Published in final edited form as:

Pain. 2009 December 15; 147(1-3): 210. doi:10.1016/j.pain.2009.09.008.

Drug Dependent Sex Differences in Periaqueductal Gray Mediated Antinociception in the Rat

Erin N. Bobeck, Amy L. McNeal, and Michael M. Morgan

Washington State University Vancouver

Abstract

Mu-opioid receptor (MOPr) agonists, such as morphine, produce greater antinociception in male compared to female rats. The ventrolateral periaqueductal gray (vlPAG) appears to contribute to this sex difference despite fewer vlPAG output neurons projecting to the rostral ventromedial medulla in male compared to female rats. This greater projection in female rats suggests that non-opioid activation of vlPAG output neurons should produce greater antinociception in female compared to male rats. This hypothesis was tested by comparing the time course and antinociceptive potency of microinjecting MOPr agonists (morphine, DAMGO, fentanyl) and non-opioid compounds (bicuculline, kainic acid) into the vlPAG of female and male rats. Microinjection of morphine or DAMGO produced antinociception that had a slow onset (peak from 15–30 min) and long duration (60 min) compared to the antinociception produced following microinjection of fentanyl, bicuculline, or kainic acid (peak effect at 3 min; duration less than 30 min). No sex-differences in the time courses were evident. All five compounds caused a dose-dependent antinociception when microinjected into the vlPAG. Antinociceptive potency was significantly greater in male compared to female rats following microinjection of morphine, DAMGO, and bicuculline, but not following microinjection of fentanyl or kainic acid. In no case did activation of the vlPAG produce greater antinociception in female compared to male rats. These findings demonstrate that the vlPAG can produce comparable antinociception in female and male rats, but antinociception produced by inhibition of GABAergic neurons (whether by morphine or the GABA_A receptor antagonist bicuculline) produces greater antinociception in males.

Keywords

analgesia; descending modulation; morphine; pain; opioid

Introduction

Mu-opioid receptor (MOPr) agonists, such as morphine, are commonly used to treat pain. Morphine antinociception tends to be greater in males compared to females in humans and other species [4,8,14,25]. Although the mechanism underlying this sex-difference in antinociception has not been determined, the descending nociceptive modulatory system that

Correspondence author: Erin Bobeck, Department of Psychology, 14204 NE Salmon Creek Ave, Vancouver, WA 98686, Phone: 360 546-9742, Fax: 360 546-9038, erin.bobek@email.wsu.edu.

Conflicts of Interest

The authors had no conflicts of interest related to the work presented in this manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

includes the ventrolateral periaqueductal gray (vlPAG) appears to contribute [16,17,20,21]. The vlPAG modulates nociception via a pathway that involves the rostral ventromedial medulla (RVM) and the dorsal horn of the spinal cord [2]. Microinjection of morphine directly into the vlPAG produces antinociception [11,12,30] and blockade of MOPr in the vlPAG attenuates antinociception produced by systemic morphine administration [3,18,40].

The finding of greater morphine antinociception in males is surprising given that the vlPAG to RVM projection is much denser in female compared to male rats [21]. However, female rats have lower MOPr expression within the vlPAG than male rats and morphine activation of vlPAG neurons projecting to the RVM is greater in male compared to female rats [3,19,23]. These findings indicate that female rats have a much greater descending pathway not activated by MOPr agonists compared to male rats. An interesting question is whether these non-morphine activated projections contribute to nociceptive modulation. If these output neurons contribute to antinociception, then direct activation of vlPAG output neurons should produce greater antinociception in female compared to male rats. This hypothesis was tested by comparing the antinociceptive effects of microinjecting an excitatory amino acid (kainic acid), a GABA_A receptor antagonist (bicuculline) and MOPr agonists (morphine, DAMGO, and fentanyl) into the vlPAG of female and male rats.

MOPr agonists and bicuculline produce antinociception by disinhibiting tonically active GABAergic neurons, but MOPr agonists primarily inhibit presynaptic release whereas bicuculline prevents postsynaptic binding [39]. Thus, comparing the effects of morphine and bicuculline will reveal whether sex differences are mediated by opioid sensitive GABAergic neurons or output neurons (see Figure 1 circuit). The effect of activating output neurons not in this opioid/GABAergic circuit will be evaluated by directly activating vlPAG output neurons with the excitatory amino acid, kainic acid.

