Sexually dimorphic effects of morphine and MK-801: sex steroid-dependent and -independent mechanisms

DEBORAH N. D’SOUZA,1 RICHARD E. HARLAN,1,3 AND MEREDITH M. GARCIA2,3

Departments of 1Structural and Cellular Biology and 2Otolaryngology, and 3Neuroscience Program, Tulane University School of Medicine, New Orleans, Louisiana 70112

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D’Souza, Deborah N., Richard E. Harlan, and Meredith M. Garcia. Sexually dimorphic responses to morphine and dizocilpine: sex steroid-dependent and -independent mechanisms. J Appl Physiol 92: 493–503, 2002; 10.1152/japplphysiol.00565.2001.—Rats show gender differences in responses to morphine and the N-methyl-D-aspartate receptor antagonist dizocilpine (MK-801); the role of sex steroids in mediating these differences is unclear. We tested the overall hypothesis that circulating gonadal steroids determine the gender differences in morphine- and MK-801-induced behavior and c-Fos expression. Morphine caused a greater expression of c-Fos in the striatum of intact males than of that females, which was independent of sex steroids. MK-801 completely inhibited morphine-induced c-Fos in intact females but only caused partial inhibition in intact males; castrated males showed complete inhibition, which was reversed by testosterone, but gonadal steroids had no effect on this response in females. In thalamus, there was a large sex difference in the response to MK-801 that was independent of gonadal steroids. Behavioral responses to morphine were greater in males, but responses to MK-801 were greater in females; both were sex steroid independent. These findings show significant sex differences in response to morphine and MK-801 that are mediated by sex steroid-dependent and -independent mechanisms, which may be important in treatment outcomes of drug addiction.

By Stewart and Rodaros (57) no gender differences were observed in the stimulant effects of morphine. Sex differences in the respiratory effects of morphine in humans have also been reported (20), although in a recent retrospective study by Zacny (64), no gender differences were reported in miosis or rate of respiration after intravenous administration of morphine. In this same study, however, the subjective effects of morphine were reported to be greater in female subjects than in males. Sex differences in patterns of drug abuse, including abuse of opiates, also have been reported, with approximately twice as many men as women using illicit drugs (6, 39, 51, 56). The role of steroid hormones in these gender differences is controversial and may be species specific and dependent on the effect studied (3, 16, 31, 33, 48, 49). Further information on sex differences and the roles of gonadal steroids in the responses to morphine may provide more tailored approaches to pain relief and to treatment of drug abuse.

Previous studies have demonstrated that some of the effects of morphine can be attenuated by antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors (e.g., 4, 22, 41, 42). Gender differences have also been reported in the effects of the noncompetitive NMDA receptor antagonist dizocilpine maleate (MK-801). Honack and Loscher (30) reported greater behavioral effects in females, who showed hyperlocomotion, head weaving, and ataxia after an injection of MK-801 (0.1 mg/kg ip), as opposed to males, who showed almost no behavioral effects in response to a similar dose of the drug. However, in deer mice, the inhibitory effects of MK-801 on analgesic responses are greater in males than in females (41). These data thus suggest that some of the MK-801-induced effects are sensitive to gender. This issue is important because of the wide use of MK-801 in studies on NMDA receptors.

Earlier studies from this and other laboratories have shown that morphine induces expression of immediate-early genes (IEG), especially c-Fos, in the rat caudate putamen (CPu) and thalamus (4, 11, 22, 25, 42). MK-801 and the competitive NMDA receptor antagonist NPC-17742 attenuate morphine-induced c-Fos expres-
sion in the rat forebrain (5, 22, 42), and our laboratory has shown that this attenuation is sexually dimorphic (22). In the present study, we tested several hypotheses. The overall hypothesis is that circulating gonadal steroids determine the gender differences in the behavioral and c-Fos responses to morphine, MK-801, and their combination. Thus castrated rats of both sexes were compared, to test the hypothesis that some gender differences are independent of circulating gonadal steroids. In addition, rats were either left gonadally intact or castrated and replaced with the gender-appropriate gonadal steroid, to test the hypothesis that some sex differences depend on circulating steroids. This approach also allowed us to test the hypothesis that responses to morphine, MK-801, or their combination are modified by gonadal steroids, in either gender.

