6-1-1995

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Research Report for

Psychology Undergraduate Honors Program

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June 1, 1995

Abstract

The possibility that the excitatory amino acid (EAA) antagonist dextrorphan may have clinical benefit in combination with opiates for neuropathic pain was investigated. In this study a subeffective dose of dextrorphan (15 mg/kg) was combined with several doses of morphine (1.5, 3.0 and 6.0 mg/kg) and assessed in an animal model of central nervous injury, the tail flick response of the acute (24) spinal rat. The results indicated that, at doses which were individually ineffective, the combination of dextrorphan and morphine (15 and 1.5 mg/kg, respectively) produced a significant antinociceptive response. The same dose of dextrorphan also increased the antinociceptive response of 3.0 and 6.0 mg/kg of morphine. These findings suggest that coadministration of low doses of an NMDA antagonist and an opiate, might have clinical benefit for the relief of pain with reduced risk of undesirable side effects.

## Introduction

Although the opiates, particularly morphine, are the primary drug treatment for the alleviation of severe, chronic pain (i.e., pain prolonged beyond normal recovery time for tissue damage), their therapeutic benefit is significantly limited. First, opiate medications produce a number of unpleasant (e.g., constipation, nausea) side effects as well as respiratory depression, a potentially lethal effect. (Marshall & Longnecker, 1990). In addition, these drugs are ineffective against chronic pain that arises from disease or damage to the central or peripheral nervous system (Arner & Meyerson, 1988). This is known as neuropathic pain and results in behavioral hyperalgesia, an enhanced responsiveness to noxious (or, injurious) stimuli, such as heat or tactile pressure, in the vicinity of the tissue damage. Clinical neuropathy occurs, for instance, after an amputated limb (i.e. phantom limb pain) and as a symptom of shingles, in which sensory nerves near the skin are irritated by the herpes virus. Because of the disadvantages of opiate drug therapy, both its limited efficacy and side effects, substantial efforts have been made to develop alternative analgesic agents.

There is now compelling evidence from neurophysiological and behavioral research in the last 5 to 10 years, that the excitatory amino acids (EAAs) are involved in the etiology of chronic,

neuropathic pain syndromes. The EAAs are a class of neurotransmitters represented endogenously in the central nervous system. L-glutamate appears to be the primary EAA related to neuropathic pain. (Dickenson, 1990). The EAAs have been shown to mediate neuropathic pain by selectively activating the N-methyl-D-aspartate (NMDA) receptor complex (i.e., binding to the NMDA ligand recognition site) located in the dorsal horn neurons of the spinal cord.

Accordingly, it has been proposed that drugs which antagonize the EAAs (i.e., block the activation of the NMDA receptor complex) may provide new treatment options (Advokat & Rutherford, 1995). This is supported by findings that such agents produce a selective antinociceptive[1] effect in various animal models of neuropathic pain (Coderre & Melzack, 1991; Dubuisson & Dennis, 1977; Ren, et. al, 1992; Yamamoto & Yaksh, 1992). Among those clinically available, such as dextromethorphan, memantine and the tricyclic antidepressants, the dissociative anesthetic ketamine has been studied the most extensively for the treatment of chronic pain. But ketamine also produces a number of undesirable cardiovascular and psychotomimetic side effects, which somewhat limit its therapeutic usefullness (Wolf & Advokat, 1995).

Recent reports indicate that dextrorphan, a metabolite of dextromethorphan, is also a noncompetitive[2] antagonist of the

excitatory amino acid glutamate at the N-methyl-D-aspartate (NMDA) receptor. Since dextrorphan appears to have minimal side effects it may be a useful addition to the treatment for neuropathic pain. This prediction is supported by investigations of animal models of peripheral and central nervous injury in which hyperalgesic responses were selectively reduced with systemic and intrathecal administrations of dextrorphan (Wolf & Advokat, 1995). Furthermore, the parent compound, dextromethorphan, may have a modest, though benefical, efficacy against neuropathic pain in human subjects.

