Estrogen Status Gates Effects of Kappa-Opioid Receptor on Temporomandibular Joint–Responsive Neurons at the Spinomedullary Junction in Female Rats

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Aims: To determine whether estrogen status alters κ-opioid inhibition of nociceptive processing by affecting temporomandibular joint (TMJ) input to neurons in the trigeminal subnucleus caudalis [Vc]/C1-2 region at the spinomedullary junction in female rats. **Methods:** TMJ-responsive neurons were recorded in laminae I–II of the Vc/C1-2 region at the spinomedullary junction of ovariectomized female rats treated for 2 days with low-dose estradiol (LE group; 2 mg/day) or high-dose estradiol (HE group; 20 mg/day). Under isoflurane anesthesia, TMJ neurons were activated by adenosine triphosphate (ATP; 1 mM, 20 µl), which was injected into the joint space before and after cumulative doses of a κ-opioid receptor (KOR) agonist (U50488) given systemically (0.03, 0.3, and 3 mg/kg, intravenously) or by local application to the dorsal surface of the Vc/C1-2 region (1 and 10 nmol/30 µl). Analysis of variance and Newman-Keuls test were performed to compare the data. **Results:** Systemic U50488 caused a dose-related inhibition of ATP-evoked neuronal activity in HE rats and reduced the size of the neuronal cutaneous receptive field (RF), while effects in LE rats were not significant. Systemic U50488 reduced the spontaneous activity of TMJ-responsive neurons to similar levels in LE and HE groups. Locally applied U50488 inhibited ATP-evoked neuronal activity in HE rats, but not in LE rats. Systemic and local administration of the KOR antagonist nor-binaltorphinine (nor-BNI) partially reversed the decrease in Rmag induced by U50488, but had no effect on neurons from LE rats. **Conclusion:** These results indicate that KOR-dependent effects on TMJ-responsive neurons in the superficial laminae of the Vc/C1-2 region in female rats are differentially modified by high and low estrogen status. The site of action for estrogen-induced modulation of TMJ neuronal activity by KOR likely includes second-order neurons in the Vc/C1-2 region. *J Oral Facial Pain Headache 2017;31:275–284. doi: 10.11607/ofph.1820*

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Temporomandibular disorders (TMD) occur in a heterogenous patient group with core complaints of temporomandibular joint (TMJ) and masticatory muscle pain.[1,2] Chronic TMD is more prevalent in women than in men,[3] and estrogen status is a risk factor for TMD[4] and other musculoskeletal pain conditions.[5] Estrogen has widespread effects on neural function and has been reported to influence pain processing by acting at multiple levels of the neuroaxis.[6,7] Estrogen status also influences pain processing by modulating the analgesic efficacy of opioids[8] and alters the ability to recruit endogenous µ-opioid receptor pathways in response to experimental jaw muscle pain in women,[9] as well as the ability of exogenous morphine to inhibit TMJ input to trigeminal subnucleus caudalis (Vc) neurons in female rats.[10] Estrogen status may also influence trigeminal pain processing by altering mechanisms mediated by the κ-opioid receptor (KOR). KOR activation has been reported to provide greater pain relief after tooth removal in women than in men[11] and to cause TMJ-evoked, pain-like behavior in female rats to vary over the estrous cycle.[12] However, the relationship between estrogen status and κ-opioid analgesia in craniofacial pain remains poorly defined.

KORs are densely distributed in the superficial laminae of the Vc/C1-2 region at the spinomedullary junction of female rats.[13] The Vc/C1-2 region receives direct input from primary afferent neurons that supply the TMJ and jaw muscles[14,15] and expresses a high density of estrogen receptors (ERs).[16,17] Although estrogen status significantly alters the encoding properties of TMJ-responsive neurons in the superficial laminae of the Vc/C1-2 region,[18,19] it is not known whether estrogen status also modifies κ-opioid modulation of TMJ neuronal activity. The aim of this study was to determine whether estrogen status alters κ-opioid inhibition of nociceptive processing by affecting TMJ input to neurons in the Vc/C1-2 region at the spinomedullary junction in female rats.

Materials and Methods

The study protocols were approved by the Committee of Research Facilities for Laboratory Animal Science, National Defense Medical College (Japan), and conformed to established guidelines set by The National Institutes of Health for the care and use of laboratory animals (PHS Law 99-158; revised, 2002). Animals were housed in cages (three rats per cage) with free access to food and water. Cages remained in climate- and light-controlled protected units (25°C, 12-hour light/dark cycle with light on at 7:00 am) for at least 5 days before experiments.

Animals

General and Endocrine Procedures

Ovariectomized, female Sprague-Dawley rats (250–320 g, n = 43) were purchased from SLC (Shizuoka, Japan). Rats were treated for 2 days with 17α-estradiol-3-benzoate (E2; Sigma) at either a low dose (LE group, n = 22; 2 μg subcutaneously) or a high dose (HE group, n = 21; 20 μg subcutaneously) to mimic the low and high estrogen status of normal cycling rats.[20] On the day of the experiment, a vaginal smear was taken to determine the estrogen status, as previously reported.[19] Data were collected without prior knowledge of E2 treatment.

