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Pain condition and sex differences in the descending noradrenergic system following lateral hypothalamic stimulation

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ABSTRACT

The lateral hypothalamus (LH) is known to modulate nociception via the descending noradrenergic system in acute nociception, but less is known about its role in neuropathic pain states. In naïve females, LH stimulation produces opposing effects of α-adrenoceptors, with α_2-adrenoceptors mediating antinociception, while pronociceptive α_1-adrenoceptors attenuate the effect. Whether this opposing response is seen in neuropathic conditions or in naïve males is unknown. We used a mixed factorial design to compare male and female rats with chronic constriction injury (CCI) to naïve rats, measured by Total Paw Withdrawal (TPW) responses to a thermal stimulus. Rats received one of three doses of carbachol to stimulate the LH followed by intrathecal injection of either an α_1- or an α_2-adrenoceptor antagonist (WB4101 or yohimbine, resp.) or saline for control. Overall, naïve rats showed a more pronounced opposing alpha-adrenergic response than CCI rats ($p < 0.04$). Naïve male and female rats demonstrated antinociception following α_1-adrenoceptor blockade and hyperalgesia following α_2-adrenoceptor blockade. Male CCI rats also showed dose dependent effects from either WB4101 or yohimbine ($p < 0.05$), while female CCI rats had significant antinociception from WB4101 ($p < 0.05$), but no effect from yohimbine. These results support the idea that peripheral nerve damage differentially alters the descending noradrenergic modulatory system in male and female rats, and notably, that female CCI rats do not show antinociception from descending noradrenergic input. These findings are suggestive that clinical therapies that recruit the descending noradrenergic system may require a different approach based on patient gender.

Introduction

The lateral hypothalamus (LH) plays an important role in descending modulation in acute nociception (Holden et al., 2001, 2009; Esmaeili et al., 2016), persistent inflammation (Jeong and Holden, 2009; Ezzatpanah et al., 2015; Jahangirvand etal., 2016; Yazdi et al., 2016), and hyperalgesia from nerve injury (Holden et al., 2014; Wardach et al., 2016). The LH modifies nociception in part through connections with the A7 catecholamine cell group, a group of spinally descending noradrenergic neurons, as shown by anatomical study (Clark and Proudfit, 1991) and behavioral studies (Yeomans et al., 1992; Yeomans and Proudfit, 1992; Holden and Proudfit, 1998; Holden et al., 1999; Nuseir and Proudfit, 2000). The LH sends projections to the A7 cell group (Leite-Almeida et al., 2006; Holden et al., 2018) and some of these projections likely contain substance P (Holden et al., 2002).

We used two models of pain to determine the role of the LH in descending modulation. Naïve rats model acute nociception and rats with chronic constriction injury (CCI) model hyperalgesia seen in neuropathic pain (Bennett and Xie, 1988; Attal et al., 1990; Kim et al., 1997). We found that stimulating the LH with the cholinergic agonist carbachol produces antinociception in both naïve and CCI rats, although pain condition and sex differences occur (Holden et al., 2014). We have also shown that, in naïve females, antinociception from LH stimulation occurs in part from norepinephrine acting at α_2-adrenoceptors in the spinal cord dorsal horn, but that concurrent pronociception also occurs from norepinephrine acting at α_1-adrenoceptors (Holden and Naleway, 2001; Jeong and Holden, 2009). This concurrent pronociception likely dampens the antinociceptive effect. We do not know whether this opposing effect occurs in naïve males, or in males and females with chronic pain conditions.

We conducted whole animal experiments to examine the role of the LH in alpha-adrenergic descending modulation of nociception in male and female rats in models of acute nociception and hyperalgesia. We stimulated the LH with one of three doses of carbachol followed by

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intrathecal (IT) injection of α -antagonists either in naïve rats, or in rats with CCI. Both male and female rats were used. A thermal stimulus was applied to the left hind paw and time to paw withdrawal (TPW) was measured.

Experimental procedures

The Institutional Animal Care Committee at the University of Michigan approved the experimental protocol used in this study. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to minimize animal suffering, reduce the numbers of animals used, and use alternatives to in vivo experiments.

Animals

Male and female Sprague-Dawley rats (275–400 g; Charles River, Portage, MI, USA) were used in the study. All rats were maintained on a 12 -h day/night schedule with free access to food and water. To reduce the risk of mirror image effects on the non-ligated paw in the CCI protocol (Kim and Chung, 1997), we used separate control animals rather than having each animal serve as its own control. The numbers of rats per group ranged from 9-15. Variability in group numbers was due to misplacement of either the cerebral microinjector cannula or the IT cannula. 524 rats were used in the analyses, and each rat was used only once.

