Physiological properties of pain-modulating neurons in rostral ventromedial medulla in female rats, and responses to opioid administration

Gwen Hryciw, Caitlynn C. De Preter, Jennifer Wong, Mary M. Heinricher

School of Dentistry, Portland, OR, USA
Departments of Biomedical Engineering, Portland, OR, USA
Behavioral Neuroscience, Portland, OR, USA
Neurological Surgery, Portland, OR, USA
Oregon Health & Science University, Portland, OR, USA

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ABSTRACT

Functional pain disorders disproportionately impact females, but most pain research in animals has been conducted in males. While there are anatomical and pharmacological sexual dimorphisms in brainstem pain-modulation circuits, the physiology of pain-modulating neurons that comprise a major functional output, the rostral ventromedial medulla (RVM), has not been explored in female animals. The goal of this study was to identify and characterize the activity of RVM cells in female, compared to male, rats. ON- and OFF-cells were identified within the RVM in females, with firing properties comparable to those described in males. In addition, both ON- and OFF-cells exhibited a sensitized response to somatic stimuli in females subjected to persistent inflammation, and both ON- and OFF-cells responded to systemically administered morphine at a dose sufficient to produce behavioral antinociception. These data demonstrate that the ON-/OFF-cell framework originally defined in males is also present in females, and that as in males, these neurons are recruited in females in persistent inflammation and by systemically administered morphine. Importantly, this work establishes a foundation for the use of female animals in studies of RVM and descending control.

Introduction

Chronic pain disorders disproportionately impact females, and while studies in healthy humans indicate that there are likely few sex differences in basal pain threshold, males and females may experience pain differently (Fillingim et al., 2009; Mogil, 2012; Racine et al., 2012a). One factor that could contribute to sex differences in pain experience is sexual dimorphisms in brainstem pain-modulation circuits. The rostral ventromedial medulla (RVM) is a major functional output of the best-studied pain-modulating circuit. The RVM has been well-characterized anatomically, physiologically, pharmacologically, and functionally in male animals. Although there is some evidence for anatomical and pharmacological sexual dimorphism in brainstem pain-modulating circuits (Bobeck et al., 2009; Boyer et al., 1998; Loyd & Murphy, 2006; 2009; Tershner et al., 2000), the physiology of pain-modulating neurons in females has been almost entirely unexplored.

A large body of evidence based almost exclusively on findings in males indicates that the RVM modulates nociceptive transmission through projections to the spinal and trigeminal dorsal horns. Two classes of neurons, termed “ON-cells” and “OFF-cells”, have been identified physiologically in males: activity of ON-cells increases, whereas activity of OFF-cells ceases prior to behavioral responses evoked by noxious stimuli (Fields et al., 1983a). These two cell classes respectively amplify and suppress nociceptive transmission. A shift in the balance between ON- and OFF-cell population output can therefore produce enhanced or diminished nociception and pain behaviors (Heinricher & Fields, 2013; Heinricher et al., 2009). RVM receives information via sensory pathways, including noxious somatic input, forming a recurrent circuit (Chen & Heinricher, 2019a; Chen & Heinricher, 2019b). Input from higher structures to RVM forms a circuit through which cognitive

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Abbreviation:
RVM, rostral ventromedial medulla; PAG, periaqueductal gray; CFA, complete Freund’s adjuvant.

1 These authors contributed equally to this work.

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and emotional factors can influence pain (Heinricher & Fields, 2013).

Given the evidence for anatomical and pharmacological differences in this brainstem pain-modulating circuit between males and females, it is surprising that few studies have considered the physiological properties of pain-modulating neurons in females (Craft et al., 2004; Rojas-Piloni et al., 1998). Both human and animal literature show that there is a similar organization in periaqueductal gray (PAG) to RVM connectivity in males and females (Kong et al., 2010; Loyd et al., 2007; Loyd & Murphy, 2006). However, some sexual dimorphisms in PAG-RVM circuitry have been identified. There is a greater number of PAG-RVM output neurons in female than male rats, but at the same time, a smaller percentage of this population is activated in females during inflammation or following systemic morphine administration (Loyd et al., 2007; Loyd & Murphy, 2006). There are also differences in opioid effects in both PAG and RVM, with most authors reporting lesser potency or efficacy of localized application of mu-opioid agonists in females (Bernal et al., 2007; Bobeck et al., 2009; Boyer et al., 1998; Loyd et al., 2007; Loyd & Murphy, 2006; 2009; Loyd et al., 2008; Tershner et al., 2000). The purpose of the present study was to identify and fully characterize the activity of RVM cells in female compared to male animals.

