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NMDA antagonist modulation of morphine antinociception in female vs. male rats

Rebecca M. Craft*, Darla A. Lee

Department of Psychology, Washington State University, Box 644820, Pullman, WA 99164-4820, USA

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Abstract

NMDA antagonists may be useful for their potential to increase or prolong opioid analgesia while attenuating the development of opioid tolerance and dependence. The purpose of this study was to determine whether there are sex differences in NMDA antagonist modulation of morphine antinociception. Adult female and male Sprague–Dawley rats were injected s.c. with saline or one dose of MK-801 (0.005, 0.01, 0.02, or 0.04 mg/kg), dextromethorphan (5, 10, or 20 mg/kg), or LY235959 (0.5, 1.0, or 2.0 mg/kg) in combination with saline or one dose of morphine (1.8, 3.2, or 5.6 mg/kg), and tested on the 50 °C hotplate and tail withdrawal assays 15–120 min post-injection. At the doses examined, only LY235959 produced any antinociception when administered alone. MK-801 attenuated morphine antinociception on both assays, but only at sporadic (inconsistent) dose-combinations. Dextromethorphan increased morphine antinociception on the hotplate but not tail withdrawal assay, at all three morphine doses in males, but only the higher morphine doses in females. In contrast, LY235959 modulated morphine antinociception on both assays; the lowest dose attenuated, and higher doses enhanced morphine antinociception, but the particular morphine doses and assay in which these effects occurred depended on the sex of the subject. Thus, all three NMDA antagonists modulated morphine antinociception in female and male rats, but the direction of this modulation depended on the particular antagonist examined, the nociceptive test, the dose of antagonist and of morphine, and time post-injection.

Keywords: Sex differences; Opioid; Analgesia; Dextromethorphan; Dizocilpine; LY235959

1. Introduction

Combinations of NMDA antagonists and opioid agonists are of clinical interest because NMDA antagonists may potentiate opioid analgesia while preventing or slowing the development of opioid tolerance and dependence (Bisaga and Popik, 2000; Trujillo, 2000). In rodents and humans, however, reports of NMDA antagonist modulation of the acute analgesic effects of opioid agonists vary from enhancement to no effect to attenuation (Kest et al., 1992; Plesan et al., 1999; Caruso, 2000; Heiskanen et al., 2002; Hoffman et al., 2003). The apparent complexity of these drug interactions indicates that further systematic evaluation will be required to determine under what conditions NMDA antagonists reliably enhance opioid analgesia.

Sex differences in opioid analgesia are commonly reported in rodent studies. For example, mu agonists are often found to be more potent or effective in male than in female rats (for review, see Craft, 2003). The few human studies in which sex differences in opioid analgesia have been explicitly examined demonstrate greater or longer-lasting analgesia in women than in men (Gear et al., 1996a,b; Sarton et al., 2000; Mogil et al., 2003), greater analgesia in men (Cepeda and Carr, 2003; Zacny and Beckman, 2004), or no sex differences (Mogil et al., 2003; Fillingim et al., 2004). Sex differences in behavioral and neurochemical effects of NMDA antagonists also have been reported (in rodents); for example, female rats are more sensitive than males to MK-801-induced locomotor activation and ataxia (Blanchard et al., 1992; Hönack and Löscher,

* Corresponding author. Tel.: +1 509 335 5040; fax: +1 509 335 5043.
E-mail address: craft@wsu.edu (R.M. Craft).

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1993). Given the sex differences in behavioral effects of opioid agonists and NMDA antagonists given alone, it is likely that combinations of these drugs will produce differential effects in males vs. females. In fact, several studies have demonstrated sex differences in NMDA antagonist modulation of morphine antinociception. Lipa and Kavaliers (1990) found that MK-801 antagonized morphine antinociception in male but not female deer mice. More recently, several NMDA antagonists were found to potentiate the antinociceptive effects of morphine in female but not male rats (Holtman et al., 2003). In a more comprehensive study in mice, however, sex differences in NMDA antagonist modulation of morphine antinociception were found to depend on the dose of morphine, the type of NMDA antagonist, and time post-injection (Nemmani et al., 2004).

The purpose of the present study was to further examine sex differences in NMDA antagonist modulation of morphine antinociception in the rat. Based on the fact that sex differences in opioid effects are often quantitative rather than qualitative (that is, opioids may be less potent in females, but still produce the same effects as in males), we hypothesized that sex differences in NMDA antagonist modulation of morphine antinociception would be primarily quantitative. To test this hypothesis, we examined multiple doses of NMDA antagonists alone and in combination with multiple doses of morphine. We tested two common non-competitive NMDA antagonists, one of which is approved for use in humans (dextromethorphan (DEX)), and the other of which has been examined extensively in animal studies (MK-801), plus one competitive antagonist (LY235959 (LY)), as it has recently been suggested that competitive NMDA antagonists are more reliable modulators of morphine antinociception than are non-competitive antagonists (Nemmani et al., 2004). Because estrous stage may affect both morphine potency (Stoffel et al., 2003) and glutamate receptor density (Palermo-Gallagher et al., 2003), estrous stage of females was documented so that its potential influence also could be evaluated. To determine whether NMDA antagonist modulation of morphine antinociception depended on the interval between administration of the two drugs, an additional experiment was conducted in which LY was administered 1 h before morphine, instead of concurrently.

2. Methods

2.1. Subjects

Adult male and female Sprague–Dawley rats, bred in-house from Taconic stock (Germantown, NY) were used. Rats were 3–5 months old at the time of testing (approximately 400–500 g males, 250–300 g females). Males and females were housed in separate rooms, 2–3/cage, in a temperature-controlled vivarium. Food and water were available ad libitum except during testing. The light:dark cycle was 12:12 h, with lights on at 0600 h. Rats were tested between 0900 and 1500 h. All protocols used in this study were approved by the WSU IACUC (LARC #2354), and met the guidelines set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Apparatus

Nociceptive testing was conducted using a digital hotplate (Columbus Instruments, Columbus, OH) set at 50±0.1 °C and a water bath (Precision Scientific, Chicago, IL) set at 50±0.5 °C.