All rats were lightly anesthetized for the experiments and received one of three doses of carbachol microinjection in the LH. These microinjections were followed immediately by IT injection of either an α_1 –antagonist, an α_2 –antagonist, or saline for control. The groups consisted of naïve males, naïve control males, naïve females, naïve control females, CCI males, CCI control males, CCI females, and CCI control females. The control rats all received LH stimulation, but were given saline IT.

CCI. procedure

This procedure has been outlined in detail elsewhere (Holden et al., 2014). Briefly, each rat was anesthetized with isoflurane, and the left common sciatic nerve exposed at mid-thigh level. Four ligatures (4.0 chromic gut) were tied loosely around the nerve about 1 mm apart and standardized by observing the initial twitching of the paw as the ligature was tightened. The muscle was sutured with 4.0 chromic gut and the incision closed with wound clips. Each rat received a subcutaneous injection of buprenorphine (0.3 mg/ml) at a dose of 0.05 mg/kg, recovered and returned to its cage. Fourteen days elapsed between CCI ligation and the conduct of the experiments to allow for development of hyperalgesia, based on the protocol of Bennett and Xie (1988).

Analgesiometric testing procedures

To determine the effect of LH stimulation and IT antagonist application on thermal nociception, the paw withdrawal test was used. In this procedure, the hairy surface of the left hind paw was exposed to a focused beam of radiant heat using an analgesiometer (37360, Ugo Basile, Italy). The radiant beam was adjusted to a maximum intensity of 145 mW/cm^2 and the time interval between the onset of skin heating and the withdrawal response was measured electronically. In the absence of a response, skin heating was terminated after 15 s to prevent burning. The longer the TPW, the greater the antinociceptive effect. Baseline response latencies were approximately 6–8 s. Temperature was measured with a rectal probe at baseline and at 50 min. Heart rate, blood pressure, and mean arterial pressure were measured pre-injection and following the final latency measurement using a tail cuff and Coda monitor (Kent Scientific; Torrington, CT, USA).

Carbachol microinjection in the LH followed by microinjection of IT antagonists

Male and female Sprague-Dawley rats were randomly assigned to groups. Each was prepared for microinjection in the LH as follows: each rat was lightly anesthetized with sodium pentobarbital (35 mg/kg, IP) and the scalp and dorsal neck infused with 1% lidocaine (0.15 ml). If the rat vocalized or moved without stimulation, supplemental pentobarbital could be given, but was rarely required.

In preparation for IT drug delivery, a 32 gauge intrathecal catheter constructed from PE-10 polyethylene tubing (Micor, Allison Park, PA) was inserted through an incision in the cisterna magna and the tip positioned over the lumbar enlargement. IT drugs were dissolved in physiological saline and filtered through a 0.2-μm filter immediately before injection. The drug was then injected in two consecutive 15 μl doses using an electronic syringe pump at a rate of 30 μl/min.

The rat was then placed in a stereotactic apparatus in preparation for LH stimulation. A 2-cm incision was made through the scalp, and the muscle and fascia retracted. A 23-gauge stainless steel guide cannula was lowered through a burr hole into the region of the left LH defined by the following stereotactic coordinates: AP −1.5 mm from bregma, lateral +1.6 mm, vertical +2.2 mm, incisor bar set at −2.5 mm. A 30-gauge stainless steel injection cannula was connected to a 10 μl syringe by a length of PE-10 polyethylene tubing filled with either saline or a solution of carbachol of either 125, 250 or 500 nmol in normal saline injected in a volume of 0.5 μl (Sigma Chemical Co., St. Louis, MO). All solutions were made fresh daily and filtered through a 0.2 μm syringe prior to use. The injection cannula was then inserted and extended approximately 3 mm beyond the end of the guide cannula.

Following a baseline measurement, one of three carbachol doses (125, 250, or 500 nmol in a volume of 0.05 μl; Holden et al., 2014) was then microinjected into the LH and TPW determined at 1, 5, and 10 min post microinjection. At 11 min post-microinjection, either the α_1-adrenoceptor antagonist WB4101 (37 μg, 97 nmol/ 15 μl; Sigma), the α_2-adrenoceptor antagonist yohimbine (38 μg, 97 nmol/15 μl; Sigma) or saline for control was then injected IT (Holden and Naleway, 2001). TPWs were measured at one min post IT injection and then every five minutes for 45 min.