Induction of c-Fos expression in the midline-intralaminar thalamic nuclei and the striatum suggests that morphine activates thalamostriatal circuits. Indeed, direct evidence for this hypothesis has recently been presented (26). Although the role of this circuit in the behavioral or physiological responses to morphine has not been determined, it is possible that this circuit is involved in the rewarding properties of morphine. It has been reported recently that microinfusion of an antisense oligonucleotide to c-fos mRNA into the nucleus accumbens, part of the ventral striatum, reduced the rewarding effects of morphine, as determined by conditioned place preference (59). Thus a focus on potential sex differences in this circuit may shed light on the neural mechanisms of gender differences in patterns of substance abuse in humans.

METHODS

Treatment of animals. Adult male or random-cycling female Sprague-Dawley rats were housed in group cages in the vivarium of the Tulane University Medical School, with controlled temperature and light-dark cycles of 12:12 h (lights on 0600; lights off 1800). Animals were provided with unlimited access to food (Purina rat chow) and tap water. All animal studies were approved by the Tulane Medical Center Advisory Committee for Animal Resources and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Male rats were either left intact or anesthetized with methoxyflurane and castrated by removal of the testes and testicular fat with a single 2-cm midscrotal incision. Half the castrated rats were given daily injections of 2 mg/kg testosterone propionate dissolved in sesame oil for a total of 8 days. Female rats were either left intact or anesthetized with methoxyflurane, ovariecotomized via bilateral dorsal incision, and implanted subcutaneously with either empty Silastic capsules or capsules containing crystalline estradiol (5 mm releasing length, 0.24 mm wall thickness). Eight days after surgery, the animals were assigned to one of four treatment groups (4 animals/group) and injected according to the following protocol: 1) vehicle (water, ip) followed by vehicle (water, sc) (V-V); 2) vehicle followed by morphine sulfate (10 mg/kg sc) (V-M); 3) MK-801 (0.2 mg/kg, ip) followed by vehicle; or 4) MK-801 followed by morphine. The second injections were administered 30 min after the first injections. Morphine sulfate was obtained from the National Institute on Drug Abuse, and MK-801 was obtained from RBI/Sigma Chemical. Steroids were obtained from Sigma Chemical.

Behavioral analysis. Behavioral observations were made immediately after the first injection and extended until the time of perfusion (i.e., 2 h after the second injection). The intensity of Straub tail was classified as “not present,” “weak,” or “strong.” The sedative effects of morphine were classified as “typical” (rats isolated from others in the cage and generally unreactive to other rats), “intense” (rats immobile but somewhat reactive to handling), and “catatonic” (immobile and unreactive to handling, with respiratory depression). General locomotor activity and motor coordination were also noted. Results were analyzed statistically with the χ² test.

Tissue preparation. Two hours after the second injection, the rats were deeply anesthetized with an overdose of pentobarbital sodium (100 mg/kg ip; Nembutal, Abbott). The rats were then perfused with 0.025 M PBS, pH 7.2, for 3 min, followed by 3% buffered paraformaldehyde for 6 min. Brains were dissected out, blocked at the midpontine level, postfixed in paraformaldehyde for 2 h at room temperature, cryoprotected in 30% sucrose at 4°C until they sank, frozen with crushed dry ice, and stored at −70°C until they were sectioned.