Methods

Subjects

Cycling female (n = 56) and intact male (n = 53) Sprague-Dawley rats were age-matched (> 60 days) at the beginning of the experiment. Female (175–245 g) and male (280–350 g) rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and implanted with a guide cannula (23 gauge; 9 mm long) aimed at the vlPAG using stereotaxic techniques (AP: +1.7 mm, ML: $\pm$ 0.6 mm, DV: -4.6 mm for males and -4.5 mm for females from lambda). Dental cement anchored the guide cannula to two screws in the skull. A stylet was inserted into the guide cannula at the end of surgery. Rats were maintained under a heating lamp until awake and then housed individually in a room maintained on a reverse light/dark schedule (lights off at 7:00 AM). Food and water were available at all times except during testing. Rats were handled daily. Testing began at least 7 days following surgery. Estrus phase was not measured in order to prevent heightened stress in female rats. All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the guidelines of the Committee for Research and Ethical Issues for the International Association for the Study of Pain. Efforts were made to minimize the number and potential suffering of subjects by testing rats in two or three conditions on different days.

Microinjections

Morphine sulfate (A gift from the National Institute on Drug Abuse), DAMGO, fentanyl citrate, bicuculline methiodide, and kainic acid (Sigma-Aldrich; St. Louis, MO) were administered through a 31-gauge injection cannula inserted into and extending 2 mm beyond the tip of the guide cannula. To reduce confounds caused by mechanical stimulation of neurons during testing, rats received a sham injection in which the injector was inserted into the guide cannula

without drug administration one day prior to the experiment. Microinjections were administered (0.4 μ L) at a rate of 0.1 μ L/10 s. To minimize backflow of the drug up the cannula track, the injection cannula remained in place an additional 20 s. Following the injection, the stylet was replaced and the rat was returned to its home cage.

Behavioral Testing

Nociception was assessed using the hot plate test. The hot plate test consisted of measuring the latency for a rat to lick a hind paw when placed on a 52.5°C plate. The rat was removed from the hot plate if no response occurred within 50 s. Any rat with a baseline hot plate score over 30 s was removed from further testing. Each rat was tested in one (females: N = 10; males: N = 9), two (females: N = 25; males: N = 24), or three (females: N = 21; males: N = 20) conditions in a counterbalanced order with 2 to 7 days between tests. The test procedure on each day was either a time course or dose-response analysis of antinociception.

Experiment 1: Time Course

The time course for antinociception was assessed to determine whether the peak antinociceptive effect was the same for female (n = 8–12/drug) and male rats (n = 9–12/drug). Following a baseline hotplate test, one of the five drugs was microinjected into the vlPAG and nociception was assessed 3, 15, 30, and 60 min later. Doses of morphine (5 μ g), DAMGO (0.5 μ g), fentanyl citrate (3 μ g), bicuculline (25 ng), and kainic acid (5 ng) were selected to produce a near maximal response in males based on previous research [24,28,29] or preliminary tests.

Experiment 2: Dose-Response

Differences in antinociceptive potency between female and male rats were assessed by comparing dose response curves for each drug. Following a baseline hot plate test, cumulative third log doses were microinjected into the vlPAG [29]. The hot plate test was conducted 2, 7, or 15 min after each injection depending on the time course for the peak effect of the drug as determined in Experiment 1.

Three MOPr agonists were tested. Rats received four or five cumulative third log doses of morphine (1, 2.2, 4.6, 10, & 22 μ g/0.4 μ L), DAMGO (0.1, 0.22, 0.46, 1, & 2.2 μ g/0.4 μ L), or fentanyl (0.46, 1, 2.2, & 4.6 μ g/0.4 μ L). Morphine and DAMGO microinjections were administered at 20 min intervals and rats were tested on the hot plate 15 min following each injection. Differences in time of peak effect (see Experiment 1) required that cumulative doses of fentanyl be administered at 4 min intervals. Rats were tested 2 min after each injection.

Cumulative doses of the GABA_A receptor antagonist, bicuculline, and the excitatory amino acid, kainic acid, were also microinjected into the vlPAG to test the hypothesis that direct activation of vlPAG output neurons would cause greater antinociception in female compared to male rats. Both bicuculline [27] and kainic acid [30] have been shown to produce antinociception when microinjected into the vlPAG. Bicuculline was administered in third log cumulative doses of 4.6, 10, 22, & 46 ng/0.4 μ L and kainic acid in cumulative doses of 1, 2.2, 4.6, & 10 ng/0.4 μ L. Based on the results of Experiment 1, bicuculline and kainic acid microinjections were spaced 7 min apart and the hot plate test was conducted 5 min after each injection. Once animals reached the cutoff for the hot plate test (50 s) further injections were not administered and a hot plate latency of 50 s was entered for subsequent doses.