We first compared RVM neuronal activity in naïve males and females to determine whether there are any basal differences in physiological properties. Second, since women report higher prevalence of chronic pain than men (Fillingim et al., 2009; Mogil, 2012; Racine et al., 2012a), we extended these studies of RVM properties to a model of persistent, localized inflammation (injection of Complete Freund’s Adjuvant into the plantar surface of one hindpaw). Finally, since the analgesic actions of opioids are reported to depend on sex (Mogil, 2020; Nasser & Affy, 2019), we also determined the responses of RVM neurons to systemically administered morphine.

Materials and methods

All experiments followed the guidelines of the National Institutes of Health and the Committee for Research and Ethical Issues of the International Association for the Study of Pain, and were approved by the Institutional Animal Care and Use Committee at the Oregon Health & Science University. Male and female Sprague Dawley rats from Charles University. Male and female Sprague Dawley rats from Charles

Effects in both PAG and RVM, with most authors reporting lesser potency in heart rate or body temperature between males and females (Kong et al., 2010; Loyd et al., 2007; Loyd & Murphy, 2006). However, some sexual dimorphisms in PAG-RVM circuitry have been identified. There is a greater number of PAG-RVM output neurons in female than male rats, but at the same time, a smaller percentage of this population is activated in females during inflammation or following systemic morphine administration (Loyd et al., 2007; Loyd & Murphy, 2006). There are also differences in opioid effects in both PAG and RVM, with most authors reporting lesser potency or efficacy of localized application of mu-opioid agonists in females (Bernal et al., 2007; Bobeck et al., 2009; Boyer et al., 1998; Loyd et al., 2007; Loyd & Murphy, 2006; 2009; Loyd et al., 2008; Tershner et al., 2000). The purpose of the present study was to identify and fully characterize the activity of RVM cells in female compared to male animals.

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Inflammation

Persistent inflammation was induced in a subset of female animals prior to experiments. Rats were briefly anesthetized with isoflurane (4%, 4–5 min) and CFA (0.1 ml) was injected subcutaneously into the plantar surface of the right hindpaw. Rats were returned to their home cage for 3 to 6 days to model persistent inflammation, since inflammation peaks at this time (Ren, 1999; Ren & Dubner, 1999). There was no significant difference in anesthetic dose required to maintain CFA-treated females at an anesthetic depth similar to that employed for naïve females (t41 = 0.74, p = 0.46, F CFA: 57.8 ± 1.82 mg/kg, F naïve: 56.18 ± 1.19 mg/kg). There was also no effect of treatment on heart rate or body temperature (HR: t41 = 1.42, p = 0.16, Temp: t41 = 0.84, p = 0.41).

Characterization of RVM neurons under basal conditions and in persistent inflammation

All testing was performed in low ambient light conditions (<5 lx). A gold- and platinum-plated stainless-steel microelectrode was placed in the RVM to record cell activity. Signals were amplified and band-pass filtered (Neurolog, Digitimer) then transmitted to a computer for real-time spike detection and monitoring using Spike2 (CED, Cambridge, UK). EMG activity, heart rate, and paw heat-stimulus temperature were also recorded using Spike2. Identified neurons were classified as ON, OFF, or NEUTRAL-cells based on changes in firing rate associated with nocifensive withdrawal (Cleary & Heinricher, 2013; Fields et al., 1983a; Martenson et al., 2016). ON-cells are defined by a burst in activity beginning just prior to withdrawal from a noxious stimulus. OFF-cells stop firing just prior to withdrawal.