IT antagonist injection only

In a separate experiment, each group of rats was given IT injections of either yohimbine, WB4101 or saline for control without LH stimulation. This experiment was done to determine whether there was tonic noradrenergic activity at either α_1- or α_2-adrenoceptors and to assure that the effects of the previous experiment were not due simply to the presence of a cannula in the IT space. The IT cannula was placed in the IT spaced as described earlier and a baseline left paw withdrawal measurement was taken. One minute later the antagonist or saline was injected into the IT space, then paw withdrawal measurements were taken it 1 min post injection, then every 5 min for 45 min.

Microinjector and IT cannula placement verification and histology

Following testing, animals were overdosed with sodium pentobarbital and decapitated. The brains were taken and drop fixed in a solution of 10% neutral-buffered formalin. To determine the position of the microinjection sites relative to the LH, 40 μm transverse brain sections were cut from blocks of tissue that contained the visible injection cannula tract using a cryostat microtome, stained and cover slipped on slides. The placement of the microinjection cannula was determined by locating the most ventral position of the cannula tip in serial sections by bright field microscopy. Tracings of the appropriate sections were then made using the Neurolucida imaging system (Microbrightfield, Colchester, VT). The tracings were compared with drawings from the atlas of Paxinos and Watson (2009) to verify that the

cannula was within the LH. Data from cannula placements outside the LH were omitted from analysis.

For IT placement, data from cases in which the cannula was located in the ventral aspect of the subdural space, or in the spinal cord itself, were excluded from analysis.

Statistical analysis

Statistical analyses were performed using Stata, IC software (v 15.0, StataCorp LP, College Station, TX) setting 2-tailed alpha to reject the null hypothesis at 0.05. This is a 2 (sex) x 3 (IT drug) x 3(carbachol dose) x 2(naïve, CCI) x 10 (time) mixed factorial design with $n = 9–15$ animals randomized to each cell in the experimental design. These data were right-censored at 15 s, the maximum amount of exposure time allowed during this experiment. The data were square root transformed to meet the normality assumption of these statistical methods.

We submitted the root-transformed, right-censored TPW data to a fully factorialized Tobit regression model (Tobin, 1958) with fixed parameters evaluating all main effects and interaction effects, including Pain Condition (naive, CCI) x Sex (male, female) x Drug (Saline, WB, Yohimbine) x Carbachol Dose (125, 250, 500 mg) x Time (minutes 1, 5, 10, 15, 20, 25, 30, 35, 40, 45) experimental design. We accommodated for the repeated observations over time by incorporating cluster-adjusted standard errors (Rogers, 1993). Our model also included each animal's maximum observed carbachol-induced (root-transformed) TPW observed prior to the start of the experimental protocol as a covariate in order to adjust for differences observed in responses to carbachol alone (Holden et al., 2014). We adjusted for inflated Type I alpha risk in these pairwise comparisons using Benjamini & Hochberg's step-up false discovery rate (FDR) adjustment method, accepting a 10% FDR (Benjamini and Hochberg, 1995).

Results

Microinjector placement

Fig. 1 is a representation of microinjector placements for female (A) and male (B) CCI rats given 500 nmol of carbachol in the LH, the dose that was most effective across groups (data not shown). Placements were similar for naïve rats. Most of the injection sites occurred within the LH as described by Paxinos and Watson (2009). Data from rats with microinjector sites outside the LH were omitted. There were no significant differences among groups for heart rate, mean arterial pressure, blood pressure, or temperature.

Analysis of overall model

Our overall Tobit model revealed a significant 5-way interaction effect on TPW ($F_{(36,\ 3831)} = 1.47$; $p < .04$), suggesting that nociceptive condition (naïve or CCI), intrathecal drug (saline, yohimbine or WB4101), carbachol dose (125, 250, 500), and sex (male, female) all interacted to explain differences observed over time. Therefore, we held nociceptive condition constant in order to evaluate the simpler interactions and main effects of drug, dose, and sex on differences over time for both CCI and naïve nociceptive types.

