Published in final edited form as:

Neurosci Lett. 2007 December 18; 429(2-3): 105–110.

Inactivation of the Periaqueductal Gray Attenuates Antinociception Elicited by Stimulation of the Rat Medial Preoptic Area

Yi-Hong Zhang and Matthew Ennis

Department of Anatomy and Neurobiology, Neuroscience Institute, University of Tennessee Health Science Center, Memphis, TN 38163, USA

Abstract

The medial preoptic area (MPOA) is a sexually dimorphic structure that plays key roles in gonadosteroidal regulation and thermoregulation. The MPOA may be involved in sex-based differences in nociceptive processing and steroid hormones effect on pain thresholds. Consistent with this, there is evidence that MPOA can produce antinociception or hyperalgesia. MPOA stimulation inhibits spinal cord or trigeminal neuronal responses to noxious stimuli or produces analgesia, yet most of these studies utilized electrical stimulation which antidromically activates periaqueductal gray (PAG) and rostroventromedial medulla (RVM) neurons involved in descending modulation of nociception. Effects of selective activation of MPOA neurons on behavioral indices of antinociception and the site-specificity of such responses are unknown. To address these questions, we examined the influence of MPOA microinjections of d, l homocysteate (DLH) on hindlimb and tail nocifensive reflexes in lightly anesthetized rats. DLH, but not saline, microinjections into several MPOA subregions markedly increased withdrawal response latencies to noxious thermal stimuli. Antinociceptive effects of MPOA activation were abolished by microinjection of lidocaine into PAG. These results suggest that activation of MPOA neurons produces antinociception that is at least partly mediated by projections to PAG.

Keywords

medial preoptic area; hypothalamus; nociception; analgesia; periaqueductal gray

Introduction

Previous work has demonstrated sex differences as well as effects of steroid hormones on nociception and responses to analgesics [7,17,19,31,35,36]. The MPOA is a sexually dimorphic structure that contains large populations of neurons that concentrate gonadal hormones and express estrogen and androgen receptors [25]. The MPOA may therefore be important in sex-based differences in nociceptive processing and/or in mediating effects of gonadal hormones on nociception. Consistent with this, several lines of evidence indicate that MPOA is involved in descending modulation of nociception. MPOA neurons do not project directly to the spinal cord, but robustly innervate two major brainstem areas involved in descending nociceptive

Correspondence: Yi-Hong Zhang, Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, 855 Monroe Ave., Suite 515, Memphis, TN 38163, USA, Telephone: 901-448-1223, Fax: 901-448-7193, Email: yzhang32@utmem.edu.

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.

modulation, the periaqueductal gray (PAG) and rostroventromedial medulla (RVM) [5,12, 26,29,32]. MPOA neurons are responsive to noxious somatic and visceral stimuli [30], and electrical stimulation of the cat and rat MPOA has been reported to suppress spinal cord and medullary dorsal horn neuronal responses to noxious cutaneous or visceral input [8,20,21, 24]. Although these studies implicate MPOA in antinociception, there are several limitations. First, all were performed in deeply anesthetized and/or paralyzed animals and behavioral indices (e.g., withdrawal reflexes) of nociceptive thresholds could not be measured. Second, in several studies [8,24] conclusions were based primarily or exclusively on electrical stimulation of MPOA which will antidromically activate PAG and RVM neurons that project to MPOA [26,32]. Thus, the effects of MPOA stimulation may be due to direct activation of PAG or RVM neurons rather than activation of MPOA neurons. While a limited number of studies used chemical stimulation to activate MPOA neurons, conclusion were based on a limited samples and in some cases responses elicited by electrical stimulation were not replicated by chemical stimulation [21,30].

Direct behavioral studies on the role of MPOA in descending nociceptive modulation are limited. Electrical stimulation of the primate or rat MPOA was reported to produce antinociception to noxious trigeminal stimuli [9,28], while more recent work demonstrated that prostaglandin E2 (PGE2) injections into MPOA produces hyperalgesia to noxious thermal stimulation of the hindpaw in rats [1,14,15]. The MPOA is a heterogeneous structure that contains a number of cytoarchitectonically, neurochemically and functionally discrete nuclei [32]. With the exception of hyperalgesia responses [14,15], the loci of effective stimulation sites with respect to subdivisions of the MPOA were not reported. The goal of the present study was to characterize the influence of selective activation of MPOA neurons on tail and hindlimb nocifensive responses in lightly anesthetized rats. Previous studies have shown that projections to PAG are required for MPOA-evoked nociceptive, autonomic and urethrogenital responses [6,22,24]. Therefore, a second goal was to assess the involvement of the PAG in antinociceptive responses elicited by MPOA activation.