A few studies have indicated that the antinociceptive action of the EAA antagonists may be improved by concomitant administration of opioids. This combination produced a powerful suppression of neuronal excitation in a neurophysiological model of neuropathic pain (Chapman & Dickenson, 1992). In a study of the behavioral effect of such treatment, it was concluded that the antinociceptive effect of intrathecal morphine in acute mononeuropathy (peripheral injury) was improved by the addition of MK-801 (Yamamoto & Yaksh, 1992). In the other behavioral study, the antinociceptive effect of morphine pellet implantation in chronic, spinal rats was increased and prolonged by the simultaneous, subcutaneous infusion of MK-801 (Gutstein &

Trujillo, 1992). It is further noted that MK-801 itself is not acceptable for clinical use.

Accordingly, the present study examined the antinociceptive effect of morphine and dextrorphan, alone and in combination, in acute (24 h) spinally transected rats (i.e., animals sustaining damage to the central nervous system). Their reaction to a noxious thermal stimulus was assessed before and after drug administration. The data indicate that at doses which do not, individually, induce antinociception, the combination of these two drugs produced a significant antinociceptive response.

## Method

### Subjects

A total of 54 male, albino Holtzman derived rats (Harlan Sprague-Dawley Laboratories, Madison, WI) weighing 275-475 g were used as subjects. The animals were housed individually in suspended steel cages in a colony room maintained on a 12 L:12 D cycle, with dark onset at 1900 h. All animals were given unlimited access to food and water throughout the experiment. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University, Baton Rouge, LA.

### Spinal Transection

Spinal transections were performed under isoflurane /oxygen anesthesia. An incision was made behind the ears and extended caudally, after which the paraspinal muscles were retracted to expose the spinal vertebrae. A laminectomy between T6-T9 was performed, and a 1-2 mm portion of the spinal cord was removed and replaced with Gelfoam to reduce bleeding, after which the incision was sutured. After the transection, a heat lamp was placed in front of the cages in order to maintain body temperature (room temperature was kept at 78 - 80 F). On the morning after surgery, the rats' bladders were voided manually by the application of pressure to the abdomen and their hindquarters were washed. All tests were performed that afternoon (i.e., approximately 22 to 26 h after surgery).

Tail Flick Assessment

Reactivity to a noxious stimulus was evaluated with the tail flick (TF) test (IITC Life Sciences, Woodland, Hills, CA). Noxious stimulation consisted of a beam of high-intensity light focused on the tail. The response time was measured automatically and defined as the interval between onset of the heat stimulus and the abrupt flick of the tail. Each determination consisted of 3-5 trials; the mean score in seconds was taken as the response latency. In order to minimize tissue damage to the tail, animals

not responding within 14 s were removed from the apparatus and assigned a response latency of 14 s.

Drug Administration

All drug injections were administered subcutaneously (SC). Morphine (Penick Corp., Lyndherst, NJ) and Dextrophan (Research Biochemicals International, Natick, MA) were freshly prepared in 0.9% saline such that the amount injected contained the desired dose in 1.0 ml/kg.

Procedure

Separate groups of rats (N = 4-6) were pre-tested with the TF test before and 30, 60 and 90 min after SC injection of either saline alone, morphine alone (1.5, 3.0 or 6.0 mg/kg), dextrorphan alone (15 or 25 mg/kg), or a combination of both drugs, in a single solution.

Statistical Analyses

In order to analyze the drug effects, difference scores were calculated by subtracting the pre-drug TF latency from the post-drug TF latency for each rat at each time point. Separate two-way repeated measures analyses of variance were performed on the difference scores using the computer program SigmaStat (Jandel, CA) to determine the time course of drug action.