Effects of LH stimulation on alpha-adrenergic activity in naïve rats

We found no significant differences between male and female rats for carbachol dose or IT drug effects, so we combined them for the analysis. Our analysis of TPW in naïve animals revealed a significant Dose by Time interaction effect ($F_{(18,\ 1943)} = 1.98$; $p < .01$) and a Drug by Time effect ($F_{(18,\ 1943)} = 5.34$; $p < .01$, see Fig. 2). Per Fig. 2, it is clear that blocking α_1-adrenoceptors with WB4101 elicited immediate antinociception, indicating that α_1-adrenoceptors promoted nociception. We also observed faster withdrawal responses in rats given the α_2-adrenoceptor antagonist, yohimbine, compared to the control (saline) condition, indicating that α_2-adrenoceptors mediated antinociception. Follow-up pairwise comparisons between WB4101 and saline revealed significant differences after FDR adjustments including those at minutes 1 through 35, and at 45 min FDR-adjusted significant differences were also observed between yohimbine and saline at minutes 10 through 45

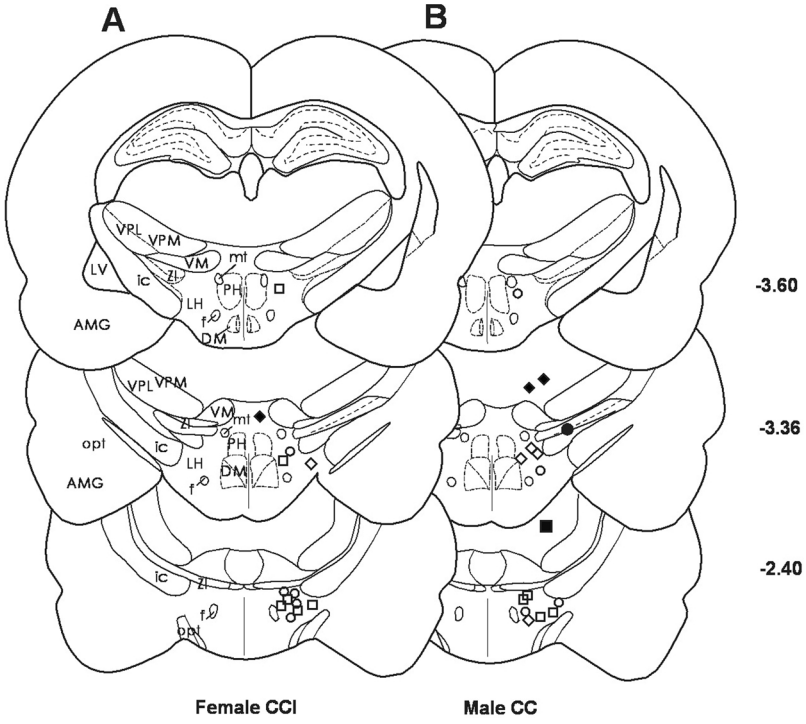

Fig. 1. Representative locations of microinjection sites in the LH for TPW for the 500 nmol dose of carbachol in female (A) and male (B) CCI rats. Most of the microinjection sites were located within the border of the LH between AP -2.40 and -3.60. The symbols represent the peak time of carbachol effectiveness at 10 min post microinjection. Symbols for TPW after microinjection of carbachol are as follows: (◇) 1–5 s; (○) 6–10 s; (□) 11–15 s. Data from injection sites located outside of the LH, as shown by solid symbols (♦,●,■), were excluded from the analysis. AMG, amygdala; DM, dorsomedial hypothalamus; ic, internal capsule; LH, lateral hypothalamus; LV, lateral ventricle; mt, mammillothalamic tract; opt, optic tract; PH, posterior hypothalamus; VM, ventromedial thalamic nucleus; VPL ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; ZI, zona incerta.

