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Anti-allodynic interactions between NMDA receptor channel blockers and morphine or clonidine in neuropathic rats

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Abstract

Previous studies suggested that combining *N*-methyl-D-aspartate (NMDA) receptor antagonists with either μ-opioid agonist morphine or α_2-adrenoreceptor agonist clonidine results in the significant synergistic enhancement of analgesic activity in the animal models of acute and neuropathic pain. When given alone, NMDA receptor antagonists, morphine and clonidine are capable of attenuating tactile allodynia associated with chronic nerve injury. The present study aimed to assess anti-allodynic effects of these compounds and to test additivity of these interactions using isobolographic analysis. Adult male Wistar rats with unilateral loose ligation of sciatic nerve developed significant tactile allodynia (between-paw difference of about 18–20 g). In separate groups of animals, dose-dependent anti-allodynic activity was confirmed for memantine (1.8–17.8 mg/kg), neramexane (1.8–17.8 mg/kg), morphine (1–10 mg/kg) and clonidine (0.01–0.1 mg/kg). In a subsequent series of experiments, memantine (or neramexane) and morphine (or clonidine) were co-administered at the fixed equi-effective dose ratios (six dose levels per drug combination). None of the tested combinations produced supra-additive, synergistic effects. In fact, memantine+clonidine, neramexane+clonidine and morphine+neramexane were producing simple additive effects, while morphine+memantine was characterized as the infra-additive combination. Thus, despite expectations based on previous studies, NMDA receptor channel blockers, memantine and neramexane, produce no synergistic interactions with either morphine or clonidine when administered acutely to rats with nerve injury-induced tactile allodynia.

Keywords: NMDA receptor antagonist; Memantine; Neramexane; Morphine analgesia; Clonidine; Rat sciatic nerve; Allodynia; Experimental nerve injury

1. Introduction

It has been suggested that combining *N*-methyl-D-aspartate (NMDA) receptor antagonists with either μ-opioid agonist morphine or α_2-adrenoreceptor agonist clonidine results in the significant enhancement of analgesic activity in the animal models of pain. For instance, both systemic and spinal administration of NMDA receptor channel blockers such as MK-801 and dextromethorphan was reported to enhance the ability of morphine to reverse hyperalgesia and tactile allodynia in neuropathic rats (Yamamoto and Yaksh, 1992; Nichols et al., 1997; Kauppila et al., 1998). Similarly, it was suggested that NMDA receptor channel blockers (MK-801) potentiated the effects of clonidine on thermal hyperalgesia in rats with sciatic nerve injury (Jevtovic-Todorovic et al., 1998). These results suggest that combinations of NMDA receptor antagonists with either morphine or clonidine may be a novel approach to manage chronic pain states. However, the nature of these interactions remains unclear.

It is well established that, when given alone either systemically and/or intrathecally, NMDA receptor antago-

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nists (Carlton and Hargett, 1995; Eisenberg et al., 1995; Chaplan et al., 1997; Medvedev et al., 2004), morphine (Bian et al., 1995) and clonidine (Yaksh et al., 1995) are capable of attenuating hyperalgesia and tactile allodynia associated with chronic nerve injury. Theoretically, combinations of these agents may produce infra-additive, additive, or supra-additive effects. In order to distinguish between these outcomes, specialized methods of statistical analysis were developed (Barkov, 1964; Tallarida et al., 1997). Previous studies in neuropathic rats suggested that supra-additive (synergistic) interactions existed between intrathecally administered morphine and clonidine (Ossipov et al., 1997) and MK-801 and clonidine (Lee and Yaksh, 1995).

The present experiments sought to test for the nature of interactions between NMDA receptor channel blockers and morphine or clonidine using sciatic nerve ligation-induced tactile allodynia in rats (Bennett and Xie, 1988). Memantine and neramexane (MRZ 2/579) were selected as representative NMDA receptor antagonists for three reasons. First, while being chemically unrelated, memantine and neramexane share a common mechanism of action demonstrating voltage-dependent blockade of NMDA receptor channel with fast unblocking kinetics and low to moderate affinity (Parsons et al., 1999; Parsons, 2001). Second, unlike MK-801 and other phencyclidine-like channel blockers, memantine and neramexane are less likely to produce behavioral toxicities within the therapeutic dose ranges. These drugs are in the current clinical use or in different stages of clinical development, respectively. Third, for one of these drugs (memantine) it was claimed that it might potentiate the anti-hyperalgesic effects of clonidine (Olney et al., 2001). When given alone, memantine attenuated thermal and mechanical hyperalgesia as well as tactile allodynia in neuropathic rats (Carlton and Hargett, 1995; Eisenberg et al., 1995; Medvedev et al., 2004).