Rats (n = 37) were sedated initially with pentobarbital sodium (50 mg/kg intraperitoneally), and catheters were placed in the femoral artery (blood pressure) and vein (opioid drugs), as well as in the jugular vein (paralytic agent). After tracheotomy, rats were respirated with oxygen-enriched room air and maintained with isoflurane (1.0% to 1.5%) anesthesia. The short-acting paralytic agent gallamine triethiodide (25 mg/kg/hour) was given only during the time of recording. Expiratory end-tidal carbon dioxide (3.5% to 4.5%), mean arterial pressure (90–120 mmHg), and body temperature (38°C) were monitored continuously and maintained within normal ranges. Rats were placed in a stereotactic frame, and the dorsal portions of the C1 and C2 vertebrae were removed to allow access to the Vc/C1-2 region. The left temporalis muscle was gently reflected to partially expose the lateral pterygoid muscle and connective tissue overlying the dorsal aspect of the posterior mandibular condyle.

The Vc/C1-2 region was explored for TMJ-responsive neurons, as described previously.[10] TMJ-responsive neurons were recorded (Fig 1a) with tungsten microelectrodes (5 megaohms [MΩ]; Frederick Haer Inc) and their activity was amplified, discriminated (model WD-2, Bio Research Center Co), stored, and analyzed offline using a PowerLab interface with LabChart software (ADInstruments). All TMJ-responsive neurons were also excited by a press or pinch stimulus applied to the facial skin, but not by brushing of the skin (ie, nociceptive specific [NS]).

Experimental Design

The recording session began ~2 hours after initial anesthesia. One neuron was recorded per rat, and all recording sites were located in the superficial laminae, < 250 μm from the dorsal surface, within 1.5 mm rostral to the level of the entrance of the C2 rootlets (see example[21]). Depth of anesthesia was determined by loss of the corneal reflex and hind paw withdrawal reflex and was similar for LE and HE rats. The high-threshold cutaneous receptive field (RF) area of each TMJ neuron was mapped using small blunt forceps (approximately 3 mm^2). The left temporalis muscle was gently reflected to partially expose the lateral pterygoid muscle and connective tissue overlying the dorsal aspect of the posterior mandibular condyle. This allowed a 26-gauge guide cannula to be placed into the TMJ capsule (approximately 3 mm deep) by a dorsal approach directed at the posterior edge of the mandibular condyle under the zygomatic arch; chemical stimuli could then be delivered via a 33-gauge inner cannula. Test solutions of phosphate-buffered saline (PBS; pH 7.4) or adenosine triphosphate (ATP; 1 mM, in PBS) were injected in 20-μl aliquots over 30 seconds to prevent tachyphylaxis.[10] The rationale for the use of ATP as a stimulus was based on several factors: ATP injections into human skin evoke acute pain[22]; ATP injections cause only transient and minor evidence of localized inflammation[23]; ATP-evoked responses can be reversed by coapplication of purinergic receptor–specific antagonists[19]; and repeated intra-TMJ injections of ATP evoke consistent neural activity with no signs of tachyphylaxis or sensitization.[10]

Two experimental designs were used. In the first design, carried out in 20 rats, the KOR agonist

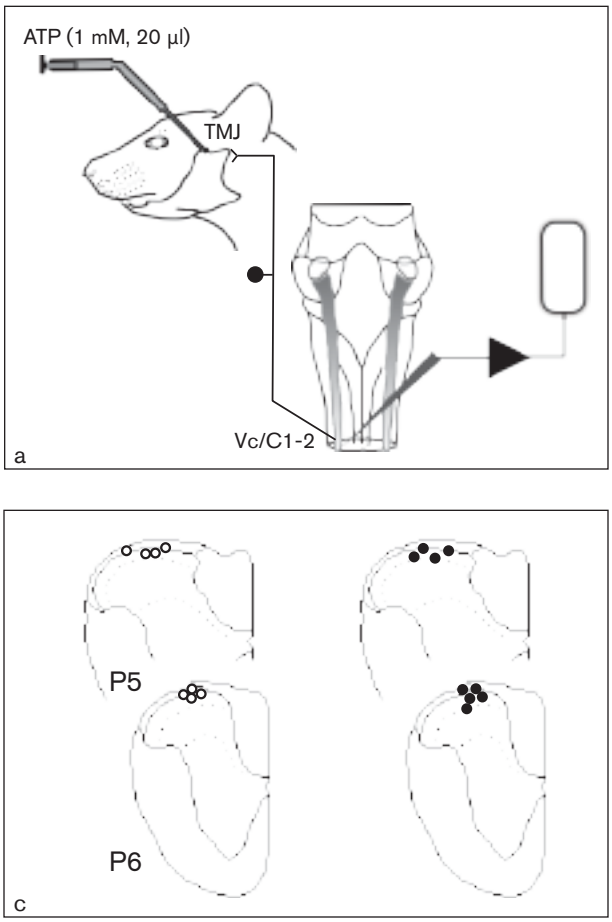


Fig 1 (a) Experimental setup for recording neurons at the Vc/C1-2 region and injecting ATP into the TMJ. Recording locations of TMJ-responsive neurons from LE and HE rats receiving **(b)** a systemic KOR agonist and **(c)** a local KOR agonist. All recording sites were located in the superficial laminae of the dorsal brainstem surface. P5 and P6 refer to approximate distance, in mm, from the obex. Open circles = LE, filled circles = HE.

U50488 (Tocris) was injected intravenously in a cumulative dose regimen (0.03, 0.3, and 3 mg/kg) at 20-minute intervals, and each dose was followed by the intra-TMJ ATP test stimulus 10 minutes later. The KOR antagonist nor-binaltorphimine (nor-BNI; 1 mg/kg) was given at the very end of the dosing regimen and 10 minutes before the final ATP test injection. In the second design, carried out in 17 rats, U50488 (1 and 10 nmol/30 µl) was applied locally at the site of recording 10 minutes prior to the ATP test stimulus. At the very end of the dosing regimen, nor-BNI (30 nmol/30 µl) was applied locally 10 minutes before the final ATP test stimulus. These doses of U50488 and nor-BNI were consistent with those reported to be effective in altering lumbar dorsal horn neural activity.[24,25] At the end of the experiment, the recording site was marked electrolytically (5 µA, 20 seconds) and later mapped onto a standard series of outlines of the rat brainstem.