2.3. Procedure

Rats were tested on the hotplate and warm water tail withdrawal tests in that order, twice, approximately 10 min apart, to obtain baseline latencies to lick a hindpaw or jump off the plate (hotplate) and to move the tail from the water (tail withdrawal). Cutoff values were 80 s (hotplate) and 20 s (tail withdrawal) to prevent tissue damage. In the first three experiments, immediately after the second baseline test, rats were injected s.c. with saline or one dose of an NMDA antagonist and saline or one dose of morphine (1.8, 3.2, or 5.6 mg/kg); latencies to respond on the hotplate and tail withdrawal tests were then recorded 15–120 min post-injection. At each time point, the hotplate test was immediately followed by the tail withdrawal test. Each of the three NMDA antagonists was examined in separate experiments (conducted by multiple personnel over approximately two years); thus, separate saline and morphine treatment groups were included in each experiment. In the first experiment (MK-801), we anticipated that drug interactions would be most complex within the first hour; thus, nociception was tested 15, 30, 45, 60, and 120 min post-injection; for the other two NMDA antagonist experiments (DEX and LY), nociception was examined 15, 30, 60, 90, and 120 min post-injection. In the final experiment, LY was administered 1 h before morphine instead of giving the two injections concurrently, and nociception was examined 15, 30, 60, 90, and 120 min after the morphine injection. Separate rats were used to test each drug and dose combination.

2.4. Estrous stage determination

Immediately after nociceptive testing, vaginal smears were taken in female rats to determine estrous stage by examination of vaginal cytology. Slides were stained with Giemsa (Sigma). Proestrus was characterized by a predominance (approximately 80% or more of epithelial cells in the sample) of nucleated epithelial cells. Estrus was characterized by dense sheets of cornified epithelial cells, diestrus-1 (metestrus) by scattered nucleated and cornified epithelial

cells plus leukocytes, and diestrus-2 by a relative lack of any cells (Freeman, 1988). Because estrous stage was sampled only once in each rat, diestrus-1 and -2 determinations were made via vaginal cytology alone and do not necessarily indicate the first and second day after estrus.

2.5. Drugs

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD), dextromethorphan hydrobromide (ICN Biomedicals, Aurora, OH), MK-801 (Sigma, St. Louis, MO), and LY235959 (Tocris, Ellisville, MO) were each dissolved in physiological saline, and administered s.c. in a volume of 1 ml/kg. Doses of each NMDA antagonist were chosen based on pilot studies and published literature, to ensure that none of the doses produced any ataxia, as apparent to a trained observer. Previous studies have reported that doses of MK-801 of 0.08 mg/kg and higher produce locomotor activation and ataxia, respectively, in rats, with greater potency in females than in males (Blanchard et al., 1992; Hönack and Löscher, 1993; Frantz and Van Hartesveldt, 1999). DEX has been shown to be sedative in male rats at doses above 20 mg/kg (Dematteis et al., 1998), and 1.0 mg/kg LY has been shown to decrease vertical but not horizontal activity in male, alcohol-sensitive rats (Vekovischeva et al., 2000).

2.6. Data analysis

To compare non-drug response latencies between males and females, data from rats treated with saline were pooled across the DEX and LY experiments – since these had identical time-effect curves – and compared using 2-way ANOVA (sex (2), time (5, repeated). Because there was considerable individual variability in baseline latency to respond, individual saline and drug latencies were then converted to % Maximum Possible Effect (%MPE) values before analysis of drug effects, using each rat's second baseline latency: [(saline or drug latency − baseline latency)/(cutoff − baseline latency)] × 100. The first baseline was not used because on the hotplate, the first baseline latency in females is significantly longer than on subsequent tests, perhaps due to initial exploratory behavior (Craft and Bernal, 2001). Sex differences in morphine antinociception were examined via 3-way ANOVA (sex (2), morphine dose (4), time (5, repeated)), also by pooling data from the DEX and LY experiments. Sex differences in NMDA antagonist modulation of morphine antinociception were examined separately for each NMDA antagonist alone and in combination with each dose of morphine (0, 1.8, 3.2, 5.6 mg/kg), via 3-way ANOVA: sex (2), NMDA antagonist dose (4–5), time (5). In addition, for ease of visualization, area-under-the-curve (AUC) values were calculated from the time course latency data (drug AUC – same-sex, mean saline AUC), and these are plotted in Figs. 1–3. AUC data were analyzed by 2-way ANOVA (sex, NMDA antagonist dose). Post-hoc analyses were conducted using independent samples *t*-tests with the Bonferroni correction, to determine at what time points (or doses, for AUC data) NMDA antagonist–morphine combinations produced significantly different antinociception compared to morphine alone. To determine whether estrous stage significantly influenced NMDA modulation of morphine antinociception in female rats, estrous stage was entered as a covariate in an ANOVA conducted on all NMDA antagonist+morphine data from female rats in each NMDA antagonist experiment. Rats that were in transition between proestrus and estrus (approximately 50% nucleated, 50% cornified epithelial cells) were included in the estrus category, as these rats are hormonally similar to estrus females (Feder, 1981). Similarly, rats in diestrus-1 and diestrus-2 were pooled in one group ("diestrus"). Rats that were in transition from diestrus-2 to proestrus were not included in the estrous analysis, as they are hormonally distinct from all other stages but are observed very rarely. For all analyses, statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Sex differences in nociceptive baselines and morphine antinociception

Analysis of pooled data from saline-treated rats in the DEX and LY experiments showed that on the hotplate test, females had longer latencies to respond than males: mean latencies from 15–120 min post-injection were 23.3±2.8 vs. 17.3±1.2 s in females vs. males, respectively (sex: $F(1,30)=11.58$, $p=0.002$). There were no sex differences in latency to respond on the tail withdrawal test: mean latencies from 15–120 min post-injection were 5.47±0.20 vs. 5.07±0.27 s in saline-treated females vs. males, respectively. Analysis of pooled morphine data from the DEX and LY experiments showed that morphine produced greater hotplate ($F(1,104)=4.12$, $p=0.05$) and tail withdrawal ($F(1,104)=7.17$, $p=0.009$) antinociception in males than in females.

3.2. NMDA antagonist modulation of morphine antinociception

When administered alone (in combination with saline), MK-801 (0.005–0.04 mg/kg) and DEX (5–20 mg/kg) did not significantly affect latency to respond on the hotplate or tail withdrawal tests, in either sex (data not shown). In contrast, LY produced small increases in latency to respond on the hotplate test, up to approximately 20% MPE at the highest dose of LY (LY dose: $F(3,56)=7.12$, $p<0.001$); post-hoc analyses revealed that this effect was significant at LY 1.0 mg/kg in both females and males (data not shown). On the tail withdrawal test, LY produced similar increases in