In these experiments, dose-effect relationships were estimated first to obtain ED_{30} value for each agent alone and then NMDA receptor channel blockers were injected jointly with either morphine or clonidine in a fraction of the dose ratio 1 : 1 for isobolographic analysis.

2. Materials and methods

2.1. Subjects

Adult male Wistar rats weighing 230–270 g upon the arrival from the breeder (“Rappolovo”, St. Petersburg, Russia) served as subjects. All experiments were conducted during the light period of a 12/12 h day–night cycle (lights on at 09:00 a.m.). Rats were individually housed with food (standard rodent chow, “Volossovo”, St. Petersburg, Russia) and filtered tap water available ad libitum. The rats were allowed to habituate to their home cage environment for at least 7 days before any manipulations were performed. Experiments were approved by the Ethics Committee of Pavlov Medical University and were performed in accordance with the recommendations and policies of the Committee for Research and Ethical Issues of IASP.

2.2. Drugs

Memantine (1-amino-3,5-dimethyladamantane hydrochloride), neramexane (1-amino-1,3,3,5,5-pentamethyl-cyclohexan hydrochloride; both from Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany), clonidine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) and morphine hydrochloride (Endocrinny Zavod, Moscow, Russia) were dissolved in distilled water and injected intraperitoneally (memantine, neramexane and clonidine) or subcutaneously (morphine) 1 h prior to the test. Injection volume was 1 ml/kg of body weight. Doses are based on the forms of drugs listed above.

2.3. Sciatic nerve ligation: general procedure

At the beginning of the experiment, baseline measurements of the 50% threshold for paw withdrawal (see below) were made for both paws of all rats on two consecutive days (Tuesday and Wednesday). Based on these data, rats with no initial difference between paws were subjected to surgical manipulations (Thursday; see below). Three paw withdrawal tests were held on Days 4 (Monday), 7 (Thursday) and 11 (Monday) post-surgery to monitor the development of tactile allodynia. Preliminary experiments have suggested that no further decrements in paw withdrawal thresholds (on the ligated side) were observed after Day 11. Thus, drug tests were administered on Mondays and Thursdays starting from Day 14 (Thursday). Different doses were administered in a pseudorandom order. Repeated vehicle (1 ml/kg) tests were conducted on all rats on Saturdays to confirm maintenance of the tactile allodynia.

2.4. Sciatic nerve ligation: surgery

Rats were anesthetized by a single i.p. injection of 60 mg/kg sodium pentobarbital. Two incisions were made — one on each thigh, parallel to the femoral bone and approximately 1.5 cm long. The common sciatic nerves were exposed by blunt dissection through biceps femoris on both sides. One paw was then designated as the “sham operated”. Contralateral paw was designated as “ligated”. The side of “ligated” paw was counterbalanced so that in half of the animals the left paw was “ligated”, whereas in another half of animals the left paw was “sham operated”. On the “ligated” paw side, proximal to the sciatic trifurcation four ligatures (4–0 silk) were tied loosely and spaced about 1 mm apart. The skin wounds were closed by 2–3 silk sutures. After surgery the animals were put into a warm air environment for approximately 3–4 h.

2.5. Sciatic nerve ligation: behavioral testing

Rats were placed into a plastic cage with a plastic string grid bottom, which allowed full access to the paws. Short habituation period (5 min) preceded the test period. The paw withdrawal thresholds were determined as described before (Chaplan et al., 1994). Paws were touched with one of a series of 8 von Frey hairs (Stoelting, IL, USA) with logarithmically incremental stiffness (0.692, 1.202, 2.041, 3.630, 5.495, 8.511, 15.136, 28.840 g). For each rat, the withdrawal thresholds on the left paw were always

evaluated first followed by the same procedure on the right paw. The tip of the hair was presented perpendicular to the mid-plantar surface avoiding the less sensitive footpads. Sufficient force was applied to cause slight buckling against the paw, and held for approximately 6–8 s. A positive response was noted either if the paw was sharply withdrawn or if flinching was seen immediately upon the removal of the hair. The testing was initiated with the 3.630 g hair. Stimuli were presented in consecutive fashion either ascending or descending. If no response were elicited by the initially selected hair (negative response), a stronger stimulus was presented. If the paw was withdrawn (positive response), the weaker stimulus was presented next. When the threshold was crossed, another 4 hairs in a same consecutive fashion were presented. All tests were performed by the experimenter blind to the treatment conditions.