To simplify the analysis of these individual difference scores across time points, the Area Under the Curve (AUC) was

determined for each rat with the aid of the computer program PHARM/PCS. The AUC (i.e., the integral) was calculated according to Simpson's rule from each xy data pair, where y = difference in TF latency and x = 30, 60, 90 min. First, a one-way ANOVA on the AUC scores was used to compare the effect of saline, dextrophan (15 mg/kg) alone and morphine (1.5 mg/kg) alone. The result of this analysis indicated whether each of these drug doses significantly affected the response. A second two-way ANOVA was used to compare the effect of the various morphine doses in the presence vs. absence of dextrorphan. Post-hoc (Student Newman-Keuls) tests were performed to determine which of the respective groups differed. Results were considered significant at $p \leq 0.05$.

## Results

The AUC values for saline, DEX (15 mg/klg) alone, MOR (1.5 mg/kg) alone and the various combinations of DEX (15 mg/kg) and MOR (1.5, 3.0, and 6.0 mg/kg) are summarized in Fig. 1. A one-way ANOVA indicated no difference among the scores for SAL, DEX alone, and MOR alone [$F(2,14) = 0.94$; $P = 0.42$]. This result confirms that these individual doses of the two drugs did not modify the TF reflex.

In addition to the 15 mg/kg dose of DEX, another group of spinal rats was tested after administration of 25 mg/kg. The AUC for this group was $162.6 \pm 104.5$. When the one-way ANOVA was

repeated with this dose, the result was not significant ($P = 0.10$). However, a non-parametric Kruskal-Wallis ANOVA, on the ranks, indicated that the 25 mg/kg dose of DEX was significantly different from saline, but not from the other three groups ($P = 0.04$). This supports the choice of the low dose for use as a threshold value.

The two-way ANOVA, comparing the groups that received MOR alone with those that received MOR + DEX showed a significant overall effect of dose [$F(2,33) = 10.4$; $P<0.001$] and of drug [$F(1,33) = 16.6$; $P<0.001$] with no interaction. This indicates that the addition of DEX significantly increased the antinociceptive effect of MOR such that the dose-response curve of the opiate was parallel, but shifted to the right.

Post-hoc tests indicated, first, that the lowest morphine dose was significantly different from the intermediate and highest morphine doses but that the latter two did not differ. Second, when DEX was added to MOR, there was still a significant difference between the highest and lowest dose, but the intermediate dose did not differ from either of these. Finally, the results showed that the addition of DEX significantly increased the antinociceptive effect of the lowest dose of morphine, but not the intermediate or highest dose.

This outcome is supported by the results of further analysis of the difference scores obtained at each time point (Fig. 2). When a two-way repeated measures ANOVA was performed for each of the three drug combinations, it was found that the time-effect curves differed significantly only for the low dose conditions [MOR = 1.5 mg/kg: $F(1,18) = 7.6$; $P = 0.022$ vs. $P = 0.068$ for each of the other conditions].

Inspection of these data suggests, however, that the antinociceptive potential of the drug combination may be underestimated because, at the last, 90 min, time point, the scores of the three MOR + DEX groups were still higher than the corresponding scores of the MOR alone subjects. If TF assessments had been continued, it is possible that the difference between these experimental conditions would have been statistically significant.

## Discussion

At the present time, the clinical treatment of neuropathic pain is unsatisfactory. This is due to the decreased efficacy and undesirable side effects of opiate medications. Although EAA antagonists may provide an effective alternative, the majority are also limited by their side effect profile. The purpose of the present study was to determine whether the combined administration of morphine and a clinically available EAA antagonist,

dextrorphan, would produce an antinociceptive response at doses which were, individually, ineffective. If so, such treatment might be clinically useful for the relief of pain with reduced side effects.

This approach was supported by a neurophysiological study in which intrathecal coadministration of submaximal doses of morphine and the EAA antagonist 7CK virtually abolished the response of spinal neurons to suprathreshold nociceptive stimulation characteristic of chronic pain (Chapman & Dickenson, 1992). In addition, behavioral evidence consistent with the results of the present study has been obtained from animal models of neuropathic pain syndromes. In one case, intrathecal administration of MK-801 was reported to produce an "additive interaction" with the antinociceptive effect of morphine in rats sustaining peripheral injury (Yamamoto & Yaksh, 1992). In another study, subcutaneous infusion of MK-801 increased and prolonged the antinociceptive effect of morphine pellets in chronic spinal rats (Gutstein & Trujillo, 1992).