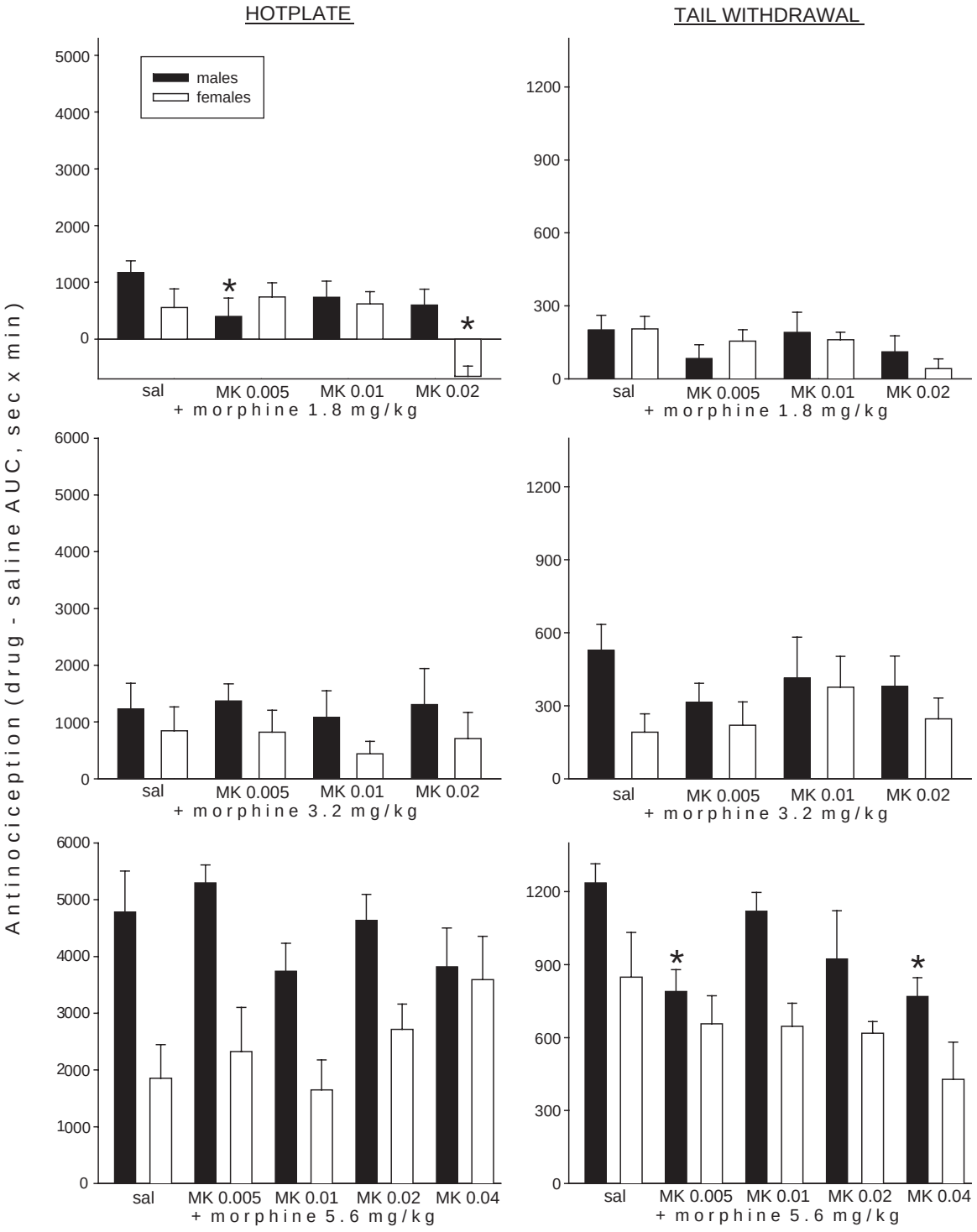

Fig. 1. MK-801 (MK) modulation of antinociception produced by 1.8 mg/kg morphine (top), 3.2 mg/kg morphine (middle), and 5.6 mg/kg morphine (bottom) in the hotplate (left panels) and tail withdrawal (right panels) tests, in female vs. male rats. Doses are in mg/kg, s.c.; MK and morphine injections were administered consecutively at time 0. Each bar is the mean+1 S.E.M. of 7–10 rats. *Significantly different from same-sex, saline+morphine (sal+morphine) controls, $p<0.05$.

latency to respond, but in males only (sex × LY dose: $F(3,56)=2.72$, $p=0.05$) (data not shown).

Fig. 1 shows the effects of the non-competitive NMDA antagonist MK-801 on antinociception produced by the low (top panels), medium (middle panels), and high (bottom panels) doses of morphine. When combined with the low dose of morphine, 1.8 mg/kg, MK-801 decreased morphine antinociception on the hotplate test, but only at sporadic doses (sex × MK-801 dose: $F(3,67)=3.80$, $p=0.015$). On the tail withdrawal test, the pattern of MK-801 attenuation