The psychophysical 50% threshold was calculated using the up down method (Dixon, 1980; Chaplan et al., 1994). For each animal the difference between paws was calculated by subtracting the log threshold value on the "ligated" paw from the log threshold value on the "sham-operated" paw (i.e., the positive values correspond to a lower threshold on the "ligated" paw).

2.6. Experiment 1: acute dose–effect relationships

Different doses of memantine (1.78–17.8 mg/kg), neramexane (1.78–17.8 mg/kg), morphine (1–10 mg/kg), and clonidine (0.01–0.1 mg/kg) and their vehicle were administered 1 h prior to the tactile allodynia test ($N=8$ per each dose).

2.7. Experiment 2: isobolographic analysis of morphine+memantine, morphine+neramexane, clonidine+memantine, and clonidine+neramexane combinations

One group of rats ($N=8$ per group) was treated with a combination of memantine and morphine administered in a fixed dose ratio (in milligrams per kilogram): 2.11/1.22, 2.95/1.70, 4.13/2.39, 5.78/3.34, 8.09/4.67, respectively. Second group was treated with a combination of neramexane and morphine administered in a fixed dose ratio (in milligrams per kilogram): 2.13/1.22, 2.98/1.70, 4.18/2.39, 5.85/3.34, 8.19/4.67, respectively. Third group of rats was treated with a combination of memantine and clonidine administered in a fixed dose ratio (milligrams per kilogram): 2.11/0.014, 2.95/0.020, 4.13/0.028, 5.78/0.039, 8.09/0.055, respectively. Fourth group of rats was treated with a combination of neramexane and clonidine administered in a fixed dose ratio (in milligrams per kilogram): 2.13/0.014, 2.98/0.020, 4.18/0.028, 5.85/0.039, 8.19/0.055, respectively.

Memantine dose of 5.78 mg/kg, neramexane dose of 5.85 mg/kg, clonidine dose of 0.039 mg/kg and morphine dose of 3.34 mg/kg were equi-effective, each producing a 30% reduction of tactile allodynia. Control tests were carried out with two saline injections instead of memantine/neramexane+morphine/clonidine treatment.

2.8. Data analysis

Data from the memantine alone, neramexane alone, morphine alone and clonidine alone experiments were converted to log dose units and analyzed using linear regression. From each linear regression, the effective doses producing 30% reduction in tactile allodynia were determined. These doses were used to construct an isobologram indicated by a line of additivity representing all dose combinations that would theoretically produce 30% reduction in tactile allodynia. Values from the actual combination studies were also converted to log dose units and analyzed using linear regression procedure. From this analysis, the dose producing 30% reduction in tactile allodynia was determined and plotted on the isobologram for comparison with the theoretically additive value (Tallarida et al., 1997).

3. Results

3.1. Experiment 1: acute dose–effect relationships

Unilateral ligation of the sciatic nerve resulted in a significant increase in responsiveness to tactile stimulation on "ligated" side as compared to "sham-operated" side. Across different treatment groups, average difference in the withdrawal thresholds was 19–21 g. Because of the log scale of the tactile stimulation, the data are presented as between-paw differences in log thresholds. Average differences in the log thresholds were 0.57–0.68. Table 1 reveals the experimentally determined (following vehicle pretreatment) and calculated (using regression analysis) baseline levels of tactile allodynia in the memantine, neramexane, morphine and clonidine treatment groups.

Administration of memantine, neramexane, morphine and clonidine produced moderate but yet dose-dependent and statistically significant reductions in tactile allodynia (Fig. 1; memantine — $F(4,28)=3.3$, $P<0.05$, neramexane — $F(4,28)=3.7$, $P<0.05$, morphine — $F(4,28)=4.2$, $P<0.01$, clonidine — $F(4,28)=4.4$, $P<0.01$). Doses of memantine, neramexane and morphine producing 30% reduction in tactile allodynia were determined using the regression analysis and are displayed in Table 1.