Materials and Methods

Experimental procedures were conducted in order to minimize animal discomfort and the number of animals used, according to an institutionally approved protocol and NIH guidelines. Male Sprague-Dawley rats (250-350 g; Harlan Co., Indianapolis, IN) were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and the right jugular vein was cannulated. Rats were mounted in a modified stereotaxic frame allowing radiant illumination of the tail and hindpaws. Core temperature was maintained at 36-37°C with a feedback-controlled heating pad. A hole was drilled in the skull above the right MPOA (0.2-0.9 mm posterior to bregma, 0.1-0.9 mm lateral to midline). Selective stimulation of MPOA neurons (6.8-7.8 mm ventral to brain surface) was accomplished by microinjections of d, l homocysteate (DLH, 10 mM in 0.9% saline, pH=7.3) from glass microinjection pipettes (40-60 µm tip diameter); saline was used as vehicle control. In some experiments, a hole was drilled above the right PAG (1.7-2.5 mm anterior to interaural line, 0.3-0.5 mm lateral to midline) and a second pipette filled with the local anesthetic lidocaine (4% in saline, pH=7.3) was lowered into the PAG (3.5-5.3 mm ventral to brain surface). All solutions contained Pontamine Sky Blue dye (2%) to mark injection sites.

Following surgery, a light level of anesthesia (corneal and flexion reflexes present) was maintained throughout by continuous infusion of pentobarbital (3-10 mg/kg/h, i.v.). Nocifensive behaviors were tested at least 60 min after surgery using a method modified from Hargreaves et al. [13]. A feedback-controlled Paw Thermal Stimulator System (Anesthesia Research Laboratory, University of California, San Diego, CA) delivered noxious radiant thermal stimuli to the dorsal hindpaw or ventral tail surface; the glass plate was maintained at 30° C. The heat source was a high-intensity projector lamp (Osram 58-8007; 8 V, 50 W).

Hindpaw withdrawal (PW) and tail flick (TF) latencies were automatically recorded to the nearest 0.1 sec. Stimulus intensity was set to produce a PW latency between 9-11 sec. Stimuli were applied at >3 min intervals and a 20-sec cut-off prevented skin damage. In most animals, only one saline or DLH microinjection was made but in some experiments several microinjections were made after withdrawal latencies returned to baseline values between injections. There were no differences ($P > 0.05$) in the magnitude of MPOA microinjection-evoked withdrawal latencies between the initial and subsequent microinjection in the same animal, therefore these data were pooled. Respiration rate was measured over 30 sec epochs at 10 min intervals. One-Way Repeated Measures Analysis of Variance (ANOVA) followed by Student-Newman-Keuls tests were used to compare PW and TF latencies from a common group of rats. Within group, repeated measures percentage data were analyzed with Wilcoxon Signed rank tests and between group percentage data were analyzed with Mann-Whitney U tests.

At the end of experiments, rats were deeply anesthetized and transcardially perfused with 100 ml of 0.9% saline followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Brains were removed, post-fixed overnight (4°C), and immersed in 20% sucrose-PB for 24 hr (4°C). Forty μ m-thick frozen sections through the MPOA and PAG were collected and counterstained with Neutral Red. In some experiments, MPOA sections were processed for Fos immunocytochemistry using an ABC visualization step (ABC-elite; 1:500; Vector Laboratories, Burlingame, CA) as previously described [3]; the Fos primary antibody (rabbit, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used at a 1:15,000 dilution and the secondary antibody (IgG goat anti-rabbit serum; Vector Laboratories, Burlingame, CA) at 1:400. Images of Fos-immunoreactive (IR) neurons were acquired with a digital camera (ORCA-ER C4742-95, Hamamatsu Photonics K.K., Hamamatsu City, Shizuoka, Japan) on a Leica microscope. Images were captured with SemplePCI software (Version 5.2.0.2404). Images were cropped, and sharpness, contrast, and brightness were adjusted with Adobe PhotoShop.