Data Analysis

Neural data were acquired and displayed by LabChart as peristimulus time histograms (PSTH) of spikes/stimulus (1-second bins), exported to a spreadsheet, and analyzed offline, as described previously.[10] Briefly, neuronal spontaneous activity (SA) was calculated as the mean spike count over a 1-minute period preceding each ATP stimulus. ATP-evoked responses from TMJ-responsive neurons were defined as response magnitude (Rmag; mean + 2 standard deviations [SDs] of SA from the spike count for each bin). The total Rmag for each stimulus was defined as the cumulative sum of spikes over contiguous bins in which the spike count minus the SA was a positive value. Response duration was defined as the time interval after stimulus onset until bins with a positive spike count occurred that exceeded the mean + 2 SDs above the SA, and until the value of bins no longer exceeded the mean + 2 SDs above the SA. Response latency was defined as the earliest time after stimulus onset for which bins exceeded the mean + 2 SDs above the SA (ie, Rmag). All neurons displayed a total Rmag to ATP that was > 50% of that to PBS.

The cutaneous RF area for each neuron was mapped three times in each animal: 10 minutes after the initial ATP test stimulus, 10 minutes after the highest dose of U50488, and 10 minutes after nor-BNI

(Figs 4a and 4b). Each RF area was mapped onto the face drawing, digitized, and quantified by planimetry and NIH ImageJ software without prior knowledge of E2 treatment.

The following variables were statistically assessed using analysis of variance (ANOVA) corrected for repeated measures: total Rmag, response duration, response latency, SA, and the cutaneous RF area. Individual comparisons were performed using Newman-Keuls test after ANOVA. A *P* value of < .05 was considered statistically significant.

KOR Analysis by Quantitative Polymerase Chain Reaction

The Vc/C1-2 region (3–6 mm caudal to the obex) was removed fresh from a separate group of LE and HE rats (250–300 g; n = 3 per group) under pentobarbital anesthesia within 1 hour of the second E2 injection. Total RNA was extracted from 30–50 mg of tissue by using an Absolutely RNA kit (Stratagene), and cDNA was synthesized from 300 ng of each sample by using an iScriptTM cDNA Synthesis kit (Bio-Rad). Quantitative polymerase chain reaction (qPCR) was performed in triplicate for 2 μL cDNA with a Chromo4TM DNA engine (Bio-Rad) using iQ SYBR Green Supermix (Bio-Rad). Data were analyzed using the cycle threshold (ΔCT) method by using a reference gene (glyceraldehyde 3-phosphate dehydrogenase [GAPDH]). The following polymerase chain reaction (PCR) conditions were used: an initial denaturation at 95°C for 3 minutes, followed by 40 cycles at 95°C for 10 seconds, 58.5°C for 30 seconds, and 72°C for 45 seconds.

Results

Systemic Administration of KOR Agonist and Antagonist

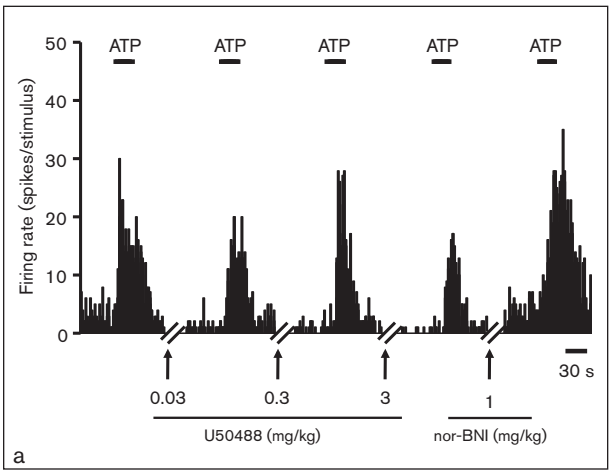
A total of 20 TMJ-responsive neurons classified as NS were recorded in the superficial laminae at the Vc/C1-2 region in LE (n = 10) and HE (n = 10) rats (Figs 1b and 1c). All TMJ-responsive neurons were spontaneously active before ATP stimulation. The average SA was determined over the course of 60 seconds immediately before each ATP stimulus and again at approximately 10 minutes after each drug injection. As shown in Figs 2a and 2b, the initial SA was comparable for neurons recorded in LE and HE rats (2.9 ± 0.6 and 4.0 ± 0.8 spikes/stimulus, respectively). After the 0.03-mg/kg dose, at which only the LE rats displayed a significant reduction in SA, both groups displayed similar reductions in SA with increasing doses of U50488 (Fig 2d).