Immunocytochemistry. The brains were cut on a freezing microtome (60-μm coronal sections) and processed for immunocytochemistry as described previously (25). Sections were washed in PBS and incubated for 1 h at room temperature in blocking serum (normal goat serum, 15 μl/ml in 0.4% Triton (TX)-PBS). The sections were then incubated in rabbit anti-c-Fos (sc-52, Santa Cruz; 1:10,000 dilution) at 4°C for 2 days. After being washed in PBS, the sections were incubated for 1 h with the secondary antibody (biotinylated goat anti-rabbit IgG, 1:400 in 0.4% TX-PBS; Vector), washed in PBS again, and incubated in ABC solution (1:100 in 0.4% TX-PBS; Vectastain Elite, Vector) for 1 h. The sections were finally washed in Tris buffer 0.05 M, pH 7.6, and then immersed in 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.005% H₂O₂ to visualize the reaction product. The reaction was stopped with PBS, and the sections were mounted from 10 mM sodium acetate containing 0.1% Dreyf's detergent onto chrom-alum subbed slides. The sections were dried using a slide warmer, dehydrated in 100% ethanol for 2 min, and cleared in Histoclear (National Diagnostics) for 10 min before coverslipping with Permount (Fisher).

Data analysis. The immunocytochemical data were examined with a Nikon Optiphot microscope. The regions of the brain examined for immediate-early gene (IEG) expression were the dorsomedial CPUs and the midline intralaminar nuclei of the thalamus. The numbers of labeled cells per unit area were counted using the NIH Image program according to the methods described previously (9). With the use of image analysis, the desired regions of interest were outlined on the brain images, i.e., within the dorsomedial CPUs (height 1.66 mm; width 1.30 mm), the reuniens nuclei (height 0.58 mm; width 0.84 mm), the rhomboid nucleus (height 0.51 mm; width 0.51 mm), the central medial nucleus (height 0.35 mm; width 1.30 mm), and the paraventricular nucleus (height 0.53 mm; width 0.66 mm), and counts were taken within the circumscribed region. The data were analyzed statistically with one-way or three-way ANOVA. For the treatment groups in which ANOVA showed significance (which was all of the groups), ANOVA was followed by post hoc analysis with the Fisher's paired least-significant difference, Scheffé’s, or Bonferroni/Dunn tests.
RESULTS

**IEG expression in the CPu.** In males, morphine caused a significant induction of c-Fos expression in the dorsomedial CPus of rats that were intact (Figs. 1A and 2A), castrated (Fig. 1A), or castrated and treated with testosterone (Fig. 1A). There was no significant difference across the three groups in the response to morphine. Thus the hypothesis that gonadal steroids modify the c-Fos response to morphine in males was not supported. MK-801 followed by vehicle did not induce c-Fos in any group (Fig. 1A). MK-801 significantly inhibited morphine-induced c-Fos expression in the dorsomedial CPus in all three groups (Fig. 1A); however, the magnitude of the MK-801-mediated inhibition was dependent on gonadal hormones. In intact male rats, MK-801 significantly decreased morphine-induced c-Fos expression, although the number of c-Fos-positive cells was still greater than in vehicle-treated rats (Figs. 1A and 2A). After castration, there was a complete inhibition of morphine-induced c-Fos expression in males (Figs. 1A and 2C); however, in castrated males that received testosterone replacement, MK-801 showed the same partial inhibition of morphine’s effects as was seen in the intact males (Figs. 1A and 2D). Thus the hypothesis that gonadal steroids modify the interaction between morphine and MK-801 was supported in males.

In females, morphine caused a significant induction of c-Fos in the dorsomedial CPus in rats that were intact, ovariectomized, and ovariectomized with estrogen treatment (Fig. 1B). Thus the hypothesis that gonadal steroids modify the c-Fos response to morphine in females was not supported. However, the magnitude of the effect of morphine was smaller in the intact females than in the intact males (Table 1; Figs. 2, A and E), and this sex difference was not eliminated by gonadectomy or with or without hormone replacement. Thus the hypothesis that the sex difference in the c-Fos response to morphine is determined by circulating gonadal steroids was not supported. MK-801 completely inhibited morphine-induced c-Fos response in all three groups of females (Fig. 1B and 2F). In females, regardless of gonadal hormone status, MK-801 inhibition of morphine-mediated c-Fos expression was significantly greater than in intact or castrated plus testosterone-treated males. However, this sex difference was eliminated by castration (Table 1). Thus the hypothesis that the sex difference in the interaction between morphine and MK-801 is mediated by gonadal steroids was supported.