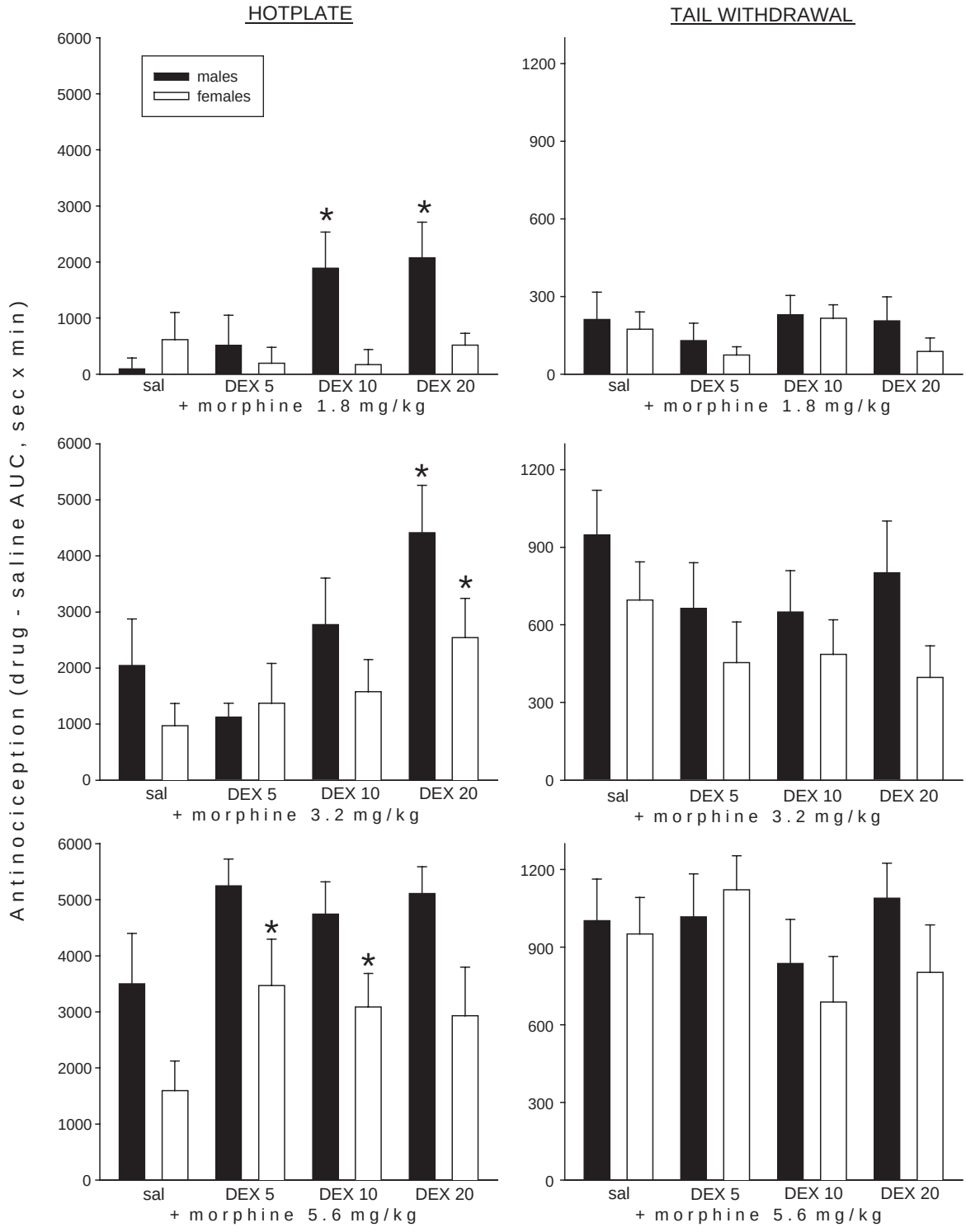

Fig. 2. Dextromethorphan (DEX) modulation of antinociception produced by 1.8 mg/kg morphine (top), 3.2 mg/kg morphine (middle), and 5.6 mg/kg morphine (bottom) in the hotplate (left panels) and tail withdrawal (right panels) tests, in female vs. male rats. Other details as in Fig. 1.

of 1.8 mg/kg morphine antinociception was similar, but none of the effects were statistically significant (Fig. 1, top right panel).

When combined with 3.2 mg/kg morphine (middle panels), MK-801 did not significantly affect morphine antinociception on the hotplate or tail withdrawal tests, based on analysis of AUC values. However, time course analysis revealed that MK-801 significantly decreased tail withdrawal antinociception in males (sex × MK dose × time: $F(12256)=2.05$, $p=0.02$; males only, MK dose × time: $F(12120)=1.93$, $p=0.04$; significant attenuation by 0.005 mg/kg MK-801 at 30–60 min post-

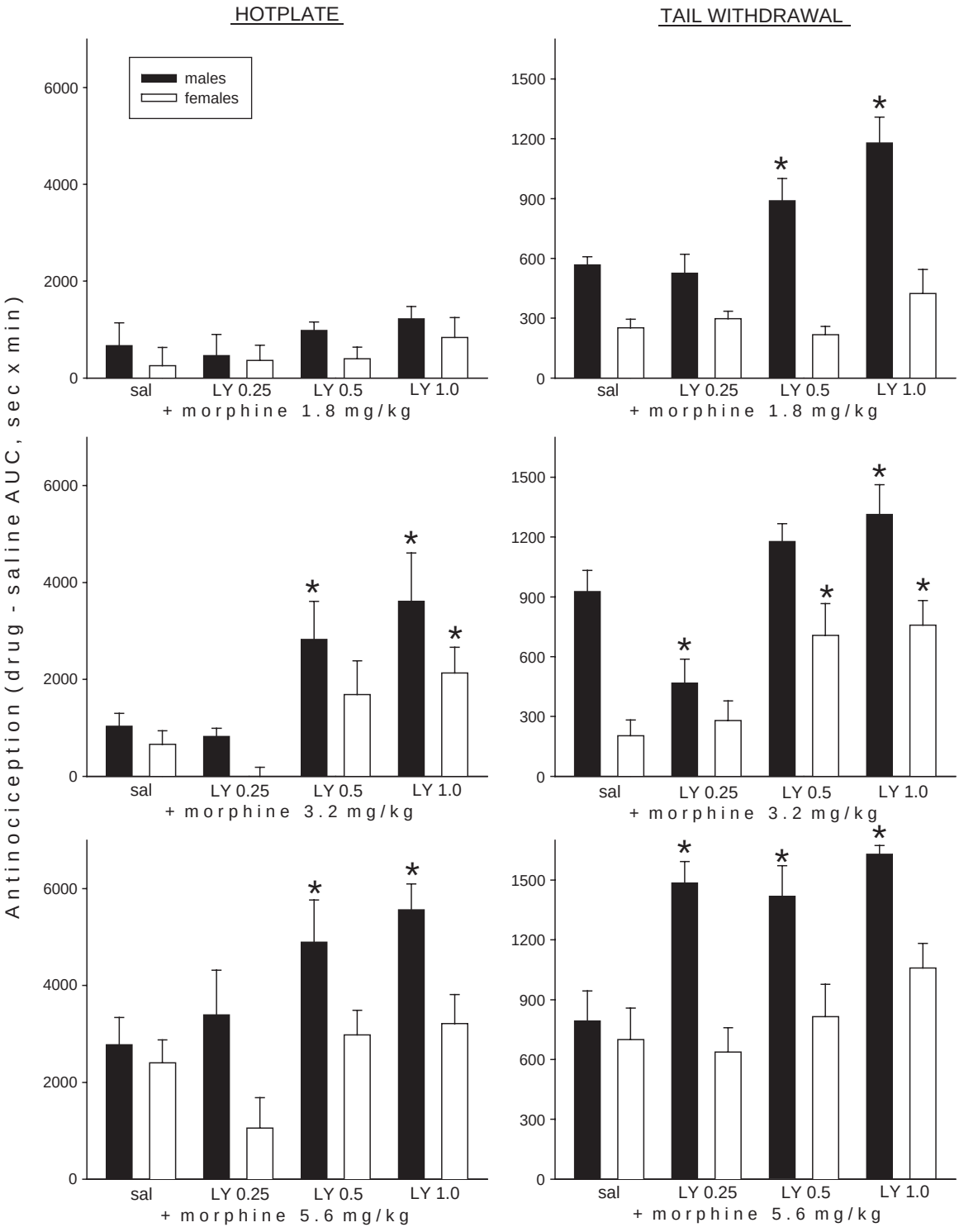

Fig. 3. LY235959 (LY) modulation of antinociception produced by 1.8 mg/kg morphine (top), 3.2 mg/kg morphine (middle), and 5.6 mg/kg morphine (bottom) in the hotplate (left panels) and tail withdrawal (right panels) tests, in female vs. male rats. Other details as in Fig. 1.

injection) (data not shown). When combined with the highest dose of morphine, 5.6 mg/kg (bottom panels), MK-801 did not significantly affect morphine antinociception on the hotplate test, but significantly decreased morphine antinociception on the tail withdrawal test ($F(4,74)=4.25$, $p=0.004$). Post-hoc analysis of AUC data yielded significant effects in males only (Fig. 1, bottom right panel); however, time course analysis revealed a similar effect in females (MK dose × time: $F(16,164)=1.75$, $p=0.042$), with MK-801 significantly decreasing morphine antinociception at 15–30 min post-injection (data not shown).