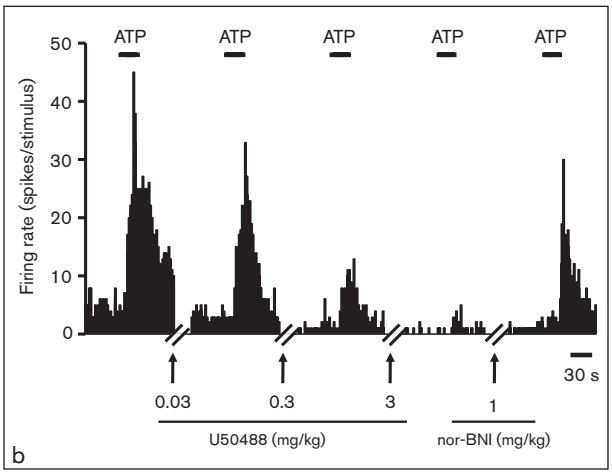
Intra-TMJ injections of ATP evoked prompt increases in total Rmag, and the response of TMJ-responsive neurons from LE rats (315 ± 65 spikes/stimulus) was significantly less than that from HE rats (847 ± 131 spikes/stimulus, $F_{1,31} = 12.5$, $P < .005$) and consistent with previous results.[19] Systemic U50488 caused a significant dose-related reduction of ATP-evoked neuronal activity in the HE group with a partial reversal after nor-BNI administration, whereas no significant drug-related effects were seen in the LE group (Figs 3a and 3b). This was a consistent finding, as 9 of 10 neurons from HE rats were inhibited by at least 50% after a high dose of U50488, while only 6 of 10 neurons from LE rats showed a reduction in total Rmag. One neuron from an LE rat displayed a progressive increase in total Rmag with increasing doses of U50488 (Fig 3a), whereas no neurons from HE rats were excited by the KOR agonist (Fig 3b). The effects of U50488 on ATP-evoked response duration generally paralleled those seen for total Rmag (Figs 3d, 3e, and 3f).

All TMJ neurons received convergent input from the TMJ and the facial skin overlying the TMJ. The high-threshold cutaneous RF areas of TMJ neurons in the LE and HE rats averaged 1.5 ± 0.2 cm^2 and 1.3 ± 0.1 cm^2, respectively, before U50488 administration. As seen in Figs 4a, 4b, and 4c, the RF area of neurons in HE rats was markedly reduced after a high dose of U50488 and partially recovered after nor-BNI administration, whereas this was only a minor reduction for LE neurons ($F_{2,36} = 19.99$, $P < .01$). To reduce the possibility that repeated mapping by pinch stimulation sensitizes cutaneous input to dorsal horn neurons,[26] subsequent mapping was made only twice more, after high dose of the KOR agonist (3 mg/kg) and after nor-BNI administration.

Local Application of KOR Agonist and Antagonist

A total of 17 TMJ-responsive neurons, classified as NS, were recorded in the superficial laminae at the Vc/C1-2 region of LE (n = 9) and HE (n = 8) rats and tested for effects of U50488 and nor-BNI applied locally at the site of recording. The average recording depths were 101 ± 33 μm and 99 ± 35 μm for LE and HE neurons, respectively. Intra-TMJ injections of ATP evoked prompt increases in total Rmag in HE rats (628 ± 107 spikes/stimulus), which were also greater than the responses of neurons from LE rats (346 ± 49 spikes/stimulus, $F_{1,22} = 6.95$, $P < .05$). Examples of the neuronal responses to ATP injection with increasing doses of U50488 are shown in Fig 5. Local application of U50488 significantly reduced the ATP-evoked Rmag of neurons from HE rats compared to that from LE rats ($F_{3,45} = 20.1$, $P < .001$, Fig 6). Local nor-BNI partially reversed the decrease in Rmag in HE rats, but had no effect on neurons in LE rats. None of the nine neurons from LE rats were reduced (Fig 6a),





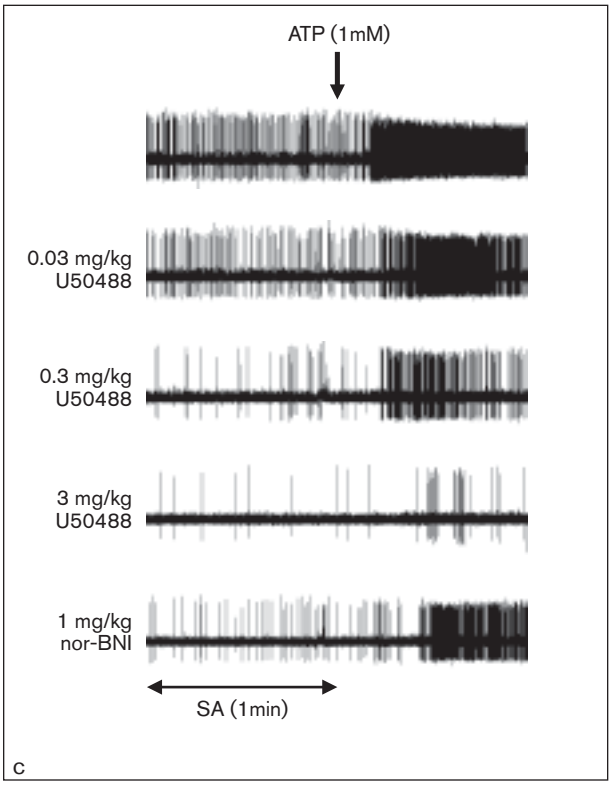
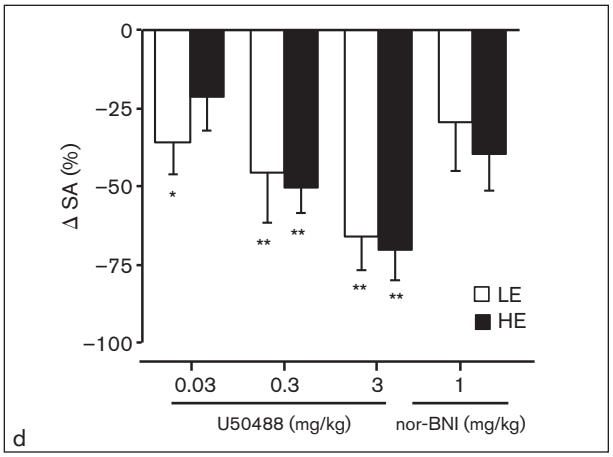