Results

The effects of DLH and saline microinjections into the right MPOA on the ipsilateral right PW (RPW), contralateral left (LPW) and TF latencies were measured. The average baseline latencies for LPW, RPW, and TF were 9.7 ± 0.2 sec, 8.6 ± 0.2 sec and 10.2 ± 0.2 sec ($n=46$), respectively. Focal injections of saline (200 nl, $n=19$ microinjections in 14 rats) did not significantly alter withdrawal latencies (Fig. 1A), with mean increases in PW and TF latencies ranging from 3.1-5.9%. By contrast, 100 nl microinjections of DLH ($n=16$ microinjections in 14 rats) significantly elevated mean withdrawal latencies by 16.5-30.1% above baseline values (Fig. 1A). Although effects were more pronounced for RPW and TF than LPW, this trend did not reach statistical significance ($P > 0.05$). 200 nl DLH microinjections ($n=35$ microinjections in 24 rats) produced comparable effects to those of 100 nl microinjections, with mean increases ranging from 17.5-33.4%; the magnitude of the increases for the respective limbs and tail did not significantly differ for 100 vs. 200 nl DLH microinjections. With 200 nl injections however, the increases in RPW and TF latencies were greater than those of LPW latencies (Fig. 1A); the RPW and TF values did not differ significantly. Increases in withdrawal latencies to the cut-off value (20 sec) was similar for 100 nl (18.7%, 3/16 rats) and 200 nl (20%, 7/35 rats) DLH injections; saline microinjection never increased withdrawal latencies to cut-off time (0/19 rats). Antinociceptive effects persisted for 8-14 min after 100 nl DLH microinjections and 10-15 min after 200 nl microinjections. Finally, DLH or saline microinjections did not alter respiratory rates ($P > 0.8$) or body temperature ($P > 0.3$).

The distribution of injection sites is shown in Figure 1 B-C. Most sites were centered in the ventral and medial portions of MPOA. Within this zone, there did not appear to be any discrete

DLH site-specificity as increased withdrawal responses were observed with microinjections into a number of MPOA nuclei, including the periventricular hypothalamic (Pe), anteroventral periventricular (AVPe), medial preoptic (MPN), ventrolateral preoptic (VLPO), and ventromedial preoptic (VMPO) nuclei. In general, however, the sites yielding the largest (>50%) increases in withdrawal latencies were concentrated in or adjacent to the AVPe, MPN and VMPO. DLH microinjections into the lateral preoptic area (n=2) produced relatively small changes (5-13% increases) in withdrawal latencies. Fos staining was used in some experiments to determine the extent of MPOA neuronal activation following microinjections (Supplementary Fig. 1). Fos-IR neurons produced by saline microinjections (200 nl, n=2) were concentrated close to the injection site, within a circular area with a diameter of less than 200 μ m, or along the track of the pipette. DLH microinjections (200 nl, n=3) produced more robust Fos expression in individual neurons and a greater spatial extent of labeling, typically within a diameter of 400-600 μ m.

MPOA projects to the PAG, and previous studies demonstrate that MPOA-evoked responses are reduced by inactivation of the PAG [6,22,24]. In the next experiments, MPOA-evoked antinociception was examined before and after lidocaine microinjections into the PAG to determine if the antinociception requires a relay through the PAG. Because MPOA projections target multiple subregions of PAG at any given rostrocaudal level of this structure [32], 3-4 dorsoventrally spaced lidocaine microinjections were made (200 nl per injection site, see Methods for details). Control experiments first investigated the effects of PAG lidocaine microinjections on withdrawal latencies. Lidocaine microinjections into PAG produced small, non-significant (n=5, $P > 0.05$) increases in withdrawal latencies, corresponding to 11.11%, 8.44% and 15.74% increases for LPW, RPW, and TF respectively (Fig. 2 A-C).

In a separate group of rats, PAG lidocaine microinjections were made immediately (1-2 min) before 200 nl DLH microinjections into MPOA (Fig. 2 A-C). In these cases (n=7), MPOA DLH microinjections produced small, non-significant increases in PW and TF latencies; the increases did not differ from those elicited by PAG lidocaine injections alone (Fig. 2 A-C). Following return of withdrawal latencies to baseline values, a second 200 nl DLH microinjection into MPOA significantly increased PW and TF latencies, and these increases were significantly greater than those elicited when DLH was microinjected in combination with lidocaine.