The present study used the spinally transected rat to assess the efficacy of combined administration of morphine and dextrorphan.[3] This preparation, involving central nervous system injury, shares many characteristics with peripheral models of neuropathic pain. Both peripheral nervous injury and spinal

transection produce a hyperalgesic decrease in limb and tail withdrawal latency, respectively, which is associated with the release of EAAs within the spinal cord and an excitation of spinal neurons (Duggan & Morton, 1988). There are also several clinical reports of central pain in spinal patients and "central pain with complete transection has been described" (Farkash and Portenoy, 1986). Several EAA antagonists, including AP5, ketamine, and dextrorphan, effective in peripheral neuropathic pain models, also produce an antinociceptive effect which is selective for spinal, relative to intact, rats (Advokat & Rutherford, 1995). Finally, morphine-induced antinociception is reduced in chronic spinal rats (Advokat & Burton, 1987), which appears "similar" to the decrease in opiate efficacy reported for neuropathic pain syndromes in human patients.

The present results, demonstrating an increase in morphine-induced antinociception with dextrorphan in spinalized rats, appear consistent with evidence from peripheral models and indicates that they may be usefully applied in the clinical treatment of neuropathic pain.

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## Author Notes

A more extensive report of the research reported in this paper will be submitted for publication in the journal Brain Research and is authored by Claire Advokat, Ph.D., and Francisco Rhein.

## Footnotes

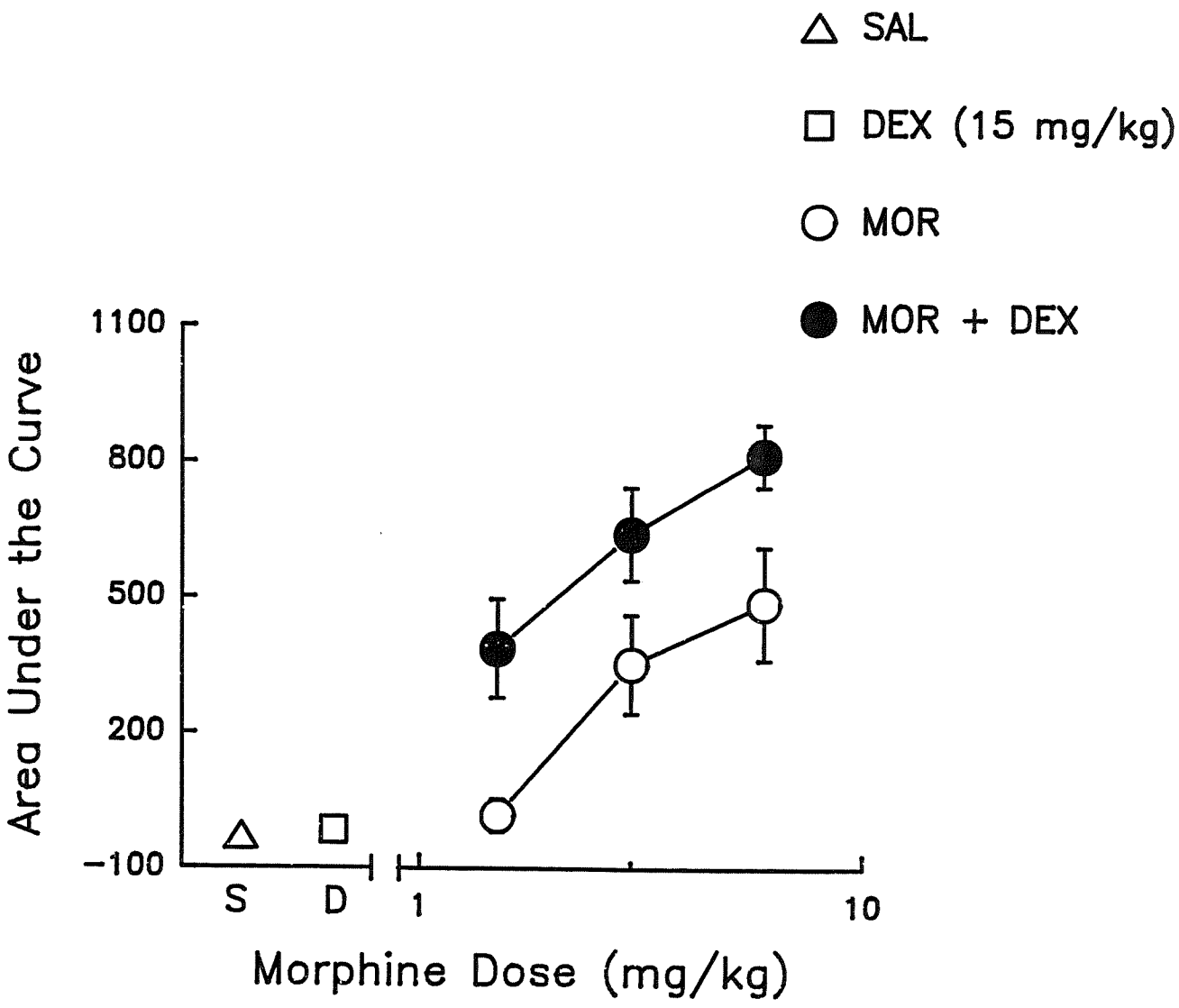
[1] Because it is not possible to determine whether non-human -- or even human -- subjects are experiencing "pain," nociception as indicated refers to a behavioral or neural response which is evoked by the activation of specialized sensory receptors (nociceptors) resulting from tissue damage (Jessell & Kelly, 1991). Hyperalgesia is a nociceptive behavioral response, which when decreased is called antinociception (effective analgesia).