Fig 2 Representative peri-stimulus time histograms of the effects of KOR agonist (U50488) on ATP-evoked responses in TMJ-responsive neurons under **(a)** LE and **(b)** HE conditions. ATP injections were delivered over 30 seconds. Horizontal bars: intra-TMJ injections of ATP (1 mM, 20 µl); 1 bin = 1 second. **(c)** Effect of cumulative doses of systemic U50488 on spontaneous activity (SA) of TMJ-responsive neurons recorded in the superficial laminae and example of change in SA after subsequent administration of nor-BNI. **(d)** Percent change in SA after systemic administration of U50488 and nor-BNI. Sample size = 10 rats per group. **P* < .05 vs before initial ATP stimulus. ***P* < .01 vs before initial ATP stimulus.



but the inhibitory effect of U50488 was noted in six of eight neurons from HE rats displaying a reduction in Rmag of > 50% (Fig 6b). Before drug application, the SA of neurons from LE and HE rats averaged 2.27 ± 0.50 spikes/stimulus and 2.50 ± 0.51 spikes/stimulus, respectively. Local application of U50488 had no significant effect on SA of TMJ-responsive neurons from either group (LE: −44.64% ± 11%, HE: −49.25% ± 17%, $P > .05$). The high-threshold cutaneous RF areas of TMJ neurons in the LE and HE rats averaged 1.5 ± 0.2 cm^2 and 1.5 ± 0.1 cm^2, respectively, before local U50488 application. High dose of U50488 significantly reduced the RF area of HE neurons (–25% ± 4%, $F_{2,30} = 12.4$, $P < .01$), while the RF area of LE neurons was not significantly affected (–7.3% ± 5.9%).

Quantitative PCR

The relative RNA expression levels of KOR (ΔCT values: LE = –12.62 ± 0.59; HE = 12.04 ± 0.57) in Vc/C1-2 tissue samples taken within 1 hour after the second of two daily LE and HE injections showed a 50% higher increase in HE rats, but this difference was not significant.