3.2. Experiment 2: isobolographic analysis of morphine+memantine, morphine+neramexane, clonidine+memantine, and clonidine+neramexane combinations

In these experiments, drugs were administered at fixed dose proportions based on the ED_{30} values presented in Table 1. There was a dose-dependent reduction in the expression of tactile allodynia for all drug combinations except for morphine+memantine (Fig. 2; morphine+memantine — $F(5,35)=0.7$, n.s., morphine+neramexane — $F(5,35)=2.5$, $P<0.05$, clonidine+memantine — $F(3,35)=4.2$, $P<0.01$, clonidine+neramexane — $F(3,35)=4.6$, $P<0.01$).

Table 1
Summary of the results of experiment 1

	Memantine	Neramexane	Morphine	Clonidine
Baseline withdrawal threshold difference (vehicle pretreatment)	0.68±0.12	0.59±0.04	0.58±0.08	0.57±0.08
Baseline withdrawal threshold difference (*Y*-axis intercept)	0.63±0.08	0.58±0.08	0.63±0.07	0.66±0.07
Calculated ED_{30} value (mg/kg)	5.78±0.92	5.85±1.07	3.34±0.96	0.039±0.006

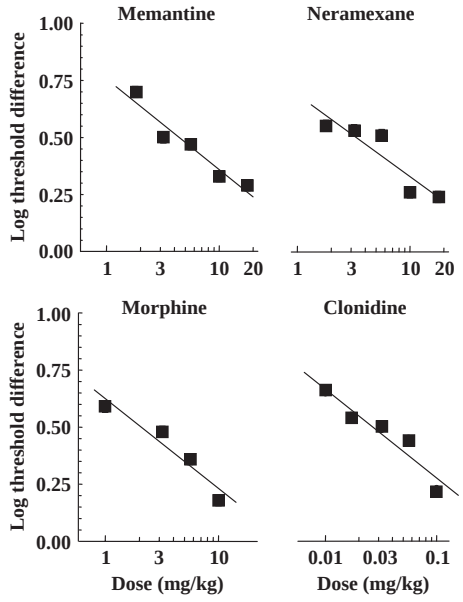

Fig. 1. Regression lines for memantine, neramexane, morphine and clonidine. Rats with unilateral sciatic nerve injury were administered different doses of memantine (1.78–17.8 mg/kg), neramexane (1.78–17.8 mg/kg), morphine (1–10 mg/kg) or clonidine (0.01–0.1 mg/kg) 1 h prior to the tactile allodynia test. Data are represented as mean group tactile response threshold difference (log) between ligated and sham-operated paws.

Calculated ED_{30} values are given in Table 2. For all tested drug combinations these experimentally derived values fell above the line of additivity indicating infra-additive combination (Fig. 3). However, the difference between theoretically predicted and experimentally derived values was significant only for morphine+memantine combination (see also Table 2, Mann–Whitney's, $P<0.05$).

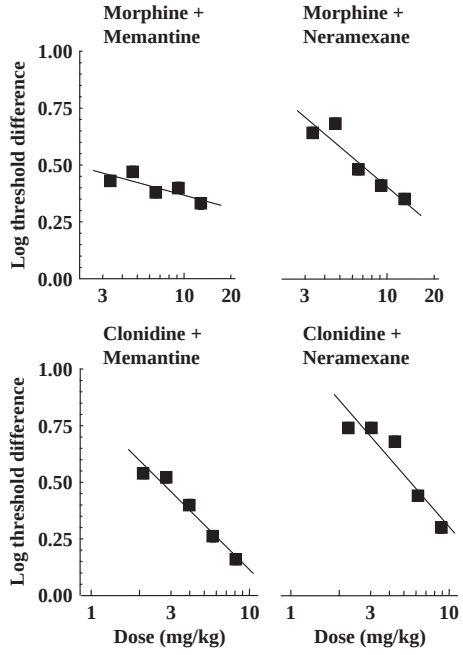

Fig. 2. Regression lines for morphine+memantine, morphine+neramexane, clonidine+memantine, and clonidine+neramexane combinations administered in a fixed dose proportion. See text for details. Total drug combination doses are plotted against *X*-axis. Data are represented as mean group tactile response threshold difference (log) between ligated and sham-operated paws.