Histology

Following testing, rats were given a lethal dose of Halothane. The brain was removed and placed in formalin (10%). Two days later the brain was sectioned coronally (100 μ m) and the location of the injection site was identified. Only those injection sites within or adjacent to

the vIPAG [31] were included in data analysis. All injections were on the right side of the vIPAG for both sexes (see Figure 2).

Data Analysis

Analysis of variance (ANOVA) on mean hot plate latency was used to evaluate differences in baseline pain sensitivity. A repeated measures ANOVA was used to assess differences in raw data between females and males across time (Experiment 1). To compare drugs, data were converted to percent maximum possible effect (%MPE) as follows: $100 \times [(test\ score - baseline)/(cutoff\ score - baseline)]$. These data were further transformed into area under the curve (AUC) for each animal. An omnibus ANOVA followed by Bonferroni post-hoc comparisons were used to detect differences in the time course for different drugs.

Dose-response curves were plotted using GraphPad (Prism) and the half maximal antinociceptive effect (D_{50}) for each group was calculated [35]. The lower limit for calculating D_{50} values was set as the mean baseline latency. The upper limit was set as the mean hot plate latency following microinjection of the highest dose. The range of the responses is indicated using 95% confidence intervals. All data were analyzed with an ANOVA. Significance was defined as an alpha level of less than .05.

Possible confounds associated with repeated testing from the within-subjects design were assessed by comparing antinociception on Trials 1, 2, and 3. Given that time course and dose response data for five different drugs were tested, comparable data points were analyzed by selecting the hot plate latency at the peak antinociceptive effect for the time course data and the dose most closely matching the males D_{50} from the dose-response data. The doses used in the two experiments closely matched one another. A between subjects ANOVA was used because not all animals were tested in all three conditions for each sex.

Results

Baseline Nociception

No significant differences in baseline hotplate latencies were evident between the sexes [$F(1, 184) = 0.264$, $p = 0.608$]. Baseline hot plate latency decreased slightly from the first (female: 19.6 ± 1.0 s; male: 16.5 ± 0.9 s) to the third test condition (female: 11.7 ± 0.6 s; male: 15.0 ± 1.1 s). Females had a mean baseline of 15.2 ± 0.5 s and males had a mean baseline of 15.7 ± 0.5 s. There also was no significant difference in baseline hot plate latencies across conditions [$F(9, 184) = 1.819$, $p = 0.067$]. Given that rats were tested with different drugs in a counterbalanced manner, variations in baseline latency were distributed across conditions.

There was a gradual, but non-significant decrease in antinociception across trials [$F(2, 224) = 1.93$, $p = 0.148$; Table 1]. This analysis compared time points for the peak effect in Experiment 1 and the dose closest to the D_{50} in Experiment 2. The lack of change in antinociception was the same in female and male rats indicated by the lack of an interaction between trial and sex [$F(2, 224) = 0.159$, $p = .853$].

Experiment 1: Time Course

The time course for antinociception following microinjection of MOPr agonists (morphine, DAMGO, fentanyl) into the vIPAG is shown in Figure 3. Although the antinociception produced by microinjecting morphine ($5\ \mu\text{g}/0.4\ \mu\text{L}$) and DAMGO ($0.5\ \mu\text{g}/0.4\ \mu\text{L}$) into the vIPAG tended to be greater in male compared to female rats across the entire time course, this difference was not statistically significant (Fig. 3A: Morphine [$F(1, 60) = 2.682$, $p = 0.122$]; Fig. 3B: DAMGO [$F(1, 68) = 1.903$, $p = 0.186$]. Microinjection of fentanyl ($3\ \mu\text{g}/0.4\ \mu\text{L}$) into

the vIPAG produced nearly identical hotplate latencies in female and male rats at all time points [Fig. 3C: $F(1, 80) = 0.273$, $p = 0.607$].