Three-way ANOVA revealed highly significant main effects of sex (P < 0.001), drug treatment (P < 0.001), and sex-drug interactions (P < 0.01) in c-Fos expression in the dorsomedial CPu.

**IEG expression in the thalamus.** Morphine alone caused a significant induction of c-Fos in the central medial nucleus in intact and castrated males (Fig. 3A) and in the rhomboid nucleus of castrated males (Fig. 4A). In female rats, morphine induced c-Fos expression in the central medial nucleus of ovariectomized rats (Fig. 3B) and in the rhomboid nucleus of intact rats (Fig. 4B). To analyze the data further for potential sex differences, we performed a separate ANOVA on each thalamic nucleus, collapsing across the hormone-treatment groups. This analysis revealed that morphine significantly induced c-Fos in the central medial nu-
Sex steroid modulation of effects of morphine or MK-801

Fig. 2. Photomicrographs of the dorsomedial CPu of intact male rats treated with morphine (A) or MK-801 plus morphine (B), castrated male rats treated with MK-801 plus morphine (C), castrated plus testosterone-treated male rats injected with MK-801 plus morphine (D), and intact (Int) female rats treated with morphine (E) or MK-801 plus morphine (F). Treatment with morphine alone induced a larger c-Fos response in intact males (A) compared with intact females (E) in the dorsomedial CPu. MK-801 significantly reduced morphine-induced c-Fos expression in intact males (B) and completely inhibited this expression in castrated males (C) and in intact females (F). LV, lateral ventricle.

nucleus in both sexes (mean = 22.9 ± 6.5 for V-V in males, 93.9 ± 15.7 for V-M in males; 17.0 ± 2.2 for V-V in females, and 72.8 ± 5.6 for V-M in females). There was no sex difference in the response to morphine. The effect of morphine was not blocked by MK-801 in males; however, the analysis is more complicated in females because MK-801 greatly induced c-Fos expression in the midline-intralaminar thalamic nuclei (see below). There was no effect of morphine in the reuniens or paraventricular nuclei in either sex (Figs. 5 and 6). Examples of the responses to the different treatments in castrated male and female rats are shown in Fig. 7.

Significant sex differences were found in the response to MK-801 and to MK-801 plus morphine in all four thalamic nuclei (Figs. 3–6; Table 2). In females, but not in males, MK-801 greatly induced c-Fos expres-
Sex steroid modulation of effects of morphine or MK-801

Table 1. Summary of treatment conditions in which the c-Fos response in the CPu was significantly greater in males than in females

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intact</th>
<th>Castrated</th>
<th>Hormone Replaced</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-M</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.05$</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>MK-M</td>
<td>$P &lt; 0.001$</td>
<td>NS</td>
<td>$P &lt; 0.05$</td>
</tr>
</tbody>
</table>

V-M, vehicle followed by morphine; MK-M, dizocilpine maleate (MK-801) plus morphine; NS, not significant.

The greater c-Fos response to MK-801 in females was independent of circulating gonadal steroids in all four nuclei (Fig. 7, C and G). Thus the hypothesis that gender differences in the c-Fos response to MK-801 are mediated by gonadal steroids was not supported. In all four nuclei of female rats, morphine significantly decreased the c-Fos expression induced by MK-801 (Fig. 3–6). However, the extent of this decrease was modulated by circulating gonadal steroids. In the reuniens, rhomboid, and central medial nuclei, the number of Fos-positive cells after MK-801 plus morphine was significantly greater in the ovariectomized rats than in the intact females or estradiol-replaced rats ($P < 0.05$). Moreover, in the absence of gonadal steroids, the combination of MK-801 plus morphine induced significantly more Fos-positive cells in these three nuclei in females than in males ($P < 0.01$; Table 2). Thus the hypothesis that gonadal steroids...
modify the interaction between morphine and MK-801 was supported in females.