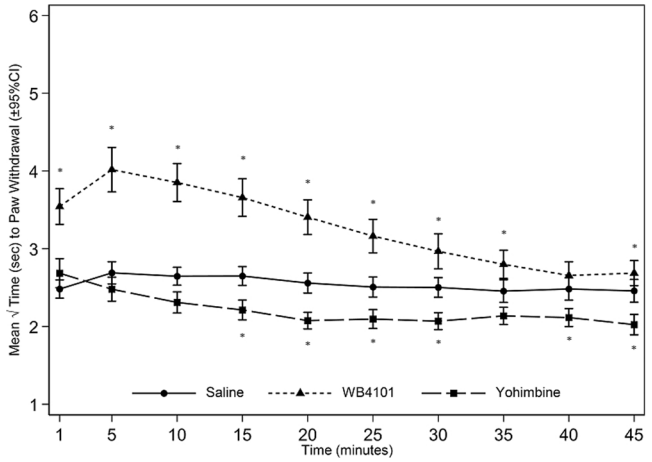

Fig. 2. Drug by Time Interaction Effect in Naïve Rats. The combined effect of IT alpha-adrenoceptor antagonists on TPW in naïve rats. Following a baseline response latency, one of three doses of carbachol was microinjected into the LH. Three paw withdrawal latencies were taken (not shown for clarity). At time 0, normal saline (circles), WB4101 (triangles), or yohimbine (squares) was then microinjected into the IT space at time 0 and the results are shown here. No differences were found for sex or carbachol dose. Male and female rats all showed significant antinociception versus control after IT WB4101 ($p < 0.01$). Yohimbine produced hyperalgesia in all groups. Asterisk (*) identifies pairwise differences that were significant using 10% False Discovery Rate adjustments across all pairwise comparisons.

Effects of LH stimulation on alpha-adrenergic activity in CCI rats

Our analysis of TPW in animals exposed to the CCI condition was more complicated, and showed a significant 4-way interaction involving Intrathecal Drug (saline, yohimbine, WB4101), Sex (male, female), Carbachol Dose (125, 250, 500) and Time ($F_{(36,1886)} = 1.92$; $p < 0.01$). To understand these effects fully, we held sex constant and examined the effects of Drug and Dose on changes over Time. Both of these simplified models revealed significant 3-way interaction effects of Drug x Dose x Time ($F_{(36, 924)} = 2.54$; $p < .01$ for females, $F_{(36, 962)} = 1.62$; $p < .05$ for males), justifying follow-on analyses within drug dose. Fig. 3 illustrates these effects, where the differences between WB4101 blockade of α_1-adrenoceptors and saline for females are fairly large, with the 500-nmol dose of carbachol producing the greatest effect. This finding is in contrast to the effects of WB4101 relative to saline for males, where the effects of WB4101 were somewhat muted. These findings indicate that the pronociceptive α_1-adrenoceptors were generally more active following LH stimulation in CCI females than in males.

Regarding the effects of yohimbine versus saline, we saw a dose-dependent effect among males, with the blockade of α_2-adrenoceptors by yohimbine producing the most consistent hyperalgesic effect following the 500-nmol dose of carbachol (Fig. 3). This effect is contrasted to female responses, in which there was essentially no significant difference with yohimbine compared to control regardless of carbachol dose (Fig. 3). These findings show that male CCI rats experienced antinociception from α_2-adrenoceptor involvement, but female CCI rats did not.

Effects of IT antagonist injection only

Analysis of TPW following IT only application of WB4101, yohimbine, or saline for control demonstrated a 4-way interaction among Sex (female, male), Pain Condition (naïve, CCI), IT drug (WB4101, yohimbine, or saline), and Time ($F_{(18, 900)} = 8.33$; $p < 0.0001$), justifying follow-on analyses within Pain Condition. Focusing naïve rat data, we found a 3-way interaction effect ($F_{(18, 485)} = 1.74$; $p < 0.05$) that showed that males and females differed across time with respect to IT drug. WB4101 producing significantly longer TPW than saline in males compared to females, while TPW for yohimbine did not differ statistically from saline control in either sex (data not shown).

Our three-way analysis focusing on CCI data for female and male rats also revealed a significant 3-way interaction effect ($F_{(18, 416)} = 7.63$; $p < 0.0001$). However, with the CCI data, both females and males demonstrated significantly longer TPW following blockade of α_1-adrenoceptors with WB4101, while neither females nor males demonstrated any significant differences between yohimbine and saline (data not shown). These findings replicate those reported from earlier work (Wagner et al., 2016).

Discussion

In the present study, we examined the role of the LH in modulating two pain conditions, naïve vs. CCI, via the descending noradrenergic system in male and female rats. We observed several key findings related to pain condition and sex differences in activity of alpha-adrenoceptors in CCI rats.

The first important finding was that responses of rats in the naïve condition were different from those of the CCI condition. This finding supports the idea that peripheral nerve damage can alter the role of descending modulatory input (Maier et al., 2010; Pergolizzi et al., 2013; Pfau et al., 2014). For example, CCI differentially upregulates α_1-adrenoceptor subtypes A, B, and D and α_2-adrenoceptor subtype A in the dorsal root ganglia (Cheng et al., 2014). Spinal nerve ligation produces upregulation of norepinephrine transporters (Rojo et al., 2012), and spinal cord impact injury can remodel the dendritic spines of lamina II neurons, which may play a role in circuit function of the dendrites and promote central sensitization (Cao et al., 2017; Tan, 2015; Mills et al., 2018).