The peak effect and duration of antinociception varied depending on the drug. For both morphine and DAMGO the peak antinociceptive effect was between 15 and 30 min with a duration of 60 min. The peak antinociceptive effect for fentanyl was 3 min, and hot plate latency returned to near baseline levels in both female and male rats by 30 min. There was no sex difference in the time of peak antinociception following microinjection of any of these drugs. The only difference between female and male rats treated with morphine and DAMGO was the duration of antinociception. The hot plate latency for female rats returned to baseline before males following morphine and DAMGO administration (Bonferroni, $p < 0.05$).

There was no statistically significant differences in the time course or peak antinociception between sexes following microinjection of 25 ng/0.4 μ L bicuculline [$F(1, 88) = 0.128$, $p = 0.724$ (Fig. 4A)] or kainic acid (5 ng/0.4 μ L) into the vIPAG [$F(1, 80) = 1.016$, $p = 0.346$ (Fig. 4B)]. The peak antinociceptive effect for both bicuculline and kainic acid was 3 min. Hot plate latency returned to near baseline levels approximately 30 min after administration of bicuculline or kainic acid. The one exception was a slight elevation in hot plate latency at 30 min for male compared to female rats injected with bicuculline (Bonferroni, $p < 0.05$).

Data were transformed into AUC to compare the magnitude of antinociception between drug conditions. There was a significant difference in the magnitude of antinociception across drug conditions [$F(4, 94) = 3.819$, $p = 0.006$]. Microinjection of fentanyl in male rats produced significantly less antinociception than microinjection of morphine or DAMGO (Bonferroni, $p < 0.05$) due to the short duration of action. No other drugs produced statistically significant differences from one another for either sex (Bonferroni, $p > 0.05$). A two-way ANOVA (sex $\times$ drug) on AUC data revealed no difference in antinociception between female and male rats [$F(1, 94) = 3.492$, $p = 0.065$].

Experiment 2: Dose-Response

Cumulative microinjections produced a dose-dependent increase in hot plate latencies for all drugs and both sexes. A sex-difference in D_{50} values was found for morphine and DAMGO, but not fentanyl (Table 2 and Fig. 5). Antinociceptive potency was 2.3 times greater in males compared to females following morphine microinjection [$F(1, 110) = 13.24$, $p = 0.0004$] and 4.25 times greater following DAMGO microinjection [$F(1, 134) = 18.00$, $p < 0.0001$]. In contrast, antinociceptive potency was not significantly different in female and male rats microinjected with fentanyl into the vIPAG [$F(1, 83) = 2.042$, $p = 0.1568$].

Antinociceptive potency was 2.6 times greater in male compared to female rats treated with the GABA_A receptor antagonist, bicuculline [$F(1, 80) = 12.06$, $p = 0.0008$, Fig. 6A]. This difference was similar to the difference between females and males following morphine administration (Table 2). In contrast, direct activation of vIPAG output neurons by microinjection of the excitatory amino acid, kainic acid, had the same effect in female and male rats [$F(1, 111) = 2.205$, $p = 0.1405$, (Fig. 6B)].

Discussion

The present data demonstrate dose-dependent antinociceptive effects to both MOPr agonists and non-opioid compounds microinjected into the vIPAG of female and male rats. Antinociceptive potency was greater in male rats compared to female rats following microinjection of morphine, DAMGO, and bicuculline, but no difference in potency was evident following microinjection of fentanyl or kainic acid. Sex differences in antinociceptive

potency were not caused by differences in time of peak effect because the time course for antinociception was similar in female and male rats for each of the drugs microinjected.

Although the vIPAG has been shown to contribute to greater morphine antinociception in male compared to female rats [17,23], we hypothesized that direct activation of vIPAG output neurons would produce greater antinociception in female rats. The projection from the vIPAG to the RVM contains more neurons in female compared to male rats [21], and this descending pathway is known to be important for morphine antinociception [2,18]. The finding that microinjection of kainic acid produces comparable antinociception in female and male rats indicates that this descending pathway can be activated equally in female and male rats. However, none of the drugs tested produced greater antinociception in female compared to male rats.

Microinjection of morphine or DAMGO into the vIPAG produced greater antinociception in male compared to female rats, as has been reported by others [17,23]. These behavioral data are in agreement with anatomical data showing greater morphine activation of vIPAG neurons projecting to the RVM in male rats [19]. Given that the vIPAG makes an important contribution to the antinociceptive effects of systemic morphine administration [18,40], it is logical that the vIPAG also contributes to the greater antinociception produced by morphine in male rats [5, 22].