Behavioral observations. After morphine administration, the animals became quiet and stuporous. They lost their righting reflex and displayed Straub tail. We looked at the presence of Straub tail during the first 60 min and at 120 min after the injection and categorized this response as "not present," "weak," or "strong." After collapsing across steroid treatment groups, there was a significant sex difference in the intensity of Straub tail in response to morphine (Table 3). At both time points, there was a significantly stronger effect on Straub tail in males than in females ($P < 0.05$). We did not test for effects of gonadal steroids.

Administration of MK-801 before morphine significantly enhanced the sedative response to morphine in both sexes (Table 3). Only 5 of the 12 male rats and none of the female rats given MK-801 plus morphine showed a "typical" response to morphine, compared with all 24 of the rats given vehicle-morphine. Moreover, MK-801 plus morphine induced catatonia in five male and eight female rats, whereas morphine by itself never induced catatonia in either sex. The frequency of "intense" sedation plus "catatonia" was significantly greater in females than in males after MK-801 plus morphine. In addition, MK-801 greatly increased the intensity and duration of Straub tail produced by morphine in female rats both during the first 60 min ($\chi^2 = 8.0, P < 0.05$) and at 120 min ($\chi^2 = 16.1, P < 0.01$) after morphine administration. Thus the combination of MK-801 plus morphine produced a more profound be-

Fig. 5. Mean ± SE number of c-Fos immunoreactive cells in the reuniens nucleus of male (A) and female (B) rats. MK-801 alone induced significant c-Fos expression in male and female rats. Morphine significantly attenuated MK-801-induced c-Fos expression in females and castrated males. This attenuation was significantly less in ovariectomized rats than in intact females or estrogen-replaced rats. The number at the bottom of each bar represents the number of animals in each group, **$P < 0.05$ vs. corresponding V-V, ***$P < 0.01$ vs. corresponding MK-V, and *$P < 0.05$ vs. MK-M in intact females.

Fig. 6. Mean ± SE number of c-Fos immunoreactive cells in the paraventricular nucleus of male (A) and female (B) rats. MK-801 alone induced a significant increase in c-Fos expression in female rats. Morphine significantly attenuated MK-801-induced c-Fos expression in females and castrated males. This attenuation was significantly less in ovariectomized rats than in intact females or estrogen-replaced rats. The number at the bottom of each bar represents the number of animals in each group, **$P < 0.05$ vs. corresponding V-V, ***$P < 0.01$ vs. corresponding MK-V, and *$P < 0.05$ vs. MK-M in intact females.
behavioral effect in females than in males. We did not test for effects of gonadal steroids.

We also saw a significant behavioral response to MK-801 in females, in which, within 20 min after the MK-801 injection, the rats started to stumble, weave their heads back and forth, wobble, and show significant hyperactivity for 2.5 h after the injection, until the time of perfusion. There were no obvious effects of gonadal hormones on behaviors induced by MK-801 in females. After the administration of MK-801 alone in males, we did not see any obvious behavioral effects. This sex difference persisted in the gonadectomized rats.

DISCUSSION

Sex differences in the behavioral and IEG responses to morphine alone were independent of circulating gonadal hormones. The larger morphine-induced c-Fos expression in the dorsomedial CPu in males was not modified by gonadal hormones. Morphine also induced a stronger behavioral effect in males than in females, and this was not altered by gonadal steroids. These findings are consistent with previous studies that have reported greater morphine-induced antinociception in males (5, 10, 13, 33, 34, 37, 41, 49, 53), which also has been reported to be independent of gonadal hormones (3, 33, 49). They are also consistent with findings that

Table 2. Summary of treatment conditions in which the c-Fos response in thalamic nuclei was greater in females than in males

