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Gender and the behavioral manifestations of neuropathic pain

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Abstract

A model of peripheral nerve injury was used to study gender differences in the development and progression of chronic constriction injury (CCI)-induced hyperalgesia and allodynia in male and female Fischer 344 FBNF1 hybrid rats. Rats were randomly assigned to one of the following treatment groups: (1) gonadally intact unligated males (♂); (2) gonadally intact ligated males ($♂_{CCI}$); (3) castrated ligated males ($♂_{CAS/CCI}$); (4) gonadally intact unligated females (♀); (5) gonadally intact ligated females ($♀_{CCI}$); and (6) ovariectomized ligated females ($♀_{OVX/CCI}$). A plantar analgesia meter and calibrated von Frey pressure filaments were used as the analgesiometric assays. In the absence of nerve injury, gonadally intact males responded significantly faster than females to a thermal nociceptive stimulus. The onset of the behavioral manifestations of unilateral ligation of the sciatic nerve did not differ as a function of sex or hormonal status (e.g., gonadally intact and gonadectomized male and female rats developed thermal hyperalgesia within 14 days post-CCI). Paw withdrawal latency (PWL) values of gonadally intact males returned to baseline control values after postligation day 14, whereas gonadally intact females, ovariectomized females and castrated males continued to elicit robust thermal hyperalgesic symptoms throughout the 35-day duration of the experiment. Allodynic responses to peripheral nerve injury were less variable across genders. These data suggest that the mechanisms underlying chronic nociceptive processing differ as a function of gender and gonadal hormone status.

Keywords: Gender; Neuropathic pain; Hyperalgesia; Allodynia; Chronic constriction injury (CCI); Estrogen; Gonadal steroid hormones

1. Introduction

A thorough understanding of the mechanisms and modulatory factors underlying neuropathic pain associated with peripheral nerve injury is imperative for the development of effective treatment strategies. The effects of gender on nociceptive processing have received increased attention recently, and epidemiologic studies acknowledge that women report more persistent pains (Ektor-Andersen et al., 1993; Unruh, 1996) and use analgesic medications more often than men (Eggen, 1993). Females also report lower pain thresholds, higher pain ratings and less tolerance to noxious stimuli than males (Berkley, 1997). Investigations of the effects of gender on nociceptive processing recognize that gender differences exist in the susceptibility of males and females to nociceptive stimuli (Bendelow, 1993; McCaffery and Ferrell, 1992); but there is a lack of consensus regarding the precise nature of these differences.

The pathophysiology of peripheral nerve injury-induced neuropathic pain disorders can be investigated using the chronic constriction injury (CCI) model of Bennett and Xie (1988). Unilateral placement of 4-0 chromic gut ligatures around the sciatic nerve evokes behavioral symptoms such as thermally mediated hyperalgesia and tactile-evoked allodynia. Within 2–4 days post-CCI, axonal swelling and large granulomas containing neutrophils, macrophages and multinucleated giant cells surround the chromic gut sutures (Clatworthy et al., 1995; Maves et al., 1993). One group of investigators reported that the chromium salts comprising the gut sutures were responsible for producing CCI-induced inflammation, thermal hyperalgesia and guarding behavior in rats (Maves et al., 1993). That CCI-induced inflammatory and immunological responses are crucial for the development of neuropathic pain following the induction of peripheral nerve injury is further substantiated by the finding that these neuropathy-induced behavioral effects are dexamethasone reversible (Clatworthy et al., 1995).

Gonadal steroids can modulate immunological function by establishing crosstalk between the endocrine and immune systems in a sexually dimorphic manner (DaSilva et al., 1993; Gaillard and Spinedi, 1998; Spinedi et al.,

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1994). For instance, female rodents have higher levels of serum immunoglobulin and prolonged antibody responses compared to males (DaSilva et al., 1993; Gaillard and Spinedi, 1998) and these inflammatory and immune reactions can be modulated by gonadal hormones (Green et al., 1999). The goal of the present study was to investigate gender-related differences in the behavioral manifestations of chronic neuropathic pain and to determine how gonadal sex hormones affect these differences. The results suggest that chronic nociceptive processing differs as a function of gender and gonadal hormones.