Morphine produces antinociception in the vIPAG by inhibition of tonically active GABAergic neurons [9,26,38,39]. Bicuculline also blocks the effect of tonically active GABAergic neurons, but does so on the postsynaptic membrane. Thus, the greater antinociception in male compared to female rats produced by microinjection of bicuculline into the vIPAG is not surprising. The difference in potency between female and male rats was very similar for rats treated with morphine and bicuculline (2.3 and 2.6 fold differences in potency, respectively) as would be expected for two drugs that produce antinociception by inhibiting GABAergic transmission. This finding indicates that sex differences are caused by differences in vIPAG output neurons as opposed to opioid sensitive GABAergic neurons.

The lack of a difference in potency between female and male rats treated with fentanyl is hard to explain, although consistent with the lack of a sex difference following systemic administration of fentanyl [1,7,32]. Sex-differences seem to be dependent on agonist, route of administration, and testing paradigm [15], but these factors were consistent for morphine and fentanyl in the present study. The main difference is that fentanyl is a high efficacy MOPr agonist, and sex-differences in antinociception are particularly variable following administration of high efficacious MOPr agonists [1,13,32,36]. Beyond these studies, research on antinociceptive sex-differences to MOPr agonists other than morphine is limited.

Other factors that distinguish fentanyl from morphine and DAMGO are the rapid onset for peak antinociception and the short duration of action. The peak antinociceptive effect of fentanyl occurred at the 3 min test compared to 15–30 min for morphine and DAMGO. The slow onset to peak antinociception following direct microinjection of morphine and DAMGO into the vIPAG is hard to explain. The rapid onset of antinociception following microinjection of bicuculline and kainic acid indicate that direct application of drugs onto vIPAG neurons should produce rapid antinociception. The fact that morphine has lower efficacy than fentanyl could contribute to differences in the time of peak antinociception. However, the time course for the high efficacy MOPr agonist DAMGO was similar to morphine, not fentanyl. High efficacy agonists need fewer receptors to produce an effect and receptor reserve has been shown to be greater in vIPAG in male compared to female rats [3]. Fentanyl may produce comparable antinociception in male and female rats because few MOPr are needed to produce

antinociception. If true, sex differences in morphine antinociception are caused by a smaller receptor reserve in female compared to male rats [22].

Although DAMGO is expected to be a high efficacy agonist, both the time course for DAMGO antinociception and the sex difference match the effects of the low efficacy agonist, morphine, not the high efficacy agonist, fentanyl. Repeated microinjections into the vIPAG of DAMGO also produces tolerance [24], an effect that is not supposed to occur with high efficacy agonists [10]. Likewise, no sex-differences have been found in DAMGO or morphine stimulated [³⁵S] GTP γ S binding within the PAG [33]. These findings suggest that DAMGO and morphine are similar in efficacy in the vIPAG.

It is possible that previous reports of sex differences in morphine potency are caused by differences in the time course for peak antinociception. We tested this hypothesis by comparing the time course for antinociception in female and male rats following microinjection of MOR agonists and non-opioid drugs. The time course for antinociception was nearly identical in female and male rats for all drugs administered. The maximal antinociceptive effects of morphine and DAMGO tended to be lower in female compared to male rats, but the time of peak antinociception was similar.

The within-subjects design allowed a lot of data to be collected from relatively few rats, but introduced possible confounds from repeated testing and the development of tolerance. The vIPAG is especially susceptible to the development of tolerance with repeated microinjections of morphine or DAMGO [6,20,24,29,37], but not bicuculline or kainic acid [28]. Thus, potential confounds from tolerance were minimized by testing rats with different drugs on different days, and allowing at least two days to pass between test days. Although both female and male rats showed a slight decrease in drug-induced antinociception from Trial 1 to 3 in the present experiment (see Table 1), this decrease was small and did not reach statistical significance. Randomly interspersing bicuculline and kainic acid trials with morphine, DAMGO, and fentanyl trials and separating any two trials by at least 48 hours surely contributed to the lack of tolerance.

Previous research has found that antinociceptive sex-differences to morphine microinjection into the vIPAG are dependent on the estrus cycle [3,34]. Although estrus phase was not measured, sex-differences in antinociception were evident in the present study. Rats in the present study were not swabbed to determine cycle phase so that female and males could be treated in the same manner.