After isolating and identifying a cell as an ON- or OFF-cell, one heat trial was performed on each hindpaw approximately 4 min apart (some trials were delayed in order to capture an ON-cell in a quiet state or an OFF-cell in an active state). Noxious heat was applied by lightly resting a Peltier device (Yale Instruments, New Haven, CT) on the plantar surface of the paw. Paw surface temperature was held at 35 °C before heat onset, and temperature then increased at a rate of approximately 1.5 °C to a maximum of 53 °C. To avoid damage to the paw, the Peltier device was removed upon limb movement, determined using EMG. von Frey fibers (4, 15, 26, 60, and 100 g) were applied to the webbing between the toes. Each fiber was applied three times to each paw, in ascending order, for 8 s. Three interdigital testing sites were alternated, with a minimum of 30 s between each trial. Longer inter-trial intervals (up to 5 min) were sometimes necessary to capture an ON-cell in a quiet state or an OFF-cell in an active state. Paw withdrawal was monitored visually as well as with EMG. In experiments using CFA, inflammation was confirmed visually in CFA-treated animals and paws were measured with calibrated calipers applied at the widest point across the dorsal-plantar surface. The treated hindpaw was significantly larger than those of untreated females (t35 = 17.84, p < 0.0001, CFA: 7.97 ± 0.16, Naïve: 4.46 ± 0.089). In experiments using systemic morphine administration, a thermal stimulus (cut-off temperature of 53 °C, 12 s) was also used.

Response of characterized RVM neurons to opioid administration

Surgical preparation was as above. Opioids were administered systemically via either a second jugular catheter (n = 10) or intraperitoneal injection (n = 25). After isolating and identifying a cell, one heat trial was performed every 5 min as described above. After a minimum of 3 trials to establish baseline cell and behavioral response, morphine sulfate was given in increments of 0.5 mg/kg every 10 min until there was no behavioral response on two of three successive heat trials (12-s cut-off). Naloxone (1 mg/kg i.v. or i.p.) was then administered, and ongoing firing and paw withdrawal-related changes in activity were recorded for a minimum of three trials. The average dose required to produce analgesia in these experiments in female animals was 1.86 mg/kg, which falls within the range of doses that are sufficient to suppress.
noxious evoked reflexes in lightly anesthetized male animals (Barbaro et al., 1986; Heinricher et al., 1999; Heinricher et al., 2001a).

**Histology**

At the end of each experiment, the recording site was marked with an electrolytic lesion. Animals were euthanized by methohexital overdose and perfused transcardially with saline and 10% formalin. Brains were removed, and the lesion site reconstructed. The RVM was defined as the nucleus raphe magnus and adjacent reticular formation medial to the lateral boundary of the pyramids at the level of the facial nucleus. For characterization of RVM physiology, a total of 21 cells from 17 males, 26 cells from 21 naive females, and 25 cells from 22 CFA-treated females were recorded (1–2 cells per animal, although only one protocol was performed in each animal, two identifiable cells were isolated in some experiments). In experiments focused on opioid responses, a total of 45 cells was recorded from 35 females (1–2 cells per animal). Cells were distributed throughout RVM in both males and females (Fig. 1).

**Data processing and analysis**

At the conclusion of each experiment, action potential waveforms were individually examined to verify correct waveform sorting. Thermal-evoked paw withdrawal latency was defined as the average time from heat onset till paw withdrawal based on EMG activity. Mechanical withdrawal thresholds for each paw were determined based on the minimum force at which a withdrawal was observed in at least two out of three trials.

Ongoing activity was defined as the average firing rate during the two 30-s periods prior to each heat trial. Evoked firing for ON-cells was defined as the total number of spikes in the longest burst during heat, or as the total number of spikes in all bursts initiated during mechanical stimulation. A “burst” was defined as the first action potential after stimulus onset until the last action potential that preceded a 2-s quiet period. However, if an ON-cell was already active prior to heat stimulus onset, then the number of action potentials in the 3-s period around the paw withdrawal was used as the evoked response. Similarly, if an ON-cell was active prior to application of the evoked response. Peak firing rate during stimulation was considered the evoked response. Peak firing rate during stimulation was also determined for ON-cells. The stimulus-evoked pause exhibited by OFF-cells was quantified as the percent suppression. In heat trials, this was the firing rate in the 3-s period around the paw withdrawal relative to the firing rate 10-s prior to heat onset. For mechanical stimulation trials, this was the firing rate in the 8-s during mechanical stimulation relative to that in the 8-s period prior to mechanical stimulation. The longest pause duration during stimulation was also determined. A “pause” was defined as the time period between one spike that was preceded within 2 s by another action potential and terminated when two action potentials occurred within 2 s. Cell response threshold was also determined by finding the force required to elicit a minimum 50% change in cell in activity in at least two out of three trials.