<table>
<thead>
<tr>
<th>Nucleus/Treatment</th>
<th>Intact</th>
<th>Castrated</th>
<th>Hormone Replaced</th>
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<tr>
<td>Central medial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-V</td>
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<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>MK-M</td>
<td>NS</td>
<td>$P = 0.002$</td>
<td>NS</td>
</tr>
<tr>
<td>Rhomboid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-V</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>MK-M</td>
<td>NS</td>
<td>$P &lt; 0.001$</td>
<td>NS</td>
</tr>
<tr>
<td>Reuniens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-V</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>MK-M</td>
<td>NS</td>
<td>$P = 0.011$</td>
<td>NS</td>
</tr>
<tr>
<td>Paraventricular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-V</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>MK-M</td>
<td>$P &lt; 0.05$</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

MK-V, MK-801 plus vehicle.
gonadal steroids do not alter sexually dimorphic opioid-induced respiratory effects in women (20), the potency of morphine in a drug discrimination test in rats (17), or the initiation or maintenance of opiate self-administration in rats (58).

The lack of effect of sex steroids on opiate-mediated analgesia, respiratory depression, and reward is in contrast to their effects on reproduction through opioidergic systems. Both endogenous opioids and opioid receptors in the hypothalamus are important in reproductive function in both sexes, and a number of studies have shown regulation of mu opioid receptor expression by estrogen in several hypothalamic nuclei; however, estrogen does not regulate opioid receptor expression in most other regions of the forebrain, including the striatum (8, 21, 32, 52). Estrogen has also been shown to have an acute effect on mu opioid receptor distribution in the medial preoptic nucleus of the hypothalamus, the bed nucleus of the stria terminalis, and the posterodorsal medial amygdala (23), and estrogen has been shown to modulate mu opioid receptor activity in cultured neurons from the arcuate nucleus, through interactions with the protein kinase A pathway (62). Thus sex steroids may exert region-specific activational effects on opioid receptors, but these effects spare the striatum. Gender differences in the analgesic and reward properties of opiates thus seem more likely to be due to the organizational effects of sex steroids rather than their activational effects. Steroids are known to exert organizational effects on the brain, which are mediated by the presence of sex steroids during certain critical periods of development. Evidence exists to suggest that this may be an underlying mechanism for gender differences in some responses to morphine (40), which may be expressed as sex-specific differences in the number and/or distribution of opioid receptors in the brain (28, 65), although this has not been examined in the striatum. Another possible mechanism for gender-specific responses to opiates could be in pharmacokinetics; however, no gender differences have been shown to exist in morphine pharmacokinetics in the rat (14, 15).

In contrast to the lack of effect of gonadal steroids on morphine-mediated c-Fos expression, we saw an androgen-dependent sex difference in the ability of MK-801 to block morphine-induced c-Fos expression in the dorsomedial CPu. An involvement of glutamate and NMDA receptors in morphine-induced IEG expression in the striatum has been reported (4, 22, 42). The cerebral cortex and the midline-intralaminar thalamic nuclei are the major sources of glutamatergic inputs to the striatum. Whereas the cerebral cortex contains low levels of mu opioid receptors, the medial thalamic nuclei contain the highest concentration of mu opioid receptors in the brain (7). The central medial thalamic nucleus projects selectively to the dorsomedial CPu (2), where systemic morphine administration induces expression of IEGs. Recently, a role for thalamostriaital projections in morphine-induced striatal c-Fos expression has been demonstrated by infusion of the mu opioid receptor antagonist β-funaltrexamine into selected midline-intralaminar thalamic nuclei (24). These results suggest that morphine-induced IEG expression in the dorsomedial CPu is subsequent to activation of thalamostriaital glutamatergic projections. This is consistent with the finding that infusion of MK-801 into the CPu blocks IEG induction by systemic morphine (4). It is also consistent with morphine-induced activation of c-Fos, especially in the central medial thalamic nucleus (25; present results), and with increased blood flow to the medial thalamus induced by the mu receptor agonist hydromorphone in humans (54). The inability of MK-801 to block morphine-induced c-Fos expression in the central medial nucleus is consistent with a thalamic effect of morphine independent of NMDA receptors. Our results of sex differences in the striatal c-Fos response to morphine suggest the possibility of sex differences in thalamostriaital mechanisns. Sex differences in the abundance of mu opioid receptors in the human thalamus have been reported (65), with females showing higher levels than males, although this difference disappeared with age. The existence of sex differences in mu opioid receptors in the thalamus and striatum of rat brain has not been investigated, to our knowledge.