Discussion

The present study demonstrates that selective activation of MPOA neurons consistently increases withdrawal latencies to noxious radiant thermal stimuli in lightly anesthetized rats. To our knowledge, this is the first demonstration that focal MPOA neuronal activation produces antinociception as determined by changes in nocifensive reflexes. MPOA-evoked antinociception was eliminated by PAG inactivation, indicating descending projections to PAG are involved. Our findings are consistent with previous reports that MPOA stimulation inhibits dorsal horn spinal cord and trigeminal neuronal responses to noxious stimuli.

DLH microinjection sites that increased withdrawal latencies were concentrated in the ventral MPOA. Within this region, antinociceptive sites were distributed across several distinct MPOA sub-nuclei. This is consistent with observations that descending projections to the PAG and RVM arise from multiple subregions of MPOA [26,32]. Stimulation sites that inhibit spinal cord or trigeminal responses to noxious stimuli were observed in different medio-lateral or dorso-ventral locations within MPOA, although specific nuclei were not reported [8,20,24]. Interestingly, PGE2 or E3 agonist microinjection sites which produced hyperalgesia were located in the ventral MPOA, and were concentrated in MPN and VMPO [14,15]; more dorsal sites in the median preoptic nucleus (MnPO) were also effective [15].

DLH microinjections into MPOA increased withdrawal latencies for both hindlimbs and the tail. However, more robust increases were observed for the ipsilateral hindpaw and tail. This is consistent with the bilateral, but ipsilaterally dominant, descending MPOA projections to PAG and RVM [26,32]. The present results, and previous studies showing that MPOA stimulation inhibits noxious sensory input to the medullary dorsal horn and to multiple levels of the spinal cord, indicate that MPOA-evoked antinociception exhibits diffuse somatotopic organization [8,21,24]. MPOA does not project to the spinal cord, but projects heavily to two major sites involved in descending modulation of nociception, the PAG and RVM. MPOA stimulation activates neurons in PAG, including those that project to RVM, and RVM [6,18, 33]. Similar to MPOA, PAG- and RVM-evoked antinociception occur over large regions of the body [29]. Our findings that lidocaine microinjections into PAG eliminated MPOA-evoked antinociception suggest that the PAG plays a critical role in this response. It should be noted however that MPOA projects to other hypothalamic nuclei (e.g., lateral hypothalamus) implicated in antinociception and effects of MPOA stimulation in the present study may have been mediated in part via these projections [2]. Our findings are in agreement with observations that PAG lesions or chemical inactivation reduces or blocks MPOA-evoked inhibition of medullary dorsal horn neuronal nociceptive responses, MPOA-evoked cardiovascular and urethrogenital responses, and effects of MPOA activation on RVM neuronal activity [6,18, 22,24]. We observed a more complete degree of suppression of MPOA-evoked responses with PAG inactivation than in the study of Mokha et al. [24], possibly because of the relatively small PAG lesions in that study. MPOA neurons also project to RVM, and RVM lesions reduce MPOA-evoked medullary dorsal horn neuronal nociceptive responses [24]. Therefore, it is possible that the actions of lidocaine in our study were partially due to effects on MPOA fibers passing through PAG en route to RVM or other structures. This seems unlikely however, as the bulk of MPOA projections to RVM appear to pass ventral to PAG [26].

MPOA is a sexually dimorphic structure containing neurons that concentrate gonadal steroids and express estrogen and androgen receptors. MPOA plays a key role in neuroendocrine and gonado-steroidal regulation and reproductive behaviors [4,10,16,22,26,34]. There are sex differences in nociceptive processing and analgesia, and changes in nociceptive thresholds and opioid-mediated analgesia occur during the estrus cycle, steroid hormone administration, and reproductive activities [7,17,19,31,35,36]. The medial preoptic nucleus (MPN), the sexually dimorphic component of MPOA, contains neurons that express estrogen or androgen receptors and project to PAG [25]. In this regard, it is noteworthy that DLH microinjections into the MPN in the present study produced antinociception. Within the MPOA, the MPN, may provide a key link mediating hormonal influences on nociceptive thresholds, and may also regulate descending nociception during maternal/reproductive behaviors. Additional studies are needed to determine if there are sex differences in MPOA-evoked antinociception.