Fig. 2 shows the effects of the non-competitive NMDA antagonist DEX on antinociception produced by the low, medium, and high doses of morphine. When combined with the low dose of morphine, 1.8 mg/kg (top panels), DEX increased morphine antinociception, but only on the hotplate test, in males (sex × DEX dose: $F(3,56)=3.30$, $p=0.027$). When combined with 3.2 mg/kg morphine (middle panels), DEX increased morphine antinociception again only on the hotplate test, but this time in both sexes (DEX dose: $F(3,64)=5.70$, $p=0.002$; no interaction with sex). On the tail withdrawal test, DEX tended to decrease tail withdrawal antinociception produced by 3.2 mg/kg morphine, but this effect was not statistically significant ($p=0.2$). When combined with 5.6 mg/kg morphine (bottom panels), DEX increased morphine antinociception—again, only on the hotplate test (DEX dose: $F(3,68)=3.96$, $p=0.012$). The effect of DEX in males was not significant in the analysis of AUC values, but this appeared to be due to a ceiling effect (5.6 mg/kg morphine alone produced a 70–80% MPE in males in this experiment). Analysis of the time course data revealed that significant increases in morphine antinociception occurred only at later time points in both sexes (90–120 min post-injection; DEX dose × time: $F(12256)=4.58$, $p<0.001$) (data not shown).

Fig. 3 shows the effects of the competitive NMDA antagonist LY on antinociception produced by the low, medium, and high doses of morphine. When combined with 1.8 mg/kg morphine (top panels), LY did not affect morphine antinociception on the hotplate test, but increased it on the tail withdrawal test, to a significantly greater extent in males than in females (sex × LY dose: $F(3,63)=5.36$, $p=0.002$). In females, only 1.0 mg/kg LY significantly increased morphine antinociception in females, and only at later time points (LY dose × time: $F(4,72)=2.70$, $p=0.04$) (data not shown).

When combined with 3.2 mg/kg morphine (Fig. 3, middle panels), LY modulated morphine antinociception on both hotplate and tail withdrawal assays, in a complex manner. On the hotplate test, LY primarily increased morphine antinociception in both sexes (LY dose: $F(3,66)=10.32$, $p<0.001$). However, time course analyses revealed that the low dose of LY, 0.25 mg/kg, tended to decrease morphine antinociception; this decrease was significant only at 15 min post-injection in males (data not shown). In contrast, increases in morphine antinociception at the higher LY doses occurred at 30 min post-injection or later (data not shown). On the tail withdrawal test, LY also produced bidirectional effects, increasing morphine antinociception at higher LY doses, but decreasing it at the low

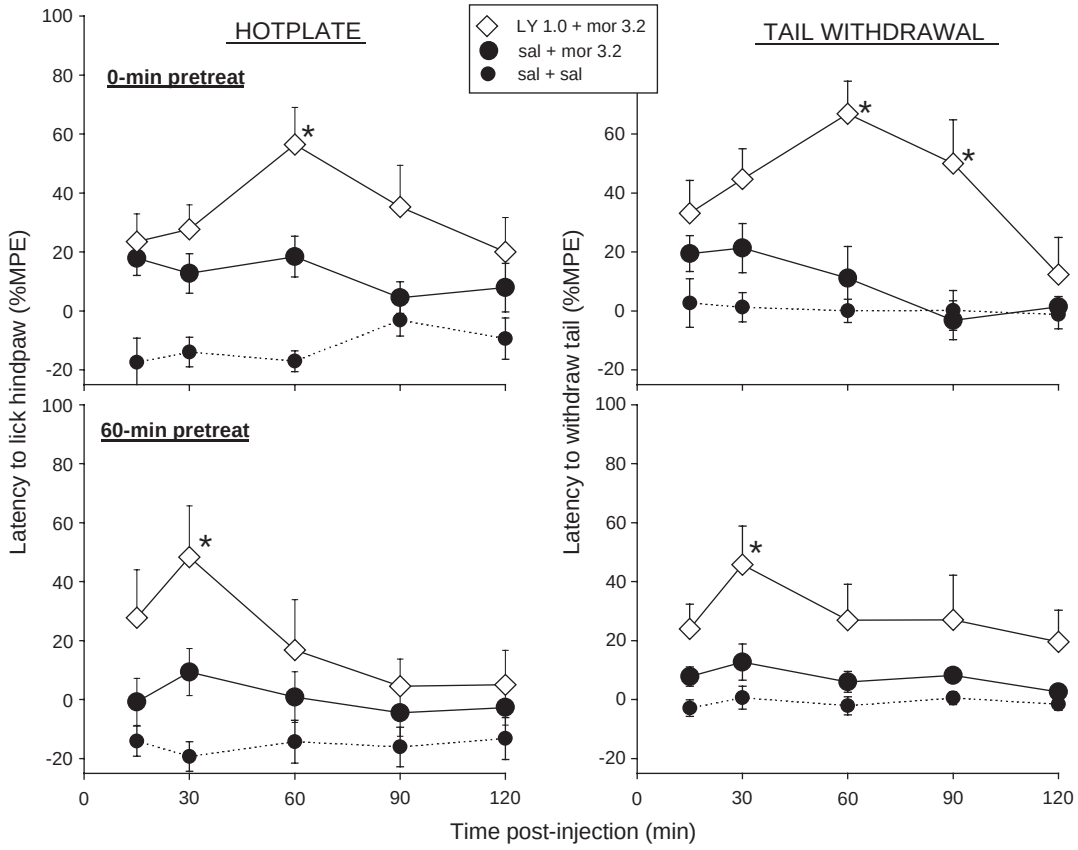

Fig. 4. Time dependence of NMDA antagonist modulation of antinociception produced by morphine in female rats. LY235959 (LY, 1.0 mg/kg) was administered immediately before (top panels) or 1 h before (bottom panels) 3.2 mg/kg morphine. The top panels are a subset of the data presented in Fig. 3. Other details as in Fig. 1.

dose of LY (LY dose: $F(3,66)=15.09$, $p<0.001$). The latter effect only occurred in males.