In sum, the present data reveal that the vIPAG can produce comparable antinociception in female and male rats, but morphine and other drugs that share a common mechanism (e.g., bicuculline) produce greater antinociception in male compared to female rats. The greater output from the vIPAG to RVM in female compared to male rats [19,21] suggests that other, non-opioid forms of antinociception (e.g., cannabinoid) may be greater in female compared to male rats.

Acknowledgments

Financial support was provided by NIH grant DA015498 and by funds provided for medical and biological research by the State of Washington Initiative Measure No. 171. The technical assistance of Gavin Meyer, Edvinas Pocius, and Lindsey Stevens is appreciated.

References

1. Bartok RE, Craft RM. Sex differences in opioid antinociception. *J Pharmacol Exp Ther* 1997;282:769–778. [PubMed: 9262340]

2. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 1984;7:309–338. [PubMed: 6143527]
3. Bernal SA, Morgan MM, Craft RM. PAG mu opioid receptor activation underlies sex differences in morphine antinociception. *Behav Brain Res* 2007;177:126–133. [PubMed: 17118467]
4. Cepeda MS, Carr DB. Women experience more pain and require more morphine than men to achieve a similar degree of analgesia. *Anesth Analg* 2003;97:1464–1468. [PubMed: 14570666]
5. Craft RM. Sex differences in analgesic, reinforcing, discriminative, and motoric effects of opioids. *Exp Clin Psychopharmacol* 2008;16:376–385. [PubMed: 18837634]
6. Craft RM, Stratmann JA, Bartok RE, Walpole TI, King SJ. Sex differences in development of morphine tolerance and dependence in the rat. *Psychopharmacology (Berl)* 1999;143:1–7. [PubMed: 10227072]
7. Craft RM, Tseng AH, McNiel DM, Furness MS, Rice KC. Receptor-selective antagonism of opioid antinociception in female versus male rats. *Behav Pharmacol* 2001;12:591–602. [PubMed: 11856896]
8. Craft RM, Ulibarri C, Leidl MD, Sumner JE. Dose- and time-dependent estradiol modulation of morphine antinociception in adult female rats. *Eur J Pain* 2008;12:472–479. [PubMed: 17869144]
9. Depaulis A, Morgan MM, Liebeskind JC. GABAergic modulation of the analgesic effects of morphine microinjected in the ventral periaqueductal gray matter of the rat. *Brain Res* 1987;436:223–228. [PubMed: 3435824]
10. Duttaroy A, Yoburn BC. The effect of intrinsic efficacy on opioid tolerance. *Anesthesiology* 1995;82:1226–1236. [PubMed: 7741298]
11. Jacquet YF, Lajtha A. Paradoxical effects after microinjection of morphine in the periaqueductal gray matter in the rat. *Science* 1974;185:1055–1057. [PubMed: 4604871]
12. Jensen TS, Yaksh TL. Comparison of the antinociceptive effect of morphine and glutamate at coincidental sites in the periaqueductal gray and medial medulla in rats. *Brain Res* 1989;476:1–9. [PubMed: 2563331]
13. Kepler KL, Standifer KM, Paul D, Kest B, Pasternak GW, Bodnar RJ. Gender effects and central opioid analgesia. *Pain* 1991;45:87–94. [PubMed: 1677751]
14. Kest B, Palmese C, Hopkins E. A comparison of morphine analgesic tolerance in male and female mice. *Brain Res* 2000;879:17–22. [PubMed: 11011001]
15. Kest B, Sarton E, Dahan A. Gender differences in opioid-mediated analgesia: animal and human studies. *Anesthesiology* 2000;93:539–547. [PubMed: 10910505]
16. Krzanowska EK, Bodnar RJ. Analysis of sex and gonadectomy differences in beta-endorphin antinociception elicited from the ventrolateral periaqueductal gray in rats. *Eur J Pharmacol* 2000;392:157–161. [PubMed: 10762669]
17. Krzanowska EK, Bodnar RJ. Morphine antinociception elicited from the ventrolateral periaqueductal gray is sensitive to sex and gonadectomy differences in rats. *Brain Res* 1999;821:224–230. [PubMed: 10064807]
18. Lane DA, Patel PA, Morgan MM. Evidence for an intrinsic mechanism of antinociceptive tolerance within the ventrolateral periaqueductal gray of rats. *Neuroscience* 2005;135:227–234. [PubMed: 16084660]
19. Loyd DR, Morgan MM, Murphy AZ. Morphine preferentially activates the periaqueductal gray-rostral ventromedial medullary pathway in the male rat: a potential mechanism for sex differences in antinociception. *Neuroscience* 2007;147:456–468. [PubMed: 17540508]
20. Loyd DR, Morgan MM, Murphy AZ. Sexually dimorphic activation of the periaqueductal gray-rostral ventromedial medullary circuit during the development of tolerance to morphine in the rat. *Eur J Neurosci* 2008;27:1517–1524. [PubMed: 18364026]
21. Loyd DR, Murphy AZ. Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: a potential circuit mediating the sexually dimorphic actions of morphine. *J Comp Neurol* 2006;496:723–738. [PubMed: 16615128]
22. Loyd DR, Murphy AZ. The role of the periaqueductal gray in the modulation of pain in males and females: are the anatomy and physiology really that different? *Neural Plast* 2009;2009:462879. [PubMed: 19197373]