The MPOA is also involved in thermoregulation and fever responses following activation of the immune system [1,14]. Injections of PGE2 or E3 agonists into MPOA produce hyperalgesia and activate RVM neurons thought to be involved in descending facilitation [14]. Taken together with the present findings and previous studies [8,21,24], these results indicate that MPOA may contain different populations of neurons that exert descending nociceptive inhibitory and facilitatory effects. As noted above, the MPOA PGE2 or E3 agonist microinjection sites that produced hyperalgesia [14,15] overlap with those in the present study. We did not observe descending facilitation or hyperalgesia. PGE2-evoked hyperalgesia in the MPOA is mediated primarily by the E3 receptor [15], which are primarily localized in MnPO and VMPO [11,27]. PGE2 excites and inhibits roughly equal numbers of MPOA neurons [23], whereas DLH would produce excitation. It is possible that PGE2-mediated hyperalgesia is mediated by inhibition of MPOA neurons, and that descending inhibitory effects predominate when both neuronal populations are simultaneously activated by DLH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Ms. Dongxia Jiang for histological assistance and support from PHS grant NS41384.

References

1. Abe M, Oka T, Hori T, Takahashi S. Prostanoids in the preoptic hypothalamus mediate systemic lipopolysaccharide-induced hyperalgesia in rats. *Brain Res* 2001;916:41–49. [PubMed: 11597589]
2. Aimone LD, Bauer CA, Gebhart GF. Brain-stem relays mediating stimulation produced antinociception from the lateral hypothalamus in the rat. *J Neurosci* 1988;8:2652–2663. [PubMed: 3249250]
3. Anseloni VZ, Ren K, Dubner R, Ennis M. A brainstem substrate for analgesia elicited by intraoral sucrose. *Neurosci* 2005;133:231–243.
4. Arendash GW, Gorski RA. Effects of discrete lesions of the sexually dimorphic nucleus of the preoptic area or other medial preoptic regions on the sexual behavior of male rats. *Brain Res Bull* 1983;10:147–154. [PubMed: 6824962]
5. Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. *Prog Neurobiol* 1995;46:575–605. [PubMed: 8545545]
6. Behbehani MM, Da Costa-Gomez TM. Properties of a projection pathway from the medial preoptic nucleus to the midbrain periaqueductal gray of the rat and its role in the regulation of cardiovascular function. *Brain Res* 1996;740:141–150. [PubMed: 8973808]
7. Berkley KJ. Sex differences in pain. *Behav Brain Sci* 1997;20:371–380. [PubMed: 10097000]
8. Carstens E, MacKinnon JD, Guinan MJ. Inhibition of spinal dorsal horn neuronal responses to noxious skin heating by medial preoptic and septal stimulation in the cat. *J Neurophysiol* 1982;48:981–991. [PubMed: 7143037]
9. Cunningham PM, Goldsmith GE, Hellon RF. Medial hypothalamic stimulation produces analgesia to facial heating in unrestrained rats. *Neurosci Lett* 1986;68:107–111. [PubMed: 3725211]
10. Edwards DA, Einhorn LC. Preoptic and midbrain control of sexual motivation. *Physiol Behav* 1986;37:329–335. [PubMed: 3488558]
11. Ek M, Arias C, Sawchenko P, Ericsson-Dahlstrand A. Distribution of the EP3 Prostaglandin E2 Receptor Subtype in the Rat Brain: Relationship to Sites of Interleukin-1–Induced Cellular Responsiveness. *J Comp Neurol* 2000;428:5–20. [PubMed: 11058221]
12. Gebhart GF. Descending modulation of pain. *Neurosci Biobehav Rev* 2004;27:729–737. [PubMed: 15019423]
13. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988;32:77–88. [PubMed: 3340425]
14. Heinricher MM, Neubert MJ, Martenson ME, Goncalves L. Prostaglandin E2 in the medial preoptic area produces hyperalgesia and activates pain-modulating circuitry in the rostral ventromedial medulla. *Neuroscience* 2004;128:389–398. [PubMed: 15350650]
15. Hosoi M, Oka T, Hori T. Prostaglandin E receptor EP3 subtype is involved in thermal hyperalgesia through its actions in the preoptic hypothalamus and the diagonal band of Broca in rats. *Pain* 1997;71:303–311. [PubMed: 9231874]
16. Hull EM, Du J, Lorrain DS, Matuszewich L. Testosterone, preoptic dopamine, and copulation in male rats. *Brain Res Bull* 1997;44:327–333. [PubMed: 9370196]
17. Ji Y, Murphy AZ, Traub RJ. Estrogen modulation of morphine analgesia of visceral pain in female rats is supraspinally and peripherally mediated. *J Pain* 2007;8:494–502. [PubMed: 17383238]
18. Jiang M, Behbehani MM. Physiological characteristics of the projection pathway from the medial preoptic to the nucleus raphe magnus of the rat and its modulation by the periaqueductal gray. *Pain* 2001;94:139–147. [PubMed: 11690727]