Table 2
Drug combinations producing 30% reduction in tactile allodynia: experimentally derived and theoretically additive amounts

	Morphine+ memantine	Morphine+ neramexane	Clonidine+ memantine	Clonidine+ neramexane
Total dose				
Experimental	10.19±3.67	7.86±1.63	3.42±0.57	4.47±0.78
Theoretical	4.56±1.94	4.60±0.94	2.91±0.38	2.95±0.58

4. Discussion

Confirming the results of earlier studies, systemic administration of morphine, clonidine and memantine produced significant anti-allodynic effects. Extending the previous findings with memantine, another NMDA receptor channel blocker, neramexane, was found to attenuate responding to tactile stimulation in neuropathic rats.

Memantine was reported before to reverse thermal and mechanical hyperalgesia as well as tactile allodynia (Carlton and Hargett, 1995; Eisenberg et al., 1995; Medvedev et al., 2004). Although these results are generally in line with the present findings, there are some procedural and other factors that may explain higher magnitude of the effects of memantine in the previous studies. First, Carlton and Hargett (1995) studied tactile allodynia in rats with tight ligation of the L5/L6 segmental nerves (the Chung model)

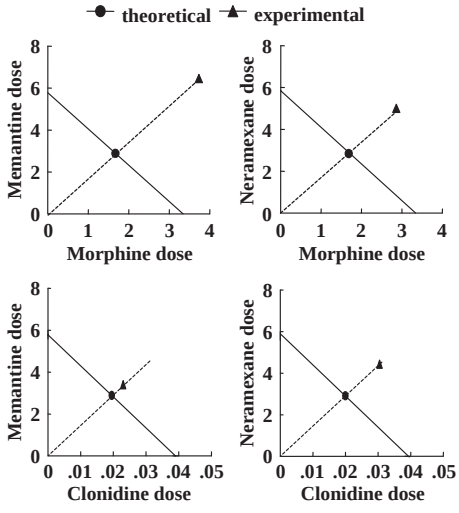

Fig. 3. Isobolograms of morphine+memantine, morphine+neramexane, clonidine+memantine, and clonidine+neramexane combinations. Drug doses are shown as milligrams per kilogram. The solid lines contain points that represent dose pairs that produce 30% reduction in tactile allodynia and that are theoretically additive. Circles on the solid lines represent such theoretically additive, equi-effective dose pairs (i.e., half of ED_{30} for one drug and half of ED_{30} for another drug). The triangle-marked points represent the experimentally determined values (i.e., actual doses of the drugs that produce 30% reduction in tactile responding) that were found to be above the theoretical additivity line.

that leaves L3 and L4 components intact. Compared with the Bennett and Xie (1988) procedure, the Chung model has some advantages such as more predictable outcome and smaller variability. However, these models were not compared directly in terms of the sensitivity to pharmacological and other manipulations. It is possible that under the present conditions higher doses of memantine would produce stronger effects. These doses were not tested because of the motor side effects (ataxia, incoordination and restlessness) that preclude efficient determination of the tactile thresholds. Nevertheless, it should be noted that already at the dose of 5 mg/kg memantine is likely to produce brain concentrations sufficient for blockade of NMDA receptors (Danysz et al., 1997). Second, it was shown that a single dose of memantine of 10 mg/kg completely reversed thermal hyperalgesia but not hyperresponsiveness to mechanical stimulation (Carlton and Hargett, 1995; Eisenberg et al., 1995). Only the higher dose of memantine (20 mg/kg) abolished mechanical hyperalgesia and allodynia (Carlton and Hargett, 1995). Similarly, another non-competitive NMDA receptor antagonist, MK-801, attenuated the nerve injury-induced tactile allodynia to a lesser degree compared to the thermal hyperalgesia (Wegert et al., 1997). Thus, it appears that various aspects of neuropathic pain syndrome in rats may be differentially affected by NMDA receptor blockade.

Morphine and clonidine were also unable to produce complete reversal of the tactile allodynia. For morphine, it is well established that its analgesic efficacy may be markedly reduced in rats with nerve ligation injury (Ossipov et al., 1995). This reduction may be important for the systemic administration of morphine because of the reduced synergy between spinal and supraspinal sites of action (Bian et al., 1999). Also, it was demonstrated that compared to thermal hyperalgesia tactile allodynia is relatively resistant to morphine treatment (Wegert et al., 1997). For clonidine, the sites of anti-allodynic activity are located at the level of the spinal preganglionic neurons. Accordingly, given systemically, clonidine is less effective compared to the spinal route of administration (Yaksh et al., 1995).