The second important finding was a difference in the responses of naïve rats to IT alpha antagonists compared to those of CCI rats. Both male and female naïve rats showed the opposing effects of α_1- and α_2-adrenoceptors similar to those we have reported previously in females (Holden and Naleway, 2001; Jeong and Holden, 2009), and that others have reported for male rats with α_2-adrenoceptor involvement with post-operative pain (Leonard et al., 2016). In the naïve model, stimulation of the LH produces antinociception in both male and female rats, albeit with carbachol dose differences (Holden et al., 2014), indicating that the net effect of opposing alpha-adrenoceptor involvement following an acute thermal stimulus is antinociception.

Such a clear opposing action was not seen in rats with the CCI condition. As expected, α_1-adrenoceptor involvement was seen in varying levels with all three doses of carbachol in both male and female CCI rats, although generally speaking, females had a greater response to α_1-adrenoceptor blockade than males, especially at the 500-nmol carbachol dose (Fig. 3). We have shown that α_1-, but not α_2-adrenoceptors are tonically active in both CCI males and females, with the CCI rats showing significantly greater responses than the naïve rats (Wagner et al., 2016). Findings from the present study, in which we gave only IT antagonists, support these earlier findings of tonic α_1-adrenoceptor activity. Furthermore, following denervation, both α_1- and α_2-adrenoceptor densities increase in the spinal cord of male rats (Roudet et al., 1993, 1994) and mRNA for the α_{1B}-adrenoceptor subtype is up-regulated in dorsal root ganglia after spinal nerve ligation in rat (Xie et al., 2001). Taken together, these findings are suggestive that, following nerve injury, pronociceptive α_1-adrenoceptors upregulate significantly, and may mediate some aspects of chronic pain from nerve injury, especially in female rats.

Contrary to our findings, α_1-adrenoceptors are antinociceptive in histamine-induced hypersensitivity following spinal nerve ligation (Wei et al., 2014), and in nitrous oxide-induced antinociception (Orii et al., 2002). The reasons for this contradiction are unclear, but likely are the result of different research models.