Behavioral and cellular data from naïve animals were averaged between the left and right paw for subsequent data analysis. Behavioral data and reflex-related cell parameters were compared between naïve males and females using unpaired *t*-tests, and between the contralateral and ipsilateral paw of CFA-treated females using paired *t*-tests. For tests with von Frey fiber stimulation, data from naïve males and females were compared using 2-factor ANOVA with repeated measures on force. Data from CFA-treated females was analyzed using a 2-factor ANOVA with paw and force as within-subject factors.

In a separate set of experiments looking at effects of morphine administration on activity of RVM neurons in females, three time periods were defined for the purpose of analysis. The “baseline” was defined as the three heat trials prior to the first dose of morphine, the “morphine” period as the final three trials prior to naloxone (two of three consecutive trials with no withdrawal within the 12-s cut-off, as described above), and the “naloxone” period was the three trials after naloxone administration that resulted in at least two paw withdrawals. Ongoing activity was defined as the average firing rate during three 30-s periods prior to the heat trial in each time period. Evoked firing for ON-cells was defined as the total number of spikes in the longest burst during

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Fig. 1. Histologically verified recording locations within the RVM. Recording sites were distributed between −1.32 and −2.90 mm (relative to the interaural line). The majority of cells were distributed between −1.52 and −2.50 mm caudal to the interaural line.
heat. In the morphine time period when the paw-withdrawal was completely lost, cell activity around the average paw-withdrawal temperature at baseline + 0.5 °C was collected to define stimulus-related cell activity. Behavioral and cellular data obtained in the baseline period were compared with the averages of the three post-morphine trials and the three post-naloxone trials using repeated-measures ANOVA. Quantitative data are presented as mean ± SEM, unless otherwise specified. Parameters with highly skewed distributions were log-transformed for analysis, and back-transformed data presented as geometric mean ± 95% confidence intervals.

RESULTS

No differences in RVM cell ongoing firing and noxious somatic stimulus-related responses in male and female animals

The first set of experiments compared the firing properties of RVM OFF- and ON-cells in female and male animals. Examples of the reflex-related changes in firing of an OFF-cell and ON-cell recorded from a female animal during heat-evoked withdrawal are shown in Fig. 2. Quantification of reflex-related changes in activity is shown in Fig. 3. There was no difference in heat-evoked OFF-cell suppression and pause duration (Fig. 3a,b) or ON-cell total evoked spikes and peak-firing rate (Fig. 3c,d) between the sexes. There was no significant difference in heat-evoked withdrawal latency (Fig. 3e). Comparison of ongoing firing rates (Fig. 4) similarly demonstrated no significant differences between males and females.

We then compared OFF- and ON-cell responses during stimulation with von Frey fibers at forces ranging from 4 to 100 g. In naive female and male animals, OFF- and ON-cells responded to forces in the frankly noxious range (60 and 100 g) that were sufficient to evoke a withdrawal reflex in either sex (Fig. 5a-d). As with heat stimulation, there was no difference between the sexes in cell responses or behavioral threshold (Fig. 5e).

Persistent inflammation following CFA injection produces mechanical but not thermal hyperalgesia in female animals

We next characterized RVM cell responses during persistent inflammation in females. Animals were treated with an injection of CFA in the right hindpaw 3 to 6 days prior to recording. We found that local administration of CFA produced mechanical hyperalgesia in the treated paw (Fig. 6a) in female animals, with a statistically significant decrease in threshold when tested 3–6 d after CFA injection. This decrease was substantial in that stimulation of the inflamed paw even with an innocuous force (≤26 g) evoked a withdrawal response in 81.8% of the animals tested, whereas this was never seen with stimulation of the contralateral paw. Females did not exhibit thermal hyperalgesia at 3–6 d post-injection (Fig. 6b), with no difference in heat-evoked withdrawal latency between the inflamed and contralateral paw. These data are consistent with prior work in males (Cleary & Heinricher, 2013).