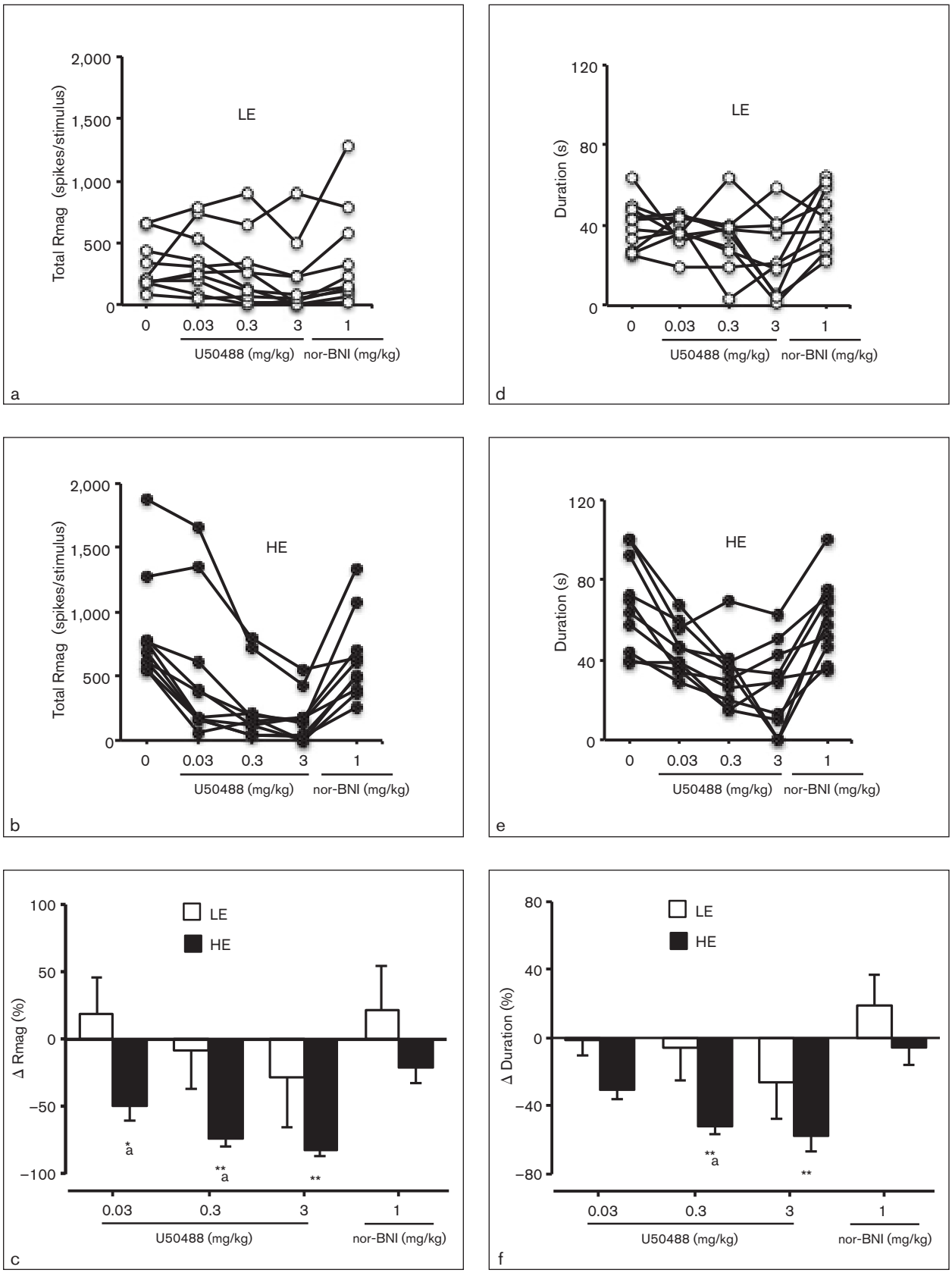


Fig 3 Effect of cumulative doses of KOR agonist on Rmag and response duration of TMJ-responsive neurons recorded in the superficial laminae. Total Rmag and response duration of individual **(a, d)** LE rats and **(b, e)** HE rats before and after systemic administration of U50488 and nor-BNI. Percent change in **(c)** total Rmag and **(f)** duration after systemic administration of U50488 and nor-BNI. Sample size = 10 rats per group. *$P < .05$. **$P < .01$ vs response to initial ATP stimulus. [a]$P < .05$ vs LE group.

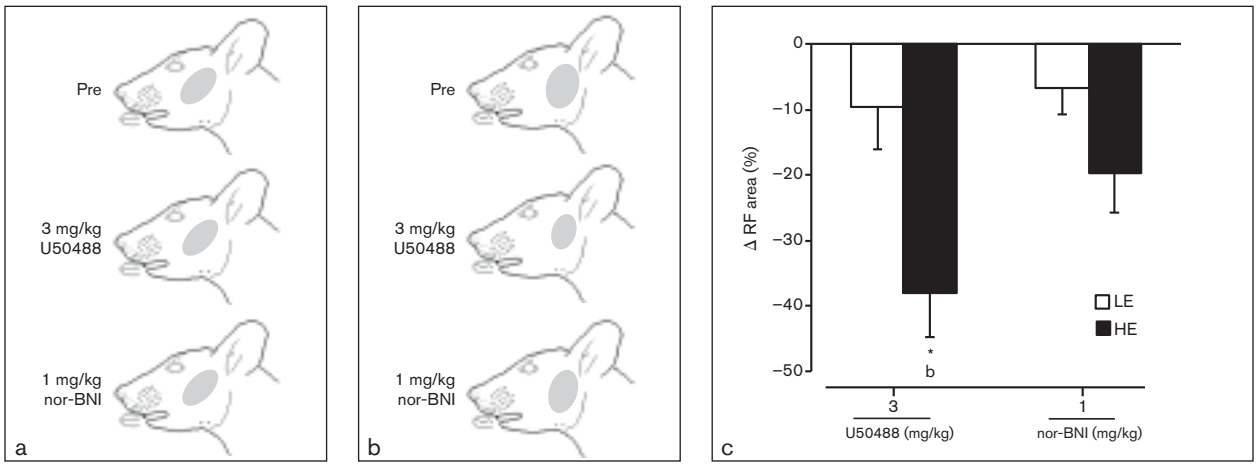


Fig 4 Effect of KOR agonist on cutaneous RF area of TMJ-responsive neurons recorded in the superficial laminae. Example of cutaneous RF of a TMJ-responsive neuron from **(a)** an LE rat and **(b)** an HE rat before and after systemic administration of U50488 and nor-BNI. **(c)** Percent change in cutaneous RF area after systemic administration of U50488 and nor-BNI. Sample size = 10 rats per group. **P* < .01 vs before KOR application.

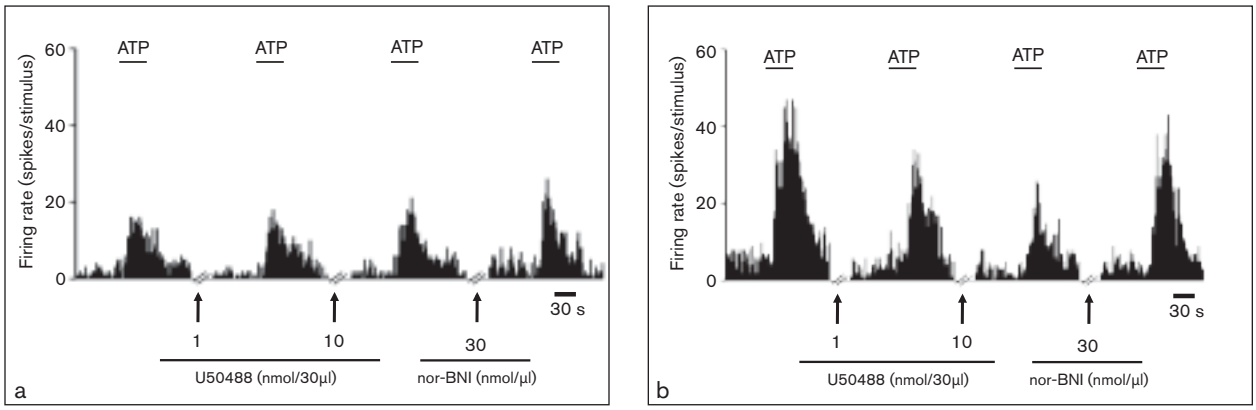


Fig 5 Representative peri-stimulus time histograms of the effects of local application of KOR agonist on ATP-evoked responses in TMJ-responsive neurons under **(a)** LE and **(b)** HE conditions. Horizontal bars: intra-TMJ injections of ATP (1 mM, 20 μl); 1 bin = 1 second.

Discussion

This study has demonstrated, for the first time, that estrogen status exerts a significant influence on KOR activation's modulatory effects on the encoding properties of TMJ-responsive neurons located in the superficial laminae in the Vc/C1-2 region. Systemic administration of KOR agonist U50488 markedly inhibited the Rmag of ATP-evoked activity and the SA in a dose-related manner under HE conditions. However, the fact that SA changes induced by systemic U50488 in TMJ-responsive neurons in LE rats were not paralleled by changes in Rmag suggests that KOR likely acted by more than one mechanism. Systemic U50488 also reduced the cutaneous RF area of neurons in HE rats, but not in LE rats, an effect consistent with a central neural site of interaction between KOR and estrogen status. These effects were at least partially reversed by systemic administration of the KOR antagonist nor-BNI.

To determine whether U50488 acted locally at the Vc/C1-2 region to alter TMJ neuronal activity, drugs were applied on the dorsal brainstem surface directly at the site of recording. Under HE conditions, local application of U50488 significantly reduced ATP-evoked responses and the size of the cutaneous RF, with only minor effects on SA. In contrast, local application of U50488 did not affect TMJ-evoked neuronal activity, SA, or the cutaneous RF area under LE conditions. Collectively, these data suggest that KOR activation acted through multiple pathways to influence TMJ nociception and that estrogen status played a role in gating this influence at the level of the Vc/C1-2 region.

KOR expression is widely distributed throughout the central nervous system (CNS), with significant levels found in regions associated with pain processing.[27,28] In the trigeminal system, KOR is expressed in 20% to 40% of trigeminal ganglion neurons,[29] while in the caudal trigeminal brainstem, KOR protein levels

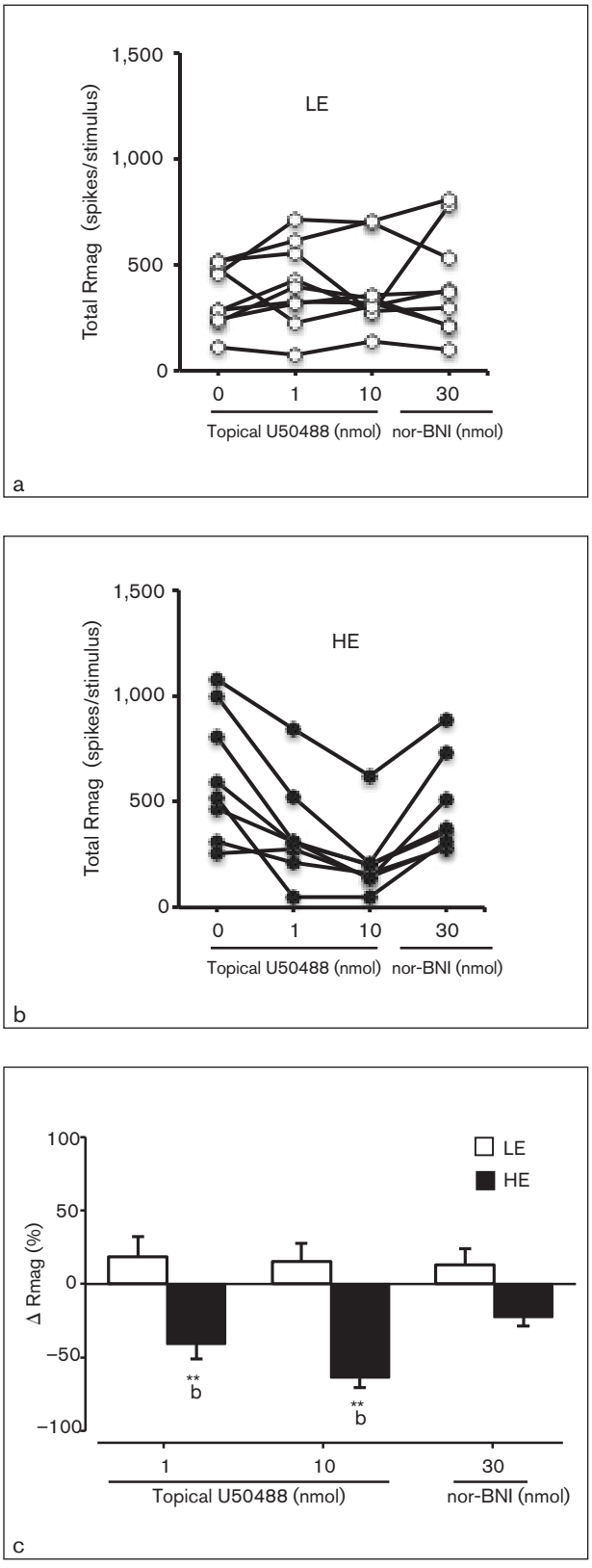


Fig 6 Effect of local application of KOR agonist on ATP-evoked responses in TMJ-responsive neurons recorded in the superficial laminae. **(a)** Total Rmag of individual LE rat. **(b)** Total Rmag of individual HE rat. **(c)** Percent change in total Rmag after U50488 and nor-BNI locally. Sample sizes: LE = 9; HE = 8. ***P* < .01 vs response to initial ATP stimulus. [b]*P* < .01 vs LE group.