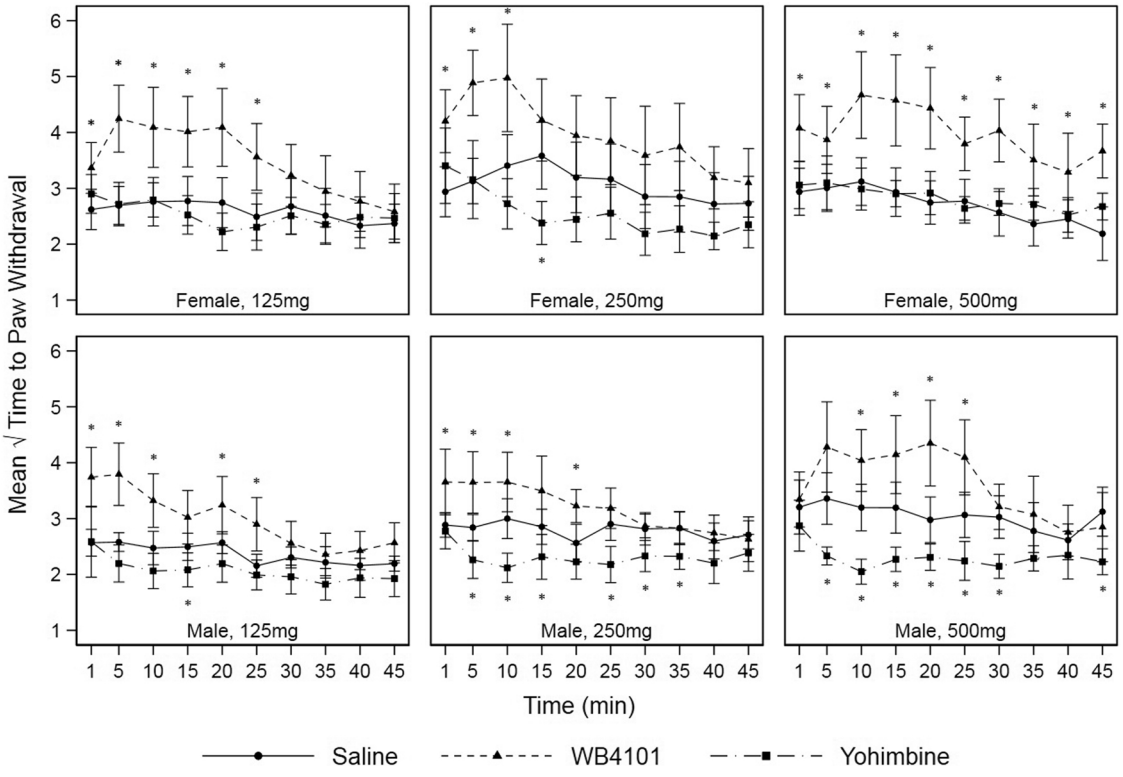

Fig. 3. Drug by Dose by Sex by Time Effects in CCI Rats. Carbachol in the LH produced significant sex and dose differences in CCI following IT administration of α–adrenergic antagonists ($p < 0.01$). Depending on dose and sex, WB4101 (triangles) produced antinociception, while yohimbine (squares) produced hyperalgesia, as compared to control (circles). Asterisk (*) identifies pairwise differences that were significant using 10% False Discovery Rate adjustments across all pairwise comparisons.

Unlike the responses of α_1-adrenoceptor blockade with WB4101, the responses to yohimbine were not the same. In male CCI rats, α_2-adrenoceptor blockade produced a dose dependent response to carbachol stimulation of the LH, with the highest dose of carbachol producing the optimal response. This finding is similar to those seen in males in models of chemotherapy-induced neuropathic pain (Nie et al., 2017), latent sensitization (Walwyn et al., 2016), spinal nerve ligation (Aira et al., 2015), and CCI (Nakamura et al., 2014). These results are suggestive that LH stimulation in male nerve injured rats produces antinociception through α_2-adrenoceptor activity that is stronger than the pronociceptive effects of α_1-adrenoceptors.

An unexpected finding was that female CCI rats had essentially no response to α_2-adrenoceptor blockade, indicating that antinociception mediated by α_2-adrenoceptors did not occur in these nerve-injured females. While α_2-adrenoceptor-mediated antinociception was seen in female rats following crush injury (Obradovic et al., 2015), it was not found following CCI in female Wistar rats (Samur et al., 2018), a model similar to that of the present study. Some have suggested that estrogen attenuates the antinociceptive effects of α_2-adrenoceptors in acute and inflammatory pain models (Nag and Mokha, 2016) and it is reasonable to assume such may be the case in CCI females. This may be so, but the issue is not straightforward. We obtained α_2-mediated antinociception in females in both acute and persistent inflammatory pain (Holden and Naleway, 2001; Jeong and Holden, 2009). And while we did not measure estrogen effects in the current study, we found no evidence of estrogen hormonal effects in previous work that included CCI females (Holden et al., 2014). In this latter experiment, the groups were small and the aim was not to test sex hormone effects per se, so we cannot completely rule out the effects of estrogen on CCI females. However, the present study demonstrated distinct differences in female CCI rats compared to male CCI and naïve male and female rats, with CCI females showing greater activity of pronociceptive α_1-adrenoceptors with no opposing effect of antinociceptive α_2-adrenoceptors. The logical conclusion from these observations is that females with nerve injury might well have less endogenous pain relief from the descending noradrenergic system.

In the present study, we used naïve rats rather than sham surgery rats. We chose naïve rats because in our previous study, we demonstrated that some male sham surgery rats were hyperalgesic (Holden et al., 2014). Others have also reported some hyperalgesic effects following incisional pain, in part from hypoxia and acidosis at the incision site (Pogatzki et al., 2002; Woo et al., 2004; Kim et al., 1997; Kang et al., 2013). However, we showed a significant difference in hyperalgesia between CCI rats and sham controls in both male and female groups. Therefore, we can conclude that the CCI rats used in the present study were hyperalgesic due to nerve damage. Furthermore, because we obtained analgesia on rats with no incision, it is lilkely that sham rats would also demonstrate some antinociceptive effect. Our aim was to compare the hyperalgesia of neuropathic pain to nociceptive pain without the potential confounding issue of incisional pain.