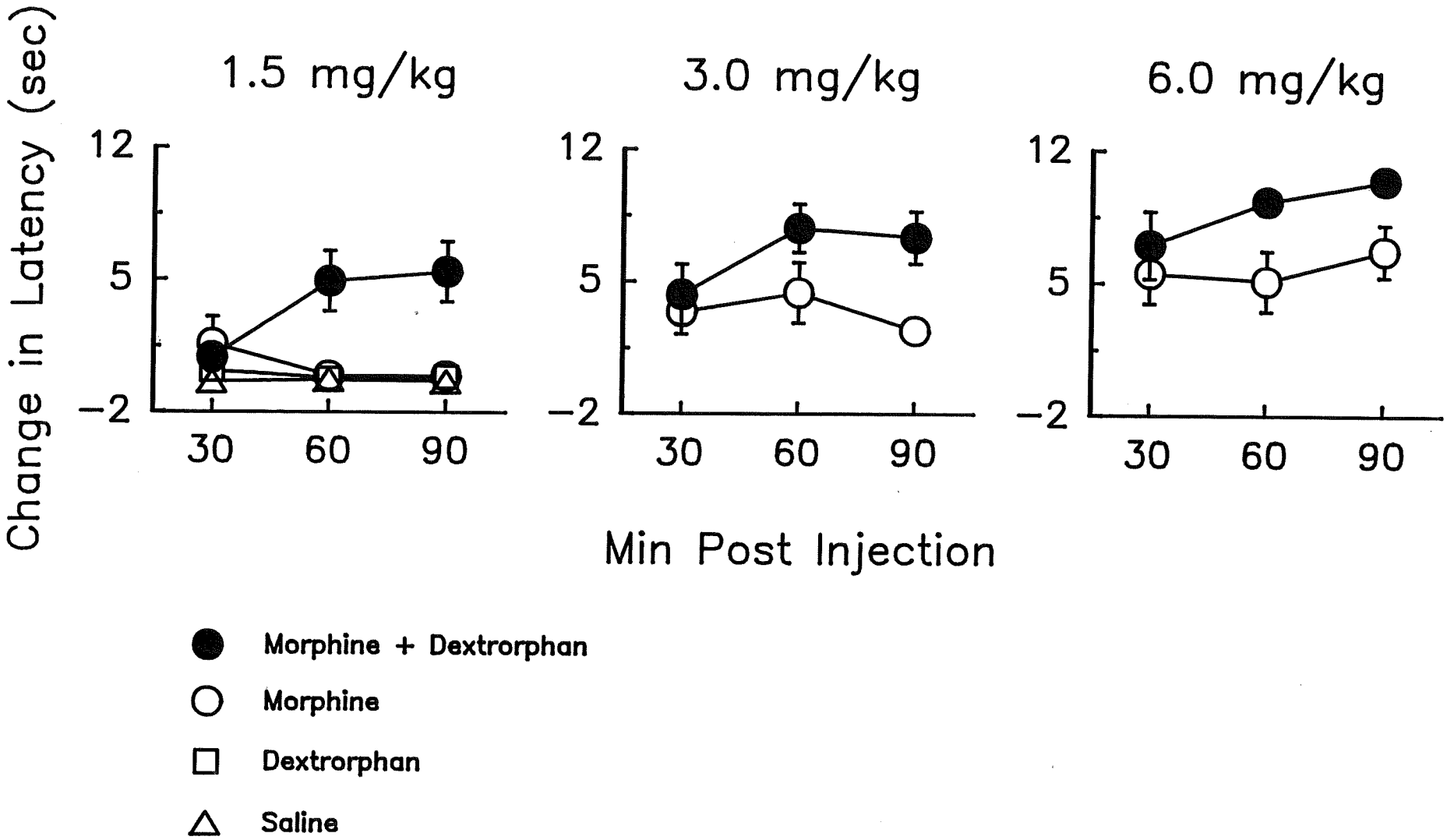
[2] Noncompetitive antagonists, including dextrorphan, ketamine and MK-801, act as NMDA receptor blockers by occupying a site within its ion channel, as opposed to competitive antagonists, which act as NMDA receptor blockers by competing for binding at its glutamate (or other EAA) ligand recognition site.