19. Kepler KL, Kest B, Kiehl JM, Cooper ML, Bodnar RJ. Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats. *Pharmac Biochem Behav* 1989;34:119–127.
20. Lumb BM. Hypothalamic influences on viscerosomatic neurones in the lower thoracic spinal cord of the anaesthetized rat. *J Physiol* 1990;424:227–244. [PubMed: 2167974]
21. Lumb BM, Cervero F. Modulation of a viscerosomatic reflex by electrical and chemical stimulation of hypothalamic structures in the rat. *Brain Res* 1989;500:400–404. [PubMed: 2605507]
22. Marson L. Lesions of the periaqueductal gray block the medial preoptic area-induced activation of the urethrogenital reflex in male rats. *Neurosci Lett* 2004;367:278–282. [PubMed: 15337249]
23. Matsuda T, Hori T, Nakashima T. Thermal and PEG₂ sensitivity of the organum vasculosum lamina terminalis region and preoptic area in rat brain slices. *J Physiol* 1992;454:197–212. [PubMed: 1474492]
24. Mokha SS, Goldsmith GE, Hellon RF, Puri R. Hypothalamic control of nociceptive and other neurons in the marginal layer of the dorsal horn of the medulla (trigeminal nucleus caudalis) in the rat. *Exp Brain Res* 1987;65:427–436. [PubMed: 3556469]
25. Murphy AZ, Hoffman GE. Distribution of gonadal steroid receptor-containing neurons in the preoptic-periaqueductal gray-brainstem pathway: a potential circuit for the initiation of male sexual behavior. *J Comp Neurol* 2001;438:191–212. [PubMed: 11536188]
26. Murphy AZ, Rizvi TA, Ennis M, Shipley MT. The organization of preoptic medullary circuits in the male rat: evidence for interconnectivity of neural structures involved in reproductive behavior, antinociception and cardiovascular regulation. *Neuroscience* 1999;91:1103–1116. [PubMed: 10391487]
27. Nakamura K, Kaneko T, Yamashita Y, Hasegawa H, Katoh H, Negishi M. Immunohistochemical Localization of Prostaglandin EP3 Receptor in the Rat Nervous System. *J Comp Neurol* 2000;421:543–569. [PubMed: 10842213]
28. Oleson TD, Kirkpatrick DB, Goodman SJ. Elevation of pain threshold to tooth shock by brain stimulation in primates. *Brain Res* 1980;194:79–95. [PubMed: 6769549]
29. Oliveras JL, Besson JM. Stimulation-produced analgesia in animals: behavioral investigations. *Progress Brain Res* 1988;77:141–157.
30. Parry DM, Semenenko FM, Conley RK, Lumb BM. Noxious somatic inputs to hypothalamic-midbrain projection neurones: a comparison of the columnar organisation of somatic and visceral inputs to the periaqueductal grey in the rat. *Exp Physiol* 2002;87:117–122. [PubMed: 11856956]
31. Ratka A, Simpkins JW. Effects of estradiol and progesterone on the sensitivity to pain and on morphine-induced antinociception in female rats. *Horm Behav* 1991;25:217–228. [PubMed: 2066081]
32. Rizvi TA, Ennis M, Shipley MT. Reciprocal connections between the medial preoptic area and the midbrain periaqueductal gray in rat: a WGA-HRP and PHA-L study. *J Comp Neurol* 1992;315:1–15. [PubMed: 1371779]
33. Rizvi TA, Murphy AZ, Ennis M, Behbehani MM, Shipley MT. Medial preoptic area afferents to periaqueductal gray medullo-output neurons: a combined Fos and tract tracing study. *J Neurosci* 1996;16:333–344. [PubMed: 8613800]
34. Shimura T, Yamamoto T, Shimokochi M. The medial preoptic area is involved in both sexual arousal and performance in male rats: re-evaluation of neuron activity in freely moving animals. *Brain Res* 1994;640:215–222. [PubMed: 8004448]
35. Vincler M, Maixner W, Vierck CJ, Light AR. Estrous cycle modulation of nociceptive behaviors elicited by electrical stimulation and formalin. *Pharmacol Biochem Behav* 2001;69:315–324. [PubMed: 11509187]
36. Wang X, Traub RJ, Murphy AZ. Persistent pain model reveals sex difference in morphine potency. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R300–306. [PubMed: 16497818]