2. Methods

All experiments were fully approved for the humane use of animals by the Institutional Animal Care and Use Committee at Northeastern Ohio Universities College of Medicine. Age-matched male and female Fischer 344 FBNF1 hybrid rats (Harlan Sprague–Dawley, Indianapolis, IN) were used in all experiments. Rats of both genders were purchased between the ages of 56 and 60 days, and all components of the investigation were conducted while the animals were between the ages of 3 and 6 months. Rats were randomly assigned to one of the following treatment groups ($n = 8$ rats/condition): (1) gonadally intact unligated males (♂); (2) gonadally intact ligated males ($♂_{CCI}$); (3) castrated ligated males ($♂_{CAS/CCI}$); (4) gonadally intact unligated females (♀); (5) gonadally intact ligated females ($♀_{CCI}$); and (6) ovariectomized ligated females ($♀_{OVX/CCI}$). All gonadally intact females were randomly cycling. Previous experience with the chronic constriction model (Bennett and Xie, 1988) has demonstrated that the development of thermal hyperalgesia following unilateral ligation of the sciatic nerve is dependent on the tightness of the constriction ligatures. For instance, we have observed that when the chromic gut ligatures are tied too tightly around the sciatic nerve, hypoalgesia, rather than hyperalgesia, will ensue. Due to the potential for variability in the surgical technique, one surgeon performed all of the CCI surgeries. Food and water were available ad libitum, and animals were individually housed in clear plastic cages containing at least 4 cm of shredded aspen bedding covering the floor of the cage throughout the entire duration of the experiment.

2.1. Surgical gonadectomies

Animals were anesthetized with sodium pentobarbital (females 40 mg/kg ip; males 45 mg/kg ip). To standardize the surgical procedure across gender cohorts, ovariectomies and castrations were performed via single abdominal incisions along the midline. The fallopian tubes were ligated with 3-0 silk sutures below the ovaries, which were then removed. The testis and the epididymis were exposed, ligated with 3-0 silk sutures at the junction of the vas deferens and spermatic blood vessels, and excised. The abdominal muscles of both the male and female rats were closed with 4-0 gut sutures and the skin wound was sealed with wound clips.

2.2. CCI surgery

Prior to CCI surgery, rats were treated with ip pentobarbital 40 mg/kg (females) or 45 mg/kg (males). The left sciatic nerve was exposed and, proximal to the trifurcation, approximately 7 mm of the common sciatic nerve was freed of adhering tissue. Four 4-0 chromic gut sutures were loosely tied around the nerve at intervals of approximately 1 mm, and ligatures were tied loosely enough so that, on visual inspection, blood flow was not obstructed (Berkley, 1997). The surgical incision was sutured and postsurgical recuperation was monitored daily.

The surgical regimen for animals assigned to the gonadectomy and CCI treatment groups was as follows: Ovariectomy or castration surgery $\rightarrow\rightarrow\rightarrow$ 10 days (for depletion of gonadal hormones) $\rightarrow\rightarrow\rightarrow$ behavioral testing on the day of CCI surgery (day 0 control) $\rightarrow\rightarrow\rightarrow$ CCI surgery $\rightarrow\rightarrow\rightarrow$ analgesiometric assays on post-CCI days 3, 14 and 35.

2.3. Paw withdrawal latency (PWL) values

A plantar analgesic meter was used to record pre- and post-CCI PWL values from the left and right hind paw of each rat. In these studies, PWL was defined as the time of initial exposure of a thermal stimulus to the plantar surface of the hind paw to the time of withdrawal of the paw from the heat source. A 20-s limit of heat exposure was imposed to preclude tissue damage to the paw. Four presurgical PWL measurements were recorded from the left and right hind paws at 10-min intervals and an average of the last three values was calculated and defined as the presurgical PWL mean (day 0 control).

To investigate gender-related differences in the onset and progression of CCI-induced thermal hyperalgesia, postsurgical PWL measurements were collected on postligation days 3, 14 and 35. All behavioral experiments were conducted between 8:00 a.m. and 11:00 a.m. On each testing day, three PWL values were recorded from the left (ligated) and right (control) hind paw of each rat at 10-min intervals, and an average of the last two measurements was calculated and defined as the postsurgical PWL mean.

2.4. Paw withdrawal threshold (PWT) values

Using calibrated von Frey filaments, pre- and postligation PWT values were obtained from all gender cohorts. The von Frey methodology was used to evaluate the sensitivity of the skin to tactile stimulation by exerting increasing pressure on the skin. Each filament is calibrated to have a characteristic bