced significant analgesia (mean tail-flick latency: 8.2 $\pm$ 0.5 s) and 50% of mice failed to respond before 10 s (cutoff time). Similar results were obtained in mice repeatedly exposed first to NMDA receptor channel blockers and then to saline instead of morphine (dizocilpine: 7.8 $\pm$ 0.5 s; memantine: 8.3 $\pm$ 0.4 s; MRZ 2/579: 8.5 $\pm$ 0.4 s).

TABLE 2

TAIL-FLICK REACTIVITY IN MICE REPEATEDLY TREATED WITH NMDA RECEPTOR CHANNEL BLOCKERS

Drug	Dose	Test
Saline	—	3.3 ± 0.1
Dizocilpine	0.3	3.5 ± 0.1
Dizocilpine	1	3.1 ± 0.1†
Memantine	10	3.4 ± 0.2
Memantine	30	3.1 ± 0.1†
MRZ 2/579	10	3.6 ± 0.1
MRZ 2/579	30	2.9 ± 0.1*†

Data are represented as mean (± SEM) tail-flick latency. Mice received injections of dizocilpine, memantine, MRZ 2/579, or saline once a day for 14 days. Tail-flick test was conducted 24 h after the last injection.

* and † $p < 0.05$ (Tukey's test) compared to saline group and low dose treatment group, respectively.

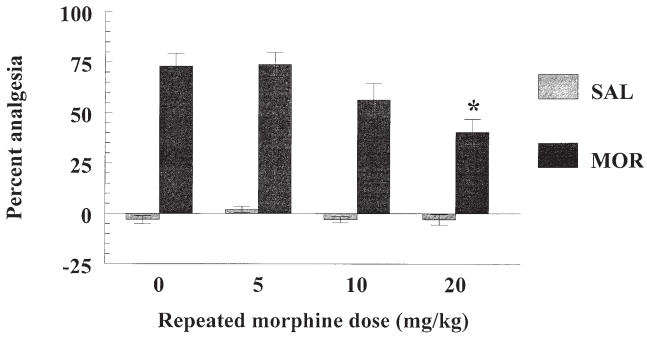

FIG. 2. Development of morphine tolerance in mice exposed to different regimens of morphine administration. Tail-flick latencies were assessed following acute 5 mg/kg of morphine injection in mice repeatedly administered with morphine (5, 10, or 20 mg/kg, SC once a day, 8 days) or saline. Data are expressed as mean (±SEM) percent of analgesia. *$p \leq 0.05$ (Tukey's test), compared to corresponding group treated with saline (instead of morphine).

Repeated administration of morphine resulted in a significant tolerance to morphine analgesia in the group that received dizocilpine, $F(1, 93) = 5.6$, $p < 0.05$, but not memantine, MRZ 2/579 or saline prior to tolerance induction protocol (Fig. 1). Analgesic activity of the test dose of morphine in the group treated with 1 mg/kg of dizocilpine was 47.1 ± 6.1% from the maximal possible effect compared to 67.1 ± 7.9% in corresponding dizocilpine-treated controls (repeated injections of saline instead of morphine).

An additional experiment conducted in the NMDA receptor channel blocker naive mice (Fig. 2) demonstrated that analgesic activity of the test dose of morphine (5 mg/kg) is significantly attenuated in mice exposed to repeated injections of 20 but not 5 or 10 mg/kg of morphine, $F(3, 76) = 5.5$, $p < 0.01$.

Social Interaction Tests

Behavioral effects of discontinued administration of NMDA receptor channel blockers were tested on day 15 (Table 3). Intensity of the social contacts with the intruder (sociability) was differentially affected by the dose regimen: low-dose regimens tended to increase the duration of social contacts, while high-dose regimens reduced the sociability. Statistical analysis confirmed significant effect only for 30 mg/kg of MRZ 2/579, $F(2, 67) = 6.4$, $p < 0.01$, but not dizocilpine or memantine. Repeated exposures to memantine and MRZ 2/579 but not dizocilpine led to the decreased rearing activity [memantine: $F(2, 67) = 6.3$, $p < 0.01$; MRZ 2/579: $F(2, 67) = 10.4$, $p < 0.01$; dizocilpine: $F(2, 67) = 2.7$, $p = 0.07$], while these mice spent more time displaying static behavioral elements [memantine: $F(2, 67) = 6.8$, $p < 0.01$; MRZ 2/579: $F(2, 67) = 3.0$, $p = 0.06$; dizocilpine: $F(2, 67) = 2.6$, $p = 0.08$]. Finally, mice treated with dizocilpine, memantine, and MRZ 2/579 exhibited less digging compared to mice treated subchronically with saline [dizocilpine: $F(2, 67) = 5.0$, $p < 0.01$; memantine: $F(2, 67) = 8.4$, $p < 0.01$; MRZ 2/579: $F(2, 67) = 4.3$, $p < 0.05$].

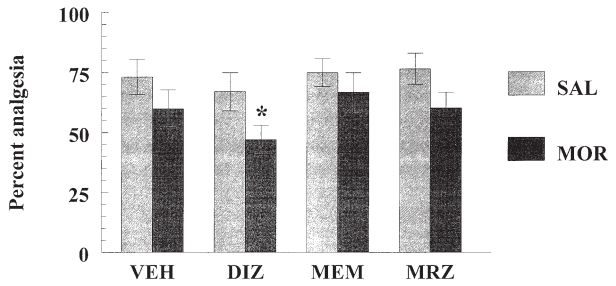

FIG. 1. Development of morphine tolerance in mice repeatedly exposed to NMDA receptor channel blockers. Tail-flick latencies were assessed following acute 5 mg/kg of morphine injection in mice repeatedly administered with morphine (MOR; 5 mg/kg, SC once a day, 8 days) or saline (SAL). Prior to the tolerance induction period, dizocilpine (DIZ, 1 mg/kg, IP), memantine (MEM, 30 mg/kg, IP), MRZ 2/579 (MRZ, 30 mg/kg, IP), or saline (SAL) were administered for 14 days, once a day. Data are expressed as mean (±SEM) percent of analgesia. *$p \leq 0.05$ (Tukey's test), compared to corresponding group treated with saline (instead of morphine).

On day 24 (morphine dependence test day), in mice with a history of repeated exposures to low-dose morphine and NMDA receptor channel blockers, there were no observed behavioral signs that could be attributed to the state of morphine withdrawal (e.g., "wet-dog"–like shaking, stretching, jumping, defense behaviors, etc.). Data on defense behaviors are presented in Table 4. No treatment group exhibited appreciable levels of aggressive or defensive behaviors either during pretests or during tests.

Meanwhile, control experiments found significant facilitation of defense behaviors in mice exposed to higher levels of repeated morphine [i.e., 10 and 20 mg/kg; Table 4; $F(3, 52) = 4.7$, $p < 0.01$]. These mice spent up to 25% of the test displaying defensive acts and postures, although there were no other withdrawal signs such as "wet-dog"–like shaking, stretching, or jumping.

DISCUSSION

Discontinuation of the 2-week long administration of NMDA receptor channel blockers revealed higher pain reactivity in mice treated with high doses of dizocilpine (1 mg/kg), memantine (30 mg/kg), and MRZ 2/576 (30 mg/kg), but no significant effect was observed at lower doses. Apparently, this facilitation of nociceptive responses faded over next 8 days, as evidenced by comparison of pain thresholds in mice

TABLE 3

BEHAVIORAL EFFECTS OF DISCONTINUATION OF 14-DAY-LONG ADMINISTRATION OF NMDA RECEPTOR CHANNEL BLOCKERS

Drug	Dose	Sociablility	Static	Rearing	Digging
Saline	—	10.3 ± 1.7	0.7 ± 0.3	18.3 ± 1.4	2.4 ± 0.4
Dizocilpine	0.3	8.0 ± 1.7	0.1 ± 0.1	13.7 ± 1.4	1.5 ± 0.5
Dizocilpine	1	10.9 ±1.5	6.1 ± 2.6	17.1 ± 1.9	0.5 ± 0.1*
Memantine	10	15.8 ± 2.5	0.3 ± 0.3	15.8 ± 1.3	1.5 ± 0.6
Memantine	30	7.7 ± 1.2	8.4 ±2.8*†	11.9 ±1.4*	0.3 ± 0.1*†
MRZ 2/579	10	14.8 ± 1.8	0.5 ± 0.2	13.9 ± 1.5	3.3 ± 1.1
MRZ 2/579	30	6.0 ± 1.0#	3.9 ± 1.8†	11.1 ± 0.8*	0.9 ± 0.2*†

Data are represented as mean (± SEM) duration of the behavior. Mice received injections of dizocilpine, memantine, MRZ 2/579, or saline once a day for 14 days. Social interaction test was conducted 24 h after the last injection.

* and † $p < 0.05$ (Tukey's test) compared to saline group and low-dose treatment group, respectively.

treated with saline instead of morphine (day 23). In addition to altered pain thresholds, drug-treated mice exhibited less digging and rearing activity but more static behavioral elements compared to mice treated subchronically with saline. These effects may be regarded as signs of NMDA receptor antagonist withdrawal syndrome. However, one should note that it is unlikely that the observed behavioral alterations (including hyperalgesia) are clinically relevant, because doses of antagonists used were very high and produced severe behavioral intoxication. Indeed, such extreme treatment regimens are not justified by the requirements set by the data on clinically relevant concentrations. For instance, cerebrospinal fluid concentrations of therapeutic doses of memantine in patients treated for Parkinson's disease and dementia are under 1 μM and are well above the concentrations needed for NMDA receptor blockade (8,27,28). Much higher brain concentrations are achieved in rats treated intraperitoneally with 10 mg/kg of memantine (8).

TABLE 4

DEFENSE BEHAVIORS IN MICE EXPOSED TO DIFFERENT REGIMENS OF MORPHINE ADMINISTRATION

Morphine	Channel Blocker	Defense
Saline	—	0.0 ± 0.0
5 mg/kg	—	0.2 ± 0.1
10 mg/kg	—	24.3 ± 9.8
20 mg/kg	—	25.5 ± 12.0
Saline	Saline	1.2 ±1.2
5 mg/kg	Saline	0.2 ± 0.2
Saline	Dizocilpine	2.5 ± 1.7
5 mg/kg	Dizocilpine	0.2 ± 0.1
Saline	Memantine	0.1 ± 0.1
5 mg/kg	Memantine	1.2 ± 0.4
Saline	MRZ 2/579	0.8 ± 0.4
5 mg/kg	MRZ 2/579	1.3 ± 0.9

Mean (±SEM) relative duration per session (%) of defensive behavioral elements. Social interaction tests were conducted 15 min after the injection of naloxone (0.1 mg/kg) in mice that were administered SC morphine or saline once a day for 9 days. Prior to the morphine administration period, dizocilpine (1 mg/kg, IP), memantine (30 mg/kg, IP), MRZ 2/579 (30 mg/kg, IP) or saline were administered for 14 days, once a day.

*$p < 0.05$ (Tukey's test), compared to corresponding group treated with saline (instead of morphine).

Nevertheless, the treatment regimens used in the present study appear to be useful for modeling of the antagonist withdrawal syndrome as well as for experiments with induction of morphine tolerance and dependence. Repeated administration of 5 mg/kg of morphine once a day for 8 days did not lead to establishment of tolerance to morphine analgesia in mice without a history of NMDA receptor channel blocker administration. Similarly, injection of naloxone did not precipitate any noticeable signs of morphine withdrawal syndrome in mice without history of NMDA receptor channel blocker administration. We did not find any published reports on the development of morphine tolerance or dependence in mice exposed to such a low-dose morphine regimen. However, a significant tolerance developed after daily 3 mg/kg of morphine injections in rats (35). In the present study, despite a relatively large number of subjects per group, there was only a nonsignificant tendency for reduced analgesic activity of morphine in the repeated morphine treatment group (Fig. 2), suggesting that the morphine regimen was the subthreshold for induction of tolerance and, thus, was applicable for the purposes of the study (see also Fig. 1). It should also be noted that the present study design excluded any possibility for the establishment of associative tolerance [see, however, Fig. 2 in (35)].

Meanwhile, statistically significant tolerance to morphine analgesia developed in mice preexposed to dizocilpine (1 mg/kg) but not memantine or MRZ 2/579. Such differences are especially intriguing, given the fact that Tiseo and colleagues (34) failed to observe intensification of morphine tolerance in rats treated for 7 days with the competitive antagonist LY274614.

It is known that chronic administration of NMDA antagonists reduces synaptic functions (5,11) and it may be argued that upon withdrawal from the NMDA receptor blockade there is a rebound period during which synaptic activity exceeds control levels. This hypothesis is partially justified by the findings on increased sensitivity of the NMDA receptor complex during the period after withdrawal from the NMDA receptor blockade [(6,23,26,29); see, however, (4)]. Thus, facilitation of morphine tolerance development in dizocilpine-experienced mice is consonant with the apparent role of NMDA receptors in the mechanisms of morphine tolerance (13). However, it becomes increasingly evident that repeated administration of NMDA receptor antagonists does not im-

prove subsequent performance in learning and memory paradigms (6,16,17).

Tolerance data should be viewed with some reserve for two reasons. First, there was no facilitation found of morphine dependence in mice with a history of administration of NMDA receptor channel blockers. Social interaction test is a sensitive behavioral test that allows registration of subtle behavioral alterations. Control experiments suggested that the development of morphine dependence was highly dependent on the repeated morphine dose (Fig. 1). Mice repeatedly exposed to 10 or 20 mg/kg of morphine spent on average up to 25% of the test displaying defensive acts and postures. Importantly, other more traditional withdrawal signs (jumping, "wet-dog"–like shaking, stretching) were not present. Increased defensiveness in morphine-withdrawn mice was dependent on the levels of morphine exposure, and is consistent with the well-known anxiogenic potential of opioid withdrawal.

Second, development of morphine tolerance was facilitated in mice treated with dizocilpine but not memantine or MRZ 2/579. These results may indicate individual differences between NMDA receptor blockers, all of which interact selectively with the NMDA receptor channel site (27,28,40). Although differences in the affinity for NMDA receptor channel site can be a likely explanation, one should note that at high doses low-affinity blockers such as memantine partially mimic behavioral effects of the high-affinity blocker, dizocilpine (12,25,31).

In conclusion, repeated administration of NMDA receptor channel blockers was characterized only by a minor behavioral withdrawal syndrome, but rendered mice more sensitive to the induction of morphine tolerance but not dependence. These effects were observed in mice exposed to behaviorally toxic doses of NMDA receptor antagonists and, thus, it is unlikely that clinical use of NMDA receptor antagonists such as memantine will be complicated by increased propensity to the induction of morphine tolerance and dependence.

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