When combined with 5.6 mg/kg morphine (Fig. 3, bottom panels), LY also modulated morphine antinociception in a complex manner. On the hotplate test, LY primarily increased antinociception (LY dose: $F(3,65)=5.99$, $p=0.001$), primarily in males. Analysis of time course data showed that in females, LY 0.25 mg/kg decreased morphine antinociception at 30–60 min post-injection, whereas LY 1.0 mg/kg increased morphine antinociception at 90–120 min (LY dose × time: $F(12\,140)=3.73$, $p<0.001$) (data not shown). In contrast, in males LY only increased antinociception produced by 5.6 mg/kg morphine (LY dose: $F(3,30)=3.49$, $p=0.03$). On the tail withdrawal test, LY also increased morphine antinociception only in males (sex × LY dose: $F(3,73)=3.06$, $p=0.034$).

3.3. Estrous cycle influence on NMDA modulation of morphine antinociception

Across the three experiments, 19.1, 31.9, and 43.2% of female rats tested with morphine (with or without an NMDA antagonist) were in proestrus, estrus, and diestrus, respectively; 5.8% (20) of the 345 smear samples were missing, unreadable, or from rats in transition from diestrus-2 to proestrus. When entered as a covariate into the ANOVA, estrous stage contributed significantly to the overall variance only in the LY experiment (hotplate: $p=0.001$; tail withdrawal: $p=0.002$). Specifically, up to 12% of the total variance in antinociception in LY+morphine-treated females could be attributed to estrous stage of females, with diestrus females showing greater antinociception than estrus females, on both the hotplate and tail withdrawal tests. Comparisons to proestrus females could not be made reliably because there were four dose combinations in which there was only one or no proestrus females. To determine whether the estrous stage influence on the LY-morphine interaction could be explained by estrous stage modulation of morphine alone (e.g., Stoffel et al., 2003), an additional analysis of covariance was conducted on data from female rats treated with morphine alone (pooled from the DEX and LY experiments). Antinociception significantly covaried with estrous stage on the hotplate ($p=0.021$), with morphine producing more antinociception in diestrus than in estrus females. Again, conclusions could not be accurately drawn about proestrus females, as there were none in the 3.2 or 5.6 mg/kg morphine groups. In contrast to the hotplate test, there was no apparent influence of estrous stage on morphine-induced tail withdrawal antinociception ($p=0.8$).

3.4. Time dependence of NMDA antagonist modulation of morphine antinociception

To determine whether NMDA antagonist modulation of morphine antinociception depended on antagonist pretreatment time, separate female rats were injected with 1.0 mg/kg LY 1 h before injecting 3.2 mg/kg morphine. Fig. 4 (top panels) shows that when administered concurrently with morphine, LY increased morphine antinociception with a peak at 60–90 min post-injection. In contrast, when administered 1 h before morphine, LY increased morphine antinociception with a peak at 30 min post-injection. LY administered alone (1 h before saline) lengthened response latencies on the hotplate and tail withdrawal tests slightly but not significantly (data not shown).

4. Discussion

Table 1 summarizes the main findings of the present study. All three NMDA antagonists modulated morphine antinociception, but the specific drug interaction depended on several variables. First, NMDA antagonist modulation varied by drug and by nociceptive test: MK-801 significantly modulated morphine antinociception primarily on the tail withdrawal test, whereas DEX modulated morphine antinociception only on the hotplate test, and LY modulated morphine antinociception on both tests. Second, the direction of the modulation varied by antagonist: whereas MK-801 decreased morphine antinociception, DEX increased it, and LY produced both effects. When this dual modulation was observed, it was the lowest dose of LY that decreased, and the higher doses that increased morphine antinociception. Additionally, decreases tended to occur earlier and increases later in the time course of drug effect, a phenomenon that has been observed previously (Plesan et al., 1999). Finally, there were sex differences in NMDA antagonist modulation of morphine antinociception: NMDA antagonists were more potent or more reliable in modulating morphine antinociception in male than in female rats. However, there were no qualitative sex differences in the

Table 1
Summary of NMDA antagonist modulation of morphine antinociception: NMDA antagonists increased (up arrows), decreased (down arrows), or did not significantly affect (–) morphine antinociception in female and male rats

	MK-801		Dextromethorphan		LY235959	
	Hotplate	Tail W/D	Hotplate	Tail W/D	Hotplate	Tail W/D
Males						
+mor 1.8	↓[a]	–	↑[a]	–	–	↑[a]
+mor 3.2	–	↓[a]	↑	–	↓ and ↑[a,b]	↓ and ↑
+mor 5.6	–	↓	↑	–	↑[a]	↑[a]
Females						
+mor 1.8	–	–	–	–	–	↑
+mor 3.2	–	–	↑	–	↑	↑
+mor 5.6	–	↓	↑	–	↓ and ↑[b]	–

[a] Sex difference: NMDA antagonist was more potent, or modulated morphine antinociception at more time points and/or NMDA antagonist doses in males than in females.

[b] Low dose of LY235959 decreased morphine antinociception, higher doses increased morphine antinociception.

drug interactions; that is, when all dose combinations were considered, in no case was modulation only observed in one sex and not the other, nor was the direction of the modulation opposite in one sex compared to the other.

Morphine by itself was more potent in males than in females, and this sex difference appeared to contribute to sex differences in NMDA modulation of morphine antinociception. That is, NMDA antagonist modulation tended to be observed in males even at low morphine doses, but in females primarily at higher morphine doses. This result suggests that some moderate level of antinociception must be achieved in order to engage systems that involve NMDA receptors, and as long as the antinociceptive effect is sufficient, these systems are engaged in both sexes. NMDA antagonist modulation of opioid antinociception has been shown previously to depend on opioid dose (Kest et al., 1992; Nemmani et al., 2004).

The present results agree in part with those of a recent comprehensive study conducted in mice, in which NMDA antagonist modulation of morphine antinociception was greater in males than in females, was morphine dose-dependent, and occurred most robustly with the competitive antagonist LY235959 (Nemmani et al., 2004). However, in this previous study, absolutely no modulation of morphine antinociception was observed in females when non-competitive antagonists – including MK-801 and DEX – were examined. It is possible that our observation of significant MK-801 and DEX modulation of morphine antinociception in females – though less consistently observed than in males – is due to the fact that we examined a relatively wide dose range of each NMDA antagonist. Additionally, we used considerably lower doses of MK-801 than in previous studies, to avoid the possible confounding factor of drug-induced motoric changes. Sex differences in NMDA antagonist-induced hyper-locomotion and ataxia (Blanchard et al., 1992; Hönack and Löscher, 1993; Frantz and Van Hartesveldt, 1999) may contribute to sex differences in NMDA antagonist modulation of antinociception. Although we chose to avoid this issue by testing relatively low doses, future studies should determine whether drug interactions on locomotion and antinociception covary. Alternatively, differences between the present study and Nemmani et al. (2004) may indicate that sex differences in NMDA antagonist modulation of morphine antinociception are more dramatic in the mouse than in the rat. It will be important to determine whether species differences truly exist in NMDA antagonist–opioid interactions, as human clinical trials have been based primarily on data from these species (Caruso, 2000; Heiskanen et al., 2002). It should be noted as well that even within rodent species, strain differences have been reported in NMDA antagonist modulation of morphine antinociception (Plesan et al., 1999), and in sex differences in opioid antinociception (Cook et al., 2000; Mogil et al., 2000); thus, it will be important to determine whether the present results generalize to other rat strains.