23. Loyd DR, Wang X, Murphy AZ. Sex differences in micro-opioid receptor expression in the rat midbrain periaqueductal gray are essential for eliciting sex differences in morphine analgesia. *J Neurosci* 2008;28:14007–14017. [PubMed: 19109484]
24. Meyer PJ, Fossum EN, Ingram SL, Morgan MM. Analgesic tolerance to microinjection of the micro-opioid agonist DAMGO into the ventrolateral periaqueductal gray. *Neuropharmacology* 2007;52:1580–1585. [PubMed: 17445843]
25. Miller PL, Ernst AA. Sex differences in analgesia: a randomized trial of mu versus kappa opioid agonists. *South Med J* 2004;97:35–41. [PubMed: 14746420]
26. Moreau JL, Fields HL. Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission. *Brain Res* 1986;397:37–46. [PubMed: 3801864]
27. Morgan MM, Clayton CC. Defensive behaviors evoked from the ventrolateral periaqueductal gray of the rat: comparison of opioid and GABA disinhibition. *Behav Brain Res* 2005;164:61–66. [PubMed: 16029902]
28. Morgan MM, Clayton CC, Lane DA. Behavioral evidence linking opioid-sensitive GABAergic neurons in the ventrolateral periaqueductal gray to morphine tolerance. *Neuroscience* 2003;118:227–232. [PubMed: 12676152]
29. Morgan MM, Fossum EN, Levine CS, Ingram SL. Antinociceptive tolerance revealed by cumulative intracranial microinjections of morphine into the periaqueductal gray in the rat. *Pharmacol Biochem Behav* 2006;85:214–219. [PubMed: 16979226]
30. Morgan MM, Whitney PK, Gold MS. Immobility and flight associated with antinociception produced by activation of the ventral and lateral/dorsal regions of the rat periaqueductal gray. *Brain Res* 1998;804:159–166. [PubMed: 9729359]
31. Paxinos, G.; Watson, SJ. The rat brain, in stereotaxic coordinates. 2. Sydney: Academic Press; 2005.
32. Peckham EM, Traynor JR. Comparison of the antinociceptive response to morphine and morphine-like compounds in male and female Sprague-Dawley rats. *J Pharmacol Exp Ther* 2006;316:1195–1201. [PubMed: 16291875]
33. Selley DE, Herbert JT, Morgan D, Cook CD, Picker MJ, Sim-Selley LJ. Effect of strain and sex on mu opioid receptor-mediated G-protein activation in rat brain. *Brain Res Bull* 2003;60:201–208. [PubMed: 12754081]
34. Shane R, Bernal SY, Rozengurtel S, Bodnar RJ. Estrus phase differences in female rats in morphine antinociception elicited from the ventrolateral periaqueductal gray. *Int J Neurosci* 2007;117:811–822. [PubMed: 17454245]
35. Tallarida, RJ. Drug Synergism and Dose-Effect Data Analysis. Boca Raton: Chapman & Hall/CRC; 2000.
36. Tershner SA, Mitchell JM, Fields HL. Brainstem pain modulating circuitry is sexually dimorphic with respect to mu and kappa opioid receptor function. *Pain* 2000;85:153–159. [PubMed: 10692614]
37. Tortorici V, Robbins CS, Morgan MM. Tolerance to the antinociceptive effect of morphine microinjections into the ventral but not lateral-dorsal periaqueductal gray of the rat. *Behav Neurosci* 1999;113:833–839. [PubMed: 10495091]
38. Vaughan CW, Christie MJ. Presynaptic inhibitory action of opioids on synaptic transmission in the rat periaqueductal grey in vitro. *J Physiol* 1997;498 (Pt 2):463–472. [PubMed: 9032693]
39. Vaughan CW, Ingram SL, Connor MA, Christie MJ. How opioids inhibit GABA-mediated neurotransmission. *Nature* 1997;390:611–614. [PubMed: 9403690]
40. Zambotti F, Zonta N, Parenti M, Tommasi R, Vicentini L, Conci F, Mantegazza P. Periaqueductal gray matter involvement in the muscimol-induced decrease of morphine antinociception. *Naunyn Schmiedeberg Arch Pharmacol* 1982;318:368–369. [PubMed: 7078669]