We also acknowledge the limitations of microinjector studies. Our microinjections of 0.5 μl tend to limit the average spread the injection (Myers and Hoch, 1978; Martin, 1991). It has also been noted that the amount of available solution decreases the farther it is from the injection site (Grossman and Stumpf, 1969; Myers and Hoch, 1978), and it is reasonable to assume that diffusion of solution does not necessarily translate to neuronal activation. For example, we have shown that microinjections in the ventral thalamus just dorsal to the LH (Fig. 1A; Holden and Pizzi, 2008) and in the internal capsule adjacent to the LH (Holden et al., 2002; 2005) produce withdrawal latencies similar to baseline. Finally, we showed previously that 62 nmol of carbachol microinjected into the LH produce withdrawal latencies similar to baseline latencies (Holden and Naleway, 2001). Given these factors, the likelihood that our findings occurred as a result of stimulating neurons outside the LH is small.

The importance of the findings from the current study are three-

fold. First, the idea of sex differences in nociceptive responses in animals as well as humans remains controversial. While a systematic literature review of pain in humans could not show a consistent pattern of pain response differences based on gender in humans (Racine et al., 2012), a review of laboratory pain sensitivity in humans demonstrated markedly increased sensitivity to pain in females compared to males (Mogil, 2012). While rat physiology may not always translate to human responses, the findings of the current study, which support the notion of increased nociceptive sensitivity of CCI female rats, indicate that sex differences deserve further investigation in clinical subjects.

The second finding relates to deep brain stimulation, which is being used for chronic pain management for cluster headaches (Cappon et al., 2019; Vukovic Cvetkovic and Jensen (2019), cervical dystonia (Ravindran et al., 2019), and in a limited manner, in phantom limb pain (Corbett et al., 2018). Our findings are suggestive that deep brain stimulation in the LH could be effective for some types of pain management and that the type of pain, stimulation dose, and sex differences are important considerations.

The third finding relates to clinical drug therapy. The serotonin and norepinephrine reptake inhibitors (SNRIs) are first-line treatments for clinical treatment of chronic pain (Hayashida and Obata, 2019). For example, the SNRI, duloxetine, is recommended for treating chemotherapy-induced neuropathic pain, but many patients still experience poorly controlled pain and impaired quality of life (Smith et al., 2013). Duloxetine works in part by blocking norepinephrine reuptake in the spinal cord dorsal horn, which keeps the neurotransmitter in the synaptic cleft longer, thereby increasing the probability that norepinephrine binds to α_2-adrenoceptors that produce analgesia (Hoshino et al., 2015). However, our findings indicate that norepinephrine will also bind to pronociceptive α_1-adrenoceptors and this binding may be part of the reason why duloxetine is not effective in all patients. Further study is warranted to see if interventions can decrease the pronociceptive effect of α_1-adrenoceptors while increasing the antinociceptive effects of α_2-adrenoceptors and to determine whether sex differences occur in human patients based on α_1- vs α_2-adrenoceptor activity.

In summary, we now know the following: 1. Male and female naïve and CCI rats demonstrate antinociception from LH stimulation (Holden et al., 2014; Ezzatpanah et al., 2015; Esmaeili et al., 2016; Yazdi et al., 2016); 2. The opposing effects of pronociceptive α_1- and antinociceptive α_2-adrenoceptors occur in naïve females (Holden land Naleway, 2001) and in females with persistent inflammatory pain (Jeong and Holden, 2009); 3. Tonic activation of α_1- but not α_2-adrenoceptors occurs in naïve and CCI rats, with a much greater effect in CCI rats (Wagner et al., 2016); 4. Both α_1- (Roudet et al., 1993, 1994; Xie et al., 2001) and α_2-adrenoceptors (Roudet et al., 1993, 1994) upregulate following nerve injury; 5. The opposing effects of pronociceptive α_1- and antinociceptive α_2-adrenoceptors were found in naïve male and female rats and male CCI rats, and carbachol dose affected male CCI rats (Figs. 2 and 3); 6. Female CCI rats did not show α_2-adrenoceptor-mediated antinociception at any carbachol dose (Fig. 3) and others have seen similar results following CCI (Samur et al., 2018). The clinical implications of this study are that deep brain LH stimulation, as well as the use of SNRIs, may be useful to relieve neuropathic pain, that sex and dose differences may occur, and in particular, females may not respond as well as males, in part because of the pronounced pronociceptive effect of α_1–adrenoceptors and the absence of α_2–mediated antinociception.

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Conflicts of interest

All authors declare any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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