Perhaps as a case in point, the present results almost entirely disagree with those of Holtman et al. (2003), who found that the NMDA antagonists MK-801, DEX, and ketamine modulated morphine antinociception to a greater extent in *female* than in male rats. Although the rat strain was the same as that used in the present study, the previous findings were based on testing with a single dose of morphine (3 mg/kg), a single nociceptive assay (tailflick), and repeated (weekly) testing. In addition, the doses of MK-801 used were approximately 10 times higher than those used in the present study, although the DEX doses were very similar. The use of a single dose of morphine does not appear to explain the discrepant findings: in the present study, although there were some morphine doses at which NMDA antagonist modulation was observed only in one sex, in all of these cases, it was males in which modulation was observed, not females. Perhaps more importantly, we tested each dose combination in different rats, whereas Holtman and colleagues tested all dose combinations in each rat. Male and female rats are known to develop differential tolerance to the same regimen of morphine administration, and males given morphine even once/week will show slight tolerance development (Craft et al., 1999). It is not known whether there are also sex differences in attenuation of morphine tolerance by NMDA antagonists, but the striking discrepancy between our results and those of Holtman et al. suggests that there may be. Future studies will be necessary to test this intriguing possibility.

A particularly puzzling result in the present study is the nociceptive test-specificity of drug interactions. Whereas MK-801 had effects primarily on the tail withdrawal test, DEX significantly affected morphine antinociception only on the hotplate test, and LY had effects on both tests. Although we had initially speculated that the hotplate and tail withdrawal tests might be used to discriminate between supraspinal and spinal mechanisms, respectively, of NMDA antagonist–morphine interactions, the inconsistent results across NMDA antagonists suggest that this hypothesis is flawed. Kozela et al. (2001) summarized previous studies in which either hotplate or tailflick/withdrawal tests were used to examine non-competitive NMDA antagonist–opioid agonist interactions in rats; although a majority demonstrates NMDA antagonist-induced increases in opioid antinociception, there are several studies that show no interaction or a decrease in opioid antinociception (with no consistency between tests). Kozela et al. (2001) go on to demonstrate that even when the same radiant heat stimulus is used, DEX (and other NMDA antagonists) increased morphine antinociception only when the heat was applied to the tail, not the paw. Such previous studies, in addition to the present one, suggest that NMDA antagonist modulation of opioid antinociception is a highly variable phenomenon, unusually dependent on specifics of the nociceptive testing procedure.

Another variable that has not been considered previously, which may influence the observation of sex differences in NMDA antagonist modulation of morphine antinociception,

is estrous stage in females. Estrous stage and ovarian hormones may influence morphine potency (for review, see Craft et al., 2004), as well as glutamate receptor pharmacology (D'Souza et al., 2003; Palermo-Gallagher et al., 2003). In the present study, only LY modulation of morphine antinociception was found to covary significantly with estrous stage. Specifically, diestrus females showed greater antinociception than estrus females. This effect may be accounted for, however, by the similar effect of estrous stage on antinociception produced by morphine alone. More specific conclusions regarding estrous stage modulation of this drug interaction will require further study in which multiple NMDA antagonists (preferably at multiple doses, since the direction of modulation may be dose-dependent) are administered alone and with morphine to females selected for proestrus, estrus, and diestrus stages. It will be important to determine whether ovarian hormone modulation of the effects of drug combinations may be explained by modulation of one or both components of the combination.

In some cases in the present study, NMDA antagonist modulation of morphine antinociception was also time-dependent, with decreases in (or no effect on) morphine antinociception being most often observed within 15 min post-injection, and increases most often observed at 30 min or later. It is possible that the apparent time-dependency of NMDA antagonist modulation of morphine antinociception simply reflected the onset of NMDA antagonist effect. That is, perhaps increases in morphine antinociception could only be observed later in the time course – peaking at 60 min post-injection – because it took that long for the NMDA antagonist to reach its target areas. The final experiment in the present study suggested that this was only partially true. When LY was administered 60 min before morphine instead of concurrently, we expected that the peak increases in morphine antinociception would be shifted approximately 60 min earlier (i.e., they would be observed at the first time point examined, 15 min post-injection)—however, they occurred at 30 min post-injection. This result suggests that NMDA antagonist enhancement of morphine antinociception is: (1) opposed by an early attenuating effect that does not subside until approximately 60 min post-injection; and/or (2) that the NMDA antagonist can only enhance morphine's effects after morphine has fully engaged the nociceptive systems on which it acts, which was at 30 min post-injection in this study.

The complexity of the drug interactions observed in the present study suggests that various mechanisms, presumably at multiple levels of the neuraxis, are engaged to different extents depending on the nociceptive response, the drug doses administered, and the time at which antinociception is evaluated after drug administration. Loci at which mu opioid–NMDA interactions are believed to occur include the ventrolateral periaqueductal gray (Kow et al., 2002), the rostral ventromedial medulla (Heinricher et al., 2001), and the spinal cord dorsal horn (Feng and Kendig, 1996; Aicher et al., 2002). Data from these previous studies suggest that NMDA receptor blockade in the mid- and hindbrain can attenuate, whereas NMDA receptor blockade in the spinal cord can enhance mu opioid antinociception. The present results – and indeed the perplexing array of results across previous studies of acute interactions of NMDA antagonists and mu opioid agonists – suggest that these opposing mechanisms are differentially engaged depending on the opioid dose, the NMDA antagonist dose, time post-injection, and nociceptive response. Given the potential clinical utility of NMDA antagonists and the widespread therapeutic use of mu opioid agonists, it will be important to determine under what conditions these drug interactions reliably occur. Contrary to previous studies, the present study suggests that NMDA antagonist modulation of morphine antinociception can be observed in rats of both sexes.

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References

Aicher SA, Goldberg A, Sharma S. Co-localization of mu opioid receptor and *N*-methyl-D-aspartate receptor in the trigeminal dorsal horn. J Pain 2002;3:203–10.

Bisaga A, Popik P. In search of a new pharmacological treatment for drug and alcohol addiction: *N*-methyl-D-aspartate (NMDA) antagonists. Drug Alcohol Depend 2000;59:1–15.