Evoked responses of RVM neurons in female animals with persistent inflammation

Stimulus-response functions for the OFF- and ON-cell responses evoked by von Frey fiber stimulation in females with persistent inflammation are shown in Fig. 7. The OFF-cell pause (cell suppression and pause duration, Fig. 7a,b) and ON-cell burst (total evoked spikes and peak firing, Fig. 7c,d) for stimulation of the inflamed and contralateral paw were compared. OFF- and ON-cells developed both increased responses to noxious (60–100 g) stimulation of the inflamed paw compared to the control paw, and novel responses to innocuous stimulation (≤26 g) of the inflamed paw (Fig. 7a,b,d,e). Thresholds were lowered for stimulation of the inflamed paw, but not the contralateral paw (Fig. 7c,f). The responses of RVM cells are thus consistent with the mechanical hypersensitivity seen in these animals.

Opioid response of RVM neurons in female animals

In a third set of experiments, we determined the response of RVM ON-, OFF-, and NEUTRAL-cells to systemic administration of morphine in female animals. NEUTRAL-cells were defined by an absence of responses to noxious-evoked withdrawal. Fig. 8 shows firing of an OFF-, ON-, and NEUTRAL-cell in baseline, after systemic administration of morphine sufficient to inhibit heat-evoked withdrawal, and following reversal of the morphine effect with naloxone. In baseline, the OFF-cell
Ongoing firing of ON- and OFF-cells. a. There was no significant difference in OFF-cell ongoing firing rate between male and female animals ($t_{19} = 1.0, p = 0.33, n = 8 M, 13F$). b. There was no significant difference in ON-cell ongoing firing rate between male and female animals ($t_{24} = 1.09, p = 0.29, n = 13 M, 13F$).

Fig. 4. Ongoing firing of ON- and OFF-cells. a. There was no significant difference in OFF-cell ongoing firing rate between male and female animals ($t_{19} = 1.0, p = 0.33, n = 8 M, 13F$). b. There was no significant difference in ON-cell ongoing firing rate between male and female animals ($t_{24} = 1.09, p = 0.29, n = 13 M, 13F$).

Fig. 5. Mechanically evoked response and withdrawal in naïve males and females. For all cell parameters, there was no significant effect of sex, although there was a significant effect of force. a. OFF-cell suppression (Sex: $F_{1,19} = 0.0057, p = 0.94; \text{Force: } F_{5,95} = 220, p < 0.0001; \text{Interaction: } F_{5,95} = 1.14, p = 0.34; n = 8 M, 13F$). b. OFF-cell pause duration (Sex: $F_{1,19} = 0.85, p = 0.37; \text{Force: } F_{5,95} = 21.47, p < 0.0001; \text{Interaction: } F_{5,95} = 0.35, p = 0.88; n = 8 M, 13F$). c. Evoked spikes in ON-cell burst (Sex: $F_{1,24} = 0.03, p = 0.88; \text{Force: } F_{5,120} = 76.21, p < 0.0001; \text{Interaction: } F_{5,120} = 2.38, p = 0.042; n = 13 M, 13F$). d. ON-cell peak firing rate (Sex: $F_{1,24} = 0.65, p = 0.43; \text{Force: } F_{5,120} = 71.87, p < 0.0001; \text{Interaction: } F_{5,120} = 1.77, p = 0.12; n = 13 M, 13F$). e. There was no significant difference in mechanical withdrawal threshold between males and females ($t_{36} = 1.12, p = 0.27, n = 17 M, 21F$).