Figure 1. Activation of MPOA increases withdrawal latencies

A: Bar graphs showing PW and TF latencies before and after microinjection of DLH (100 nl and 200 nl) or saline (200 nl) into the right MPOA. Data are mean $\pm$ SEM percent change in latency from baseline. Saline produced small, non-significant ($P > 0.05$) increases in withdrawal responses. PW and TF latencies were significantly increased by 100 or 200 nl DLH microinjections. * $P < 0.02$, ** $P < 0.01$, NS-non-significant ($P > 0.05$); ANOVA followed by Neuman-Keuls tests performed on baseline and post-injection withdrawal latencies. Mann-Whitney U and Wilcoxin Sign rank tests were used for comparison of between and within group percentage data, respectively, as indicated by brackets. * $P < 0.05$, ** $P < 0.003$, NS-non-significant. **B-C:** Distribution of DLH (200 nl) and saline (200 nl) microinjection sites. All injections were made in the right MPOA, but saline microinjections are plotted on the left for brevity. Magnitude of changes in withdrawal latencies, expressed as percent increases, are plotted as: open circles, 0-14%; grey circles, 15-30%; black stars, 31-50%; black circles, >50%. Abbreviations: 3V, 3rd ventricle; ac, anterior commissure; AVPe, anteroventral periventricular nucleus; LPOA, lateral preoptic area; MnPO, median preoptic nucleus; MPOA, medial preoptic area; MPNI, medial preoptic nucleus, lateral; MPNm, medial preoptic nucleus, medial; Pe, periventricular hypothalamic nucleus; OX, optic chiasm; SO, supraoptic nucleus; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus.

Figure 2. Inactivation of the PAG blocks MPOA-evoked antinociception

A-C: Bar graphs (mean $\pm$ SEM) showing the percent of change in left (**A**) and right (**B**) PW and TF (**C**) latencies following lidocaine injection into the PAG (LIDO), DLH into MPOA combined with lidocaine in the PAG (DLH+LIDO), and DLH alone into MPOA (DLH). The LIDO data represent one group of rats ($n=5$), and the DLH+LIDO and DLH results are from a second group of rats ($n=7$). For DLH+LIDO, 3-4 PAG lidocaine microinjections (200 nl each, see Fig. D-F and text for details) preceded the MPOA DLH microinjection. PAG lidocaine microinjections produced small, non-significant increases in latencies. When PAG-lidocaine preceded MPOA-DLH, no significant increase in latencies occurred and there were no difference between the LIDO and DLH+LIDO conditions. In the same animals, subsequent DLH microinjection into MPOA significantly increased latencies in all cases. * $P < 0.02$, NS-non-significant ($P > 0.05$); ANOVA followed by Neuman-Keuls tests performed on baseline and post-injection withdrawal latencies. Mann-Whitney U or Wilcoxin Sign Rank tests were used for comparison of percentage data, as indicated by brackets. NS-non-significant ($P > 0.05$), * $P < 0.05$. **D-F:** Lidocaine microinjection sites in PAG. Open circles, lidocaine microinjections alone; filled circles, lidocaine microinjections made prior to DLH MPOA microinjections. Abbreviation: Aq, aqueduct.