Blanchard DC, Blanchard RJ, De Padua Carobrez A, Veniegas R, Rodgers RJ, Shepherd JK. MK-801 produces a reduction in anxiety-related antipredator defensiveness in male and female rats and gender-dependent increase in locomotor behaviour. Psychopharmacology 1992; 108:352–62.

Caruso FS. MorphiDex® pharmacokinetic studies and single-dose analgesic efficacy studies in patients with postoperative pain. J Pain Symptom Manage 2000;19:S31–6.

Cepeda MS, Carr DB. Women experience more pain and require more morphine than men to achieve a similar degree of analgesia. Anesth Analg 2003;97:1464–8.

Cook CD, Barrett AC, Roach EL, Bowman JR, Picker MJ. Sex-related differences in the antinociceptive effects of opioids: importance of rat genotype, nociceptive stimulus intensity, and efficacy at the μ opioid receptor. Psychopharmacology 2000;150:430–42.

Craft RM. Sex differences in opioid analgesia: "from mouse to man". Clin J Pain 2003;19:175–86.

Craft RM, Bernal SA. Sex differences in opioid antinociception: k and 'mixed action' agonists. Drug Alcohol Depend 2001;63:215–28.

Craft RM, Stratmann JA, Bartok RE, Walpole TI, King SJ. Sex differences in development of morphine tolerance and dependence in the rat. Psychopharmacology 1999;143:1–7.

Craft RM, Mogil JS, Aloisi AM. Sex differences in pain and analgesia: the role of gonadal hormones. Eur J Pain 2004;8:397–411.

Dematteis M, Lallement G, Mallaret M. Dextromethorphan and dextrorphan in rats: common antitussives – different behavioural profiles. Fundam Clin Pharmacol 1998;12:526–37.

D'Souza DN, Harlan RE, Garcia MM. Modulation of glutamate receptor expression by gonadal steroid hormones in the rat striatum. Brain Res Bull 2003;59:289–92.

Feder HH. Estrous cyclicity in mammals. In: Adler NT, editor. Neuroendocrinology of reproduction. New York: Plenum Press; 1981. p. 279–348.

Feng J, Kendig J. The NMDA receptor antagonist MK-801 differentially modulates mu and kappa opioid actions in spinal cord in vitro. Pain 1996;66:343–9.

Fillingim RB, Ness TJ, Glover TL, Campbell CM, Price DD, Staud R. Experimental pain models reveal no sex differences in pentazocine analgesia in humans. Anesthesiology 2004;100:1263–70.

Frantz K, Van Hartesveldt C. Locomotion elicited by MK801 in developing and adult rats: temporal, environmental, and gender effects. Eur J Pharmacol 1999;369:145–57.

Freeman ME. The ovarian cycle of the rat. In: Knobil E, Neill J, editors. The physiology of reproduction. New York: Raven Press; 1988. p. 1893–928.

Gear RW, Gordon NC, Heller PH, Paul SM, Miaskowski C, Levine JD. Gender difference in analgesic response to the kappa-opioid pentazocine. Neurosci Lett 1996;205:207–9.

Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD. Kappa-opioids produce significantly greater analgesia in women than in men. Nat Med 1996;2:1248–50.

Heinricher MM, Schouten JC, Jobst EE. Activation of brainstem *N*-methyl-D-aspartate receptors is required for the analgesic actions of morphine given systematically. Pain 2001;92:129–38.

Heiskanen T, Härtel B, Dahl M-L, Seppälä T, Kalso E. Analgesic effects of dextromethorphan and morphine in patients with chronic pain. Pain 2002;96:261–7.

Hoffman VLH, Vermeyen KM, Adriaensen HF, Meert TF. Effects of NMDA receptor antagonists on opioid-induced respiratory depression and acute antinociception in rats. Pharmacol Biochem Behav 2003; 74:933–41.

Holtman JR, Jing X, Wala EP. Sex-related differences in the enhancement of morphine antinociception by NMDA receptor antagonists in rats. Pharmacol Biochem Behav 2003;76:285–93.

Hönack D, Löscher W. Sex differences in NMDA receptor mediated responses in rats. Brain Res 1993;620:167–70.

Kest B, Marek P, Liebeskind JC. The specific *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks U-50,488, but not morphine antinociception. Brain Res 1992;589:139–42.

Kow L-M, Commons KG, Ogawa S, Pfaff DW. Potentiation of the excitatory action of NMDA in ventrolateral periaqueductal gray by the mu-opioid receptor agonist, DAMGO. Brain Res 2002;935:87–102.

Kozela E, Danysz W, Popik P. Uncompetitive NMDA receptor antagonists potentiate morphine antinociception recorded from the tail but not from the hind paw in rats. Eur J Pharmacol 2001;423:17–26.

Lipa SA, Kavaliers M. Sex differences in the inhibitory effects of the NMDA antagonist MK-801 on morphine and stress-induced analgesia. Brain Res Bull 1990;24:627–30.

Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF. Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. Neurosci Biobehav Rev 2000; 24:375–89.

Mogil JS, Wilson SG, Chesler EJ, Rankin AL, Nemmani KVS, Lariviere WR, et al. The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. Proc Natl Acad Sci U S A 2003;100:4867–72.

Nemmani KVS, Grisel JE, Stowe JR, Smith-Carliss R, Mogil JS. Modulation of morphine analgesia by site-specific *N*-methyl-D-aspartate receptor antagonists: dependence on sex, site of antagonism, morphine dose, and time. Pain 2004;109:274–83.

Palermo-Gallagher N, Bidmon H-J, Zilles K. AMPA, kainate, and NMDA receptor densities in the hippocampus of untreated male rats and females in estrus and diestrus. J Comp Neurol 2003;459:468–74.

Plesan A, Hoffmann O, Xu X-J, Wiesenfeld-Hallin Z. Genetic differences in the antinociceptive effects of morphine and its potentiation by dextromethorphan in rats. Neurosci Lett 1999;263:53–6.

Sarton E, Olofsen E, Romberg R, den Hartigh J, Kest B, Nieuwenhuijs D, et al. Sex differences in morphine analgesia. Anesthesiology 2000; 93:1245–54.

Stoffel EC, Ulibarri CM, Craft RM. Gonadal steroid hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats. Pain 2003;103:285–302.

Trujillo KA. Are NMDA receptors involved in opiate-induced neural and behavioral plasticity? Psychopharmacology 2000;151:121–41.

Vekovischeva OY, Haapalinna A, Nakki R, Sarviharju M, Honkanen A, Heikkila J, et al. Enhanced locomotor stimulation by NMDA receptor antagonists in alcohol-sensitive ANT rats. Pharmacol Biochem Behav 2000;67:793–9.

Zacny JP, Beckman NJ. The effects of a cold-water stimulus on butorphanol effects in males and females. Pharmacol Biochem Behav 2004;78:653–9.