Discussion

The primary goal of this study was to identify and characterize pain-modulating neurons in the RVM in females. Since RVM is a major output of brainstem pain-modulatory circuitry that can amplify or suppress pain-transmission by actions at the dorsal horn (Heinricher & Fields, 2013; Heinricher et al., 2009), sex-related differences in the activity or organization of this system could in principle predispose females to develop chronic pain conditions. As in prior work in males, we were able to identify ON-, OFF- and NEUTRAL-cells in the RVM in females, with reflex-related activation of ON-cells and suppression of OFF-cell firing. Firing properties in females were comparable to those in males recorded in parallel experiments. In addition, both ON- and OFF-cells exhibited a “sensitized” response to somatic stimuli in females subjected to persistent inflammation, with lowered thresholds and enhanced responses to suprathreshold stimuli. Finally, both ON- and OFF-cells responded to systematically administered morphine at a dose sufficient to produce behavioral antinociception. Thus, the physiological properties of RVM neurons in females, sensitization in a persistent inflammatory state, and response to systematically administered morphine are entirely consistent with what is known of these neurons in males. Overall, these findings validate the defining features of RVM cells by extending them to females.

We first considered ongoing activity levels and noxious-evoked responses of RVM cells in naïve animals. There was no significant difference between the two sexes in cell firing parameters, showing that RVM cells in females have similar response properties to those in males under basal conditions. Thus, despite some anatomical and pharmacological differences in pain-modulation circuitry upstream of RVM and in RVM itself (Bobeck et al., 2009; Boyer et al., 1998; Loyd & Murphy, 2006; 2009; Tershner et al., 2000), the properties of RVM neurons are comparable in males and females under basal conditions. Moreover, since the RVM contributes to basal nociceptive “tone” (Heinricher et al., 1989), this observation of similar output from RVM in males and females
is consistent with our own observation of no difference between males and females in thermal or mechanical nociception, and more generally, the lack of evidence for a robust sex difference in basal nociceptive

**Fig. 6.** Mechanical but not thermal hypersensitivity in females with persistent inflammation. a. There was a significant difference between paws for mechanically-evoked paw withdrawal threshold (paired t-test, $t_{21} = 11.61, p < 0.0001, n = 22$). b. No significant difference between paws for heat-evoked paw withdrawal latency (paired t-test, $t_{19} = 0.95, p = 0.35, n = 20$).

**Fig. 7.** Shift in cell stimulus–response curve for mechanical stimulation of CFA treated paw. For all cell parameters, there was a significant effect of force, paw, and force × paw interaction. a. OFF-cell suppression: force ($F_{5,60} = 47.28, p < 0.0001$), paw ($F_{1,12} = 51.71, p = 0.00012$), force × paw ($F_{5,60} = 5.58, p = 0.0003$), $n = 13$ cells. b. OFF-cell pause duration: force ($F_{5,50} = 22.61, p < 0.0001$), paw ($F_{1,10} = 21.31, p = 0.0010$), force × paw ($F_{5,50} = 7.12, p < 0.0001$), $n = 11$. c. OFF-cell response threshold was significantly lower in the inflamed paw ($t_{12} = 7.88, p < 0.0001$). d. ON-cell burst: force ($F_{5,55} = 28.34, p < 0.0001$), paw ($F_{1,11} = 28.5, p = 0.0002$), force × paw ($F_{5,55} = 4.26, p = 0.0024$), $n = 12$. e. ON-cell peak firing rate: force ($F_{5,55} = 31, p < 0.0001$), paw ($F_{1,11} = 21.06, p = 0.0008$), force × paw ($F_{5,55} = 3.81, p = 0.0049$), $n = 12$. f. ON-cell response threshold was significantly lower in the inflamed paw ($t_{10} = 6.77, p < 0.0001$).