display moderate to high levels.[13] The Vc/C1-2 region is critical to TMJ nociceptive processing. TMJ and associated masticatory muscle afferent fibers[14,15] terminate in this region. KOR expression is high in the superficial laminae at the Vc/C1-2[13] and overlaps with that of estrogen receptor alpha (ERα).[16,17] In the spinal dorsal horn, dynorphin (an endogenous KOR agonist) and ERα are colocalized in superficial laminae neurons; this suggests a close relationship between estrogen status and KOR effects on sensory processing.[30] The present study found that U50488 reduced ATP-evoked neuronal activity and the size of the neuronal cutaneous RF in HE rats after systemic administration or local application at the site of recording. In contrast, U50488 failed to cause a significant reduction in either the ATP-evoked Rmag or the cutaneous RF area in LE rats, regardless of the route of administration. These data suggest that local KOR activation contributes substantially to the effects of KOR on TMJ nociceptive neurons at the Vc/C1-2 region, and that estrogen status plays a significant role in determining the magnitude of the effects of KOR. These data do not exclude the possibility that KOR activation in other brain regions could have contributed to the modulation of the encoding properties of TMJ-responsive neurons, and that estrogen status could have affected these pathways as well. Indeed, the SA of TMJ neurons was significantly reduced after systemic, but not local, administration of U50488 in both HE and LE animals, consistent with the notion that KOR-dependent pathways in other brain regions affect the ongoing activity of TMJ-responsive neurons. Also, the effects of KOR activation in the rostral ventromedial medulla on nociceptive behavior display marked sex differences.[31]

The relationship between sex differences, estrogen status, and κ-opioid analgesia remains controversial. Earlier human studies suggested that women were more sensitive than men to analgesic drugs with κ-opioid activity for reducing postoperative pain after tooth extraction.[11] In animal studies, nociceptive behavior evoked by intra-TMJ administration of formalin was reduced by local injection of U50488 in awake male and cycling female rats, with the greatest effect occurring in diestrous females.[12] However, recent studies have suggested that the influence of κ-opioid analgesics on nociception is dependent on the pain model used.[32] The local doses of U50488 used in the present study (1 and 10 nmol) were less than the intrathecal doses used by Lawson et al[22] and similar to those injected directly into the TMJ by Clemente et al.[12] In a previous study, systemic doses of U50488 (3 and 7 mg/kg) greatly reduced the number of Fos-positive neurons produced at the Vc/C1-2 region after intra-TMJ injection of mustard oil in proestrous females and had only minor effects in

diestrous females and males.[25] In the lumbar spinal dorsal horn, systemic administration of U50488 (1–16 mg/kg) caused a dose-dependent inhibition of thermally and mechanically evoked responses of superficial laminae neurons in spinalized male rats,[33] whereas local spinal administration of high doses of U50488 (0.19–1.9 μmol) caused an expansion of the RF area and caused either inhibition or facilitation of thermal and mechanical inputs, depending on the dose.[34]

The mechanisms that underlie estrogen-KOR interactions in TMJ nociceptive processing are likely complex and may involve peripheral as well as CNS sites of action. However, several lines of evidence suggest that CNS mechanisms are critical for this interaction. First, both estrogen status and KOR activation can greatly modify the size of the cutaneous RF area of superficial laminae neurons in the Vc/C1-2 region. It is well accepted that changes in size of the cutaneous RF area of spinal neurons depend almost exclusively on CNS mechanisms.[25,34] Second, although KOR immunoreactivity is seen on presynaptic axon terminals and postsynaptic neurons of the spinal dorsal horn[35–37] in spinal cord slice preparations with attached dorsal roots, blockade of postsynaptic action prevented KOR-induced modulation of C-fiber input to substantia gelatinosa neurons.[38] Third, KOR expression has been reported in trigeminal ganglion neurons, and local application of U50488 at the Vc/C1-2 region could have acted presynaptically to prevent TMJ-evoked responses; however, a decrease in SA of TMJ-responsive neurons was seen in both HE and LE animals, and so a presynaptic action unlikely explains the differential effects on evoked activity. However, since rats were given the surgical preparation, it is possible that there could have been SA peripherally driven by nociceptive inputs, upon which the drug caused a decrease in activity.

One possible explanation for increased responsiveness to U50488 in HE rats was due to an estrogen-induced increase in KOR expression. Previous studies reported that E2 treatment or elevated E2 levels in proestrous females were associated with increased expression of KOR in the rat spinal cord.[24] The present study found a 50% increase in the RNA expression of KOR in Vc/C1-2 tissue samples from HE rats compared to those from LE rats; however, this did not reach statistical significance. The expression of KOR may increase through KOR translocation to the cell membrane independently of any change in the RNA expression of KOR. It is not yet known if KOR protein levels at the Vc/C1-2 junction were altered by estrogen status. Although other studies have reported a decrease in KOR protein levels in trigeminal ganglia of proestrous compared to diestrous female rats, these animals received intra-TMJ injections of formalin 1 hour prior to tissue collection.[39] A second possible mechanism for enhanced responsiveness to U50488 in HE rats could have involved E2-induced expression of KOR/μ-opioid receptor (KOR/MOR) heterodimers.[40] Earlier studies found that nearly 50% of ER-positive neurons in the superficial laminae at the Vc/C1-2 region also expressed preproenkephalin.[16] Thus, it is possible the estrogen conditions that enhance the responsiveness to KOR agonists also enhance the secretion of other endogenous opioids, and together an increased formation of heterodimers leads to altered sensitivity to opioid analgesics.

Conclusions

Estrogen status is a risk factor for several chronic craniofacial pain conditions, including migraine and TMD.[1] Although estrogen modulation of trigeminal nociception may involve multiple pathways,[6,41,42] the present results suggest that estrogen-dependent KOR modulation of TMJ-responsive neurons occurs at the earliest stages of sensory processing at the Vc/C1-2 region.

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