With all of the four tested compounds producing incomplete reversal of the tactile allodynia, only ED_{30}, but not ED_{50}, values could be calculated. Based on these ED_{30} values, equi-effective dose ratios were determined and all subsequent tests were conducted with drugs administered at the fixed dose ratios. Although such studies are typically based on ED_{50} values, other effect levels are possible, provided that the level is reached by each drug (Tallarida et al., 1997).

The results revealed that none of the tested drug combinations was characterized by the supra-additive interactions between the components. These results stand in an apparent contrast to the previous studies, which suggested the possibility of synergism between NMDA receptor antagonists and morphine or clonidine.

Several reports suggested that systemic administration of NMDA receptor antagonists potentiates the effects of morphine in the acute nociception tests (Grass et al., 1996; Bespalov et al., 1998). However, outcome of such studies appears to depend significantly on the anti-nociceptive assay and the type of the NMDA receptor antagonist that was used in combination with morphine. For example, no additivity or even the reduction in morphine analgesia is observed in studies with low to moderate affinity NMDA receptor channel blockers such as memantine when noxious thermal stimulus is applied to the rat's paw (Kozela et al., 2001 and Table 1 therein). With intrathecal route of administration, both NMDA receptor antagonists and morphine exert significant anti-nociceptive activity but when these agents were given intrathecally in combination, isobolographic analysis did not reveal synergistic antinociception (Nishiyama et al., 1998). Thus, in acute nociception tests, no synergism may be found between NMDA receptor channel blockers and morphine given either systemically or intrathecally.

In rats with sciatic nerve ligation (like in the present study), intrathecal administration of the NMDA receptor channel blocker, MK-801, had simple additive interactions with anti-hyperalgesic effects of morphine (Yamamoto and Yaksh, 1992). In rats with L5/L6 spinal nerve ligations, intrathecal morphine alone was ineffective but adding NMDA receptor channel blocker, MK-801, restored its anti-allodynic and anti-nociceptive effects (Nichols et al., 1997). Supraspinal administration of MK-801 had a similar effect of enhancing the efficacy of intrathecal morphine (Pertovaara and Wei, 2003). Moreover, it was shown that in nerve-injured rats synergy between spinal and supraspinal sites of action of morphine is lost and MK-801 restores this synergy (Bian et al., 1999). Taken together, this evidence indicates that when given alone systemically administered morphine reduces tactile allodynia via acting at the supraspinal sites (Lee et al., 1995).

The present results suggested lack of synergism between systemically administered NMDA receptor channel blockers and morphine in the rats with sciatic nerve ligation injury. Moreover, isobolographic analysis indicated infra-additive interaction for these combinations. Since the primary site of systemic morphine's anti-allodynic activity is located supraspinally, it may be speculated that NMDA receptor channel blockers may act supraspinally to reduce the effects of systemic morphine and thereby prevent synergistic interactions. Indeed, activation of the brainstem NMDA receptors is required for the acute anti-nociceptive effects of morphine given systemically (Heinricher et al., 2001). It remains to be tested whether this would hold true for anti-allodynic effects of systemic morphine as well. Meanwhile, as the present study suggests, no synergism exists between morphine and NMDA receptor channel blockers given systemically to neuropathic rats.

Similarly to the experiments with morphine, combinations of NMDA receptor antagonists with clonidine were

clearly not synergistic. Again, these results are in contrast with the evidence for supra-additive (synergistic) interactions between intrathecally administered MK-801 and clonidine (Lee and Yaksh, 1995). Systemic administration of both MK-801 and memantine seemed to enhance the effects of clonidine on sciatic nerve ligation-induced thermal hyperalgesia (Jevtovic-Todorovic et al., 1998; Olney et al., 2001). In these experiments, ineffective doses of the NMDA receptor antagonist and clonidine were combined to provide complete and long-lasting reversal of hyperalgesia. However, in the present study isobolographic analysis indicated that the interactions between NMDA receptor antagonists and clonidine were limited to simple additivity.

In conclusion, despite expectations based on previous studies, NMDA receptor channel blockers, memantine and neramexane, produce no synergistic interactions with either morphine or clonidine when administered acutely to rats with nerve injury-induced tactile allodynia.

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