[3] Although this study did not compare the effect of concomitant administration of morphine and an EAA antagonist in spinal rats with that of intact rats, previous investigations provide contradictory evidence. It has been reported that antinociception is increased, is decreased, or remains unchanged with the drug combination, so that assessment of antinociception in nonneuropathic pain states and its significance to the results of this study is subject to further interpretation.

Figure 1. Dose response effect of subcutaneously administered morphine (1.5, 3.0 and 6.0 mg/kg; open circles) alone and in combination with 15 mg/kg of dextrorphan (filled circles) on the tail flick reflex of acute (24 h) spinal rats. The open triangle and square indicate, respectively, the effect of saline and dextrorphan alone. Each symbol represents the mean ± SEM of the Area Under the Curve, calculated from the data in Fig. 2.

Figure 2. Mean change in tail flick latency (± SEM) in separate groups of acute (24 h) spinally transected rats at 30, 60 and 90 min after the indicated subcutaneous drug treatments. The graph on the left summarizes the effect of saline (open triangles, n=6), 15 mg/kg of dextrorphan (open squares, n=4), 1.5 mg/kg of morphine (open circles, n=5) and dextrorphan + morphine (filled circles, n=6). The middle graph summarizes the effect of 3.0 mg/kg of morphine alone (open circles, n=6) and in combination with dextrorphan (filled circles, n=6) and the graph on the right summarizes the effect of 6.0 mg/kg morphine alone (open circles, n=5) and in combination with dextrophan (filled circles, n=6).


△ SAL
□ DEX (15 mg/kg)
○ MOR
● MOR + DEX
Area Under the Curve
1100
800
500
200
−100
S
D
1
10
Morphine Dose (mg/kg)


Change in Latency (sec)
1.5 mg/kg
3.0 mg/kg
6.0 mg/kg
12
5
−2
30
60
90
Min Post Injection
Morphine + Dextrorphan
Morphine
Dextrorphan
Saline