Potential mechanisms by which androgens regulate the striatal response to MK-801 include regulation of NMDA receptors and/or the activity of thalamostriaital
neurons. There is much evidence in the literature suggesting that estrogens regulate NMDA receptors in the brain (e.g., 27, 36). It was therefore surprising that we saw no effect of estrogens on the ability of MK-801 to block morphine-induced IEG expression in the dorsomedial CPu. Evidence suggests that androgens can regulate expression of NMDAR1 subunits in the hypothalamus (38) and MK-801 binding in the hippocampus (50). Whether similar regulation occurs in the striatum, where androgen receptors are low or absent (55), is not known. Very low levels of androgen receptor mRNA-containing cells were reported in the thalamic paraventricular nucleus and none in the central medial, rhomboid, and reuniens nuclei of the thalamus (55). However, there are various neuropeptidergic inputs from the hypothalamus to the midline-intralamina thalamic nuclei, including substance P, somatostatin, neurotensin, and β-endorphin. Androgens can regulate β-endorphin neurons in the brain (12, 46, 47) and can increase β-endorphin immunoreactivity in the fiber plexus of the thalamus (29). Thus androgens may exert effects on midline intralaminar thalamic nuclei through peptidergic inputs. Altered activity of thalamostrial neurons might explain a complete block of the morphine-induced IEG expression by MK-801 in the CPu of castrated males as opposed to an incomplete block in intact males. Similarly, estrogen-regulated neuropeptide inputs to the thalamus may underlie the decreased ability of morphine to block MK-801-induced IEG expression in the midline-intralaminar thalamus in ovariectomized rats (Figs. 5–7).

In intact female rats, the administration of MK-801 alone produced a robust increase in c-Fos expression in the cortex (22, 26), and reuniens, rhomboid, central medial, and paraventricular nuclei of the thalamus. This effect was sexually dimorphic and independent of gonadal steroids in females. MK-801 induced no obvious behavioral responses in males but induced uncoordinated hyperactivity in females. Although sex differences in MK-801-induced behavior have been reported earlier (19, 30), this is the first report that has systematically investigated the effect of gonadal hormones on IEG expression and behavioral effects in response to MK-801. We found that the behavioral sex difference in response to MK-801 persists in gonadectomized rats, with or without appropriate steroid replacement. Hockack and Loscher (30) also reported that these sex differences are not likely to be dependent on the estrous cycle or on differences in drug metabolism between males and females. MK-801 has been found to increase serotonin metabolite levels in brain regions in both male (63) and female (43) rats. However, we know of no direct comparisons of the serotonin-releasing effects of MK-801 in male vs. female rats. MK-801-induced IEG expression in the thalamus in females could be due to an activation of 5-HT (serotonin) receptors in this region of the brain. Moreover, the behaviors induced by MK-801 are similar to those induced by 5-HT agonists such as 8-OH-DPAT (61), L-tryptophan, or 5-hydroxytryptophan (60), and antagonists of the 5-HT1A receptor block the behavioral effects of MK-801 (44). These reports thus suggest that the behavioral effects of MK-801 may be modulated by serotonergic systems. Whether serotonergic mechanisms underlie the sexual dimorphism in the IEG response to MK-801 is not known.

In conclusion, we observed sex differences in MK-801 and morphine-induced behaviors and IEG expression in the brain, and some of these gender differences appear to be mediated by gonadal steroids, whereas others are independent of circulating gonadal steroids. These studies may have therapeutic implications for the treatment of drug addiction and for pain control by opiates.

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Gazzaley AH, Weiland NG, McEwen BS, and Morrison JH.