**Fig. 8.** Representative RVM cell response to systemic morphine administration in female animals. Ratemeter records (1 s bins) show the effect of systemic morphine administration on the activity of a. OFF-cell, b. ON-cell, and c. NEUTRAL-cell. Heat onset (black bars) prior to morphine administration and after naloxone administration resulted in paw withdrawal (black triangles). Analgesic doses of morphine resulted in a loss of paw withdrawal (open triangles).
Fig. 9. Effects of systemic morphine administration on ongoing cell activity and withdrawal-evoked cell behaviors in naïve females. a. Systemic morphine administration significantly changed OFF-cell ongoing activity (F(2,28 = 7.76, p = 0.0021), with post-morphine increased compared to baseline (p = 0.0043). b. Morphine significantly decreased the OFF-cell pause (F(2,28 = 25.43, p < 0.0001) with post-morphine significantly different from baseline (p < 0.0001). c. There was a significant change in ON-cell ongoing activity (F(2,28 = 5.078, p = 0.013), and morphine significantly decreased ongoing activity compared to baseline (p = 0.016). d. There was a significant difference in the total evoked spikes in the ON-cell burst (F(2,16 = 21.30, p < 0.0001) with the post-morphine time point depressed compared to baseline (p < 0.0001). e. No significant change in NEUTRAL-cell ongoing activity (F(2,28 = 0.18, p = 0.84). One-way ANOVA with repeated measures and post-hoc Dunn’s multiple comparisons test, n = 15 OFF-cells, 15 ON-cells, 15 NEUTRAL cells. There was no significant difference between baseline and naloxone for any cell measure.

Responding (Fillingim et al., 2009; Loyd et al., 2008; Mogil, 2012; Racine et al., 2012a; Wang et al., 2006).

We next looked at the effects of persistent inflammation on RVM output and behavioral sensitivity in female animals. When tested 3 to 6 days after localized injection of CFA in a single hindpaw, mechanical hyperalgesia was prominent in the CFA-treated paw, consistent with previous reports in lightly anesthetized males (Chen & Heinricher, 2019b; Cleary & Heinricher, 2013; Montagne-Clavel and Olivares, 1994; Pinto-Ribeiro et al., 2008). We did not observe thermal hyperalgesia in female animals at the time points studied here, which again is in agreement with previous findings in male animals, that thermal hyperalgesia begins to resolve within the first 24 h after CFA injection (Almarestani et al., 2011; Cleary & Heinricher, 2013; Guan et al., 2003; Okun et al., 2011; Pinto-Ribeiro et al., 2008; Ren & Dubner, 1996; Wei et al., 1999). The observation that both ON- and OFF-cells are sensitized in females, as in males, is consistent with limited evidence for substantial sex differences in CFA-induced hyperalgesia (Armendariz & Nazarian, 2018; Bradshaw et al., 2000; Graff et al., 2013; Loyd et al., 2008; Wang et al., 2006).

Despite the similarity in behavioral endpoints and RVM sensitization in persistent inflammation in females and males, there are differences in pain-modulation circuitry between the sexes that could in principle underlie observed discrepancies in prevalence and presentation of chronic pain disorders in humans. For example, Loyd and colleagues (2008) reported increased activation of PAG-RVM output neurons in males compared to females during persistent inflammation. Nonetheless, these authors also saw no differences in inflammation-induced hyperalgesia between the two sexes. One possible explanation for this discrepancy is that the similar behavioral outcome in males and females ultimately reflects comparable recruitment of RVM ON- and OFF-cells by the PAG during inflammation. Our finding that ON- and OFF-cells are sensitized in females, as in males, is consistent with this idea. This argument would further imply that molecular and anatomical differences between males and females at the level of the PAG are compensated for at the level of the RVM, leading to similar output from the PAG-RVM system and explaining comparable behavior.

We also investigated the effects of systemic opioid administration on behavioral analgesia and RVM cell responses in female animals. Opioids are thought to produce analgesia in both sexes in part by engaging the PAG-RVM descending modulatory system. Thus, sex differences in the pharmacological properties of this circuit could contribute to differential opioid effects in men and women (Bernal et al., 2007; Bobeck et al., 2009; Loyd & Murphy, 2005). However, animal and human literature raise the possibility that sex differences in analgesic effects may reflect differences in the pharmacological properties of the system and the sensitivity of the opioid receptors in males and females. For example, some authors report that opioids are more effective in males (Bernal et al., 2007; Bobeck et al., 2009; Loyd & Murphy, 2005), while others report no sex differences in the pharmacological properties of this system (Barnes et al., 2009). Notably, these findings are based on studies in rodents, and it is possible that sex differences in the pharmacological properties of this system may differ in humans.

The present results are consistent with limited evidence for sex differences in acute experimental pain in humans and rodents. Effects of sex in humans are small, with a host of confounding factors...
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