Figure 1.

Model of PAG circuitry underlying antinociception. Administration of morphine, DAMGO, fentanyl, bicuculline, or kainic acid causes excitation of vLPAG output neurons. MOPr agonists inhibit GABAergic neurons, which disinhibits vLPAG output neurons projecting to the RVM. Bicuculline disinhibits vLPAG output neurons by preventing postsynaptic GABA binding. Kainic acid directly excites vLPAG output neurons.

Figure 2.

Cannula placements for male and female rats at two rostrocaudal levels of the periaqueductal gray. Open symbols represent placements for the time course data (Experiment 1). Filled symbols are placements for dose response data (Experiment 2). Injection sites were similar for males (squares on left) and females (circles on right) for all five drugs compared. All injections were on the right side of the vlPAG, but are separated here for clarity. The distance from lambda is shown in mm at the bottom of the two sections [31].

Figure 3.

Time course for antinociception following microinjection of MOPr agonists into the vIPAG. Microinjection of morphine (A) or DAMGO (B) produced peak antinociception at 15–30 min for both female and male rats. Microinjection of fentanyl (C) caused a rapid increase in hotplate latency leading to a peak effect at 3 min for female and male rats.

Figure 4.

Time course for antinociception following microinjection of the GABA_A receptor antagonist, bicuculline, or the excitatory amino acid, kainic acid. Microinjection of bicuculline (A) or kainic acid (B) caused peak antinociception for female and male rats at 3 min. Antinociception was equal in both sexes.

Figure 5. Agonist dependent sex-differences in potency of MOPr agonists microinjected into the vIPAG. Morphine, DAMGO, and fentanyl caused a dose-dependent increase in hot plate latencies for female and male rats. Antinociceptive potency was greater in males following cumulative microinjections of morphine (A) and DAMGO (B), but not fentanyl (C).

Figure 6.

Sex-difference in antinociceptive potency following microinjection of bicuculline, but not kainic acid into the vIPAG. A dose-dependent increase in hotplate latency was evident for both female and male rats following microinjection of the GABA_A receptor antagonist bicuculline and the excitatory amino acid kainic acid. Microinjection of bicuculline (A) was more potent in male compared to female rats, but antinociceptive potency was equal following cumulative microinjections of kainic acid (B).

Table 1

Comparison of PAG mediated antinociception across trials

	Trial 1		Trial 2		Trial 3	
	Mean $\pm$ SEM	n	Mean $\pm$ SEM	n	Mean $\pm$ SEM	n
Males	35.4 $\pm$ 2.1 s	43	35.3 $\pm$ 2.4 s	39	31.7 $\pm$ 3.3 s	27
Females	34.7 $\pm$ 2.3 s	48	32.5 $\pm$ 2.8 s	40	28.2 $\pm$ 3.0 s	33

Notes: No statistical difference across trials (Bonferroni, $p > 0.05$)

SEM - standard error of the mean; n - sample size

Table 2Comparison of D₅₀ values between female and male rats

Drug	Female		Male	
	<i>D50 (C.I.)</i>	<i>n</i>	<i>D50 (C.I.)</i>	<i>n</i>
Morphine	15.68 µg (± 5.02) *	10	6.83 µg (± 2.14)	9
DAMGO	0.85 µg (± 0.28) *	12	0.20 µg (± 0.10)	11
Fentanyl	2.06 µg (± 1.57)	9	3.42 µg (± 0.90)	10
Bicuculline	32.00 µg (± 11.07) *	10	12.07 µg (± 4.39)	7
Kainic Acid	6.94 µg (± 2.31)	10	5.08 µg (± 1.10)	13

* Notes: Statistically significant from males (p < 0.05)

C.I. - 95% Confidence Interval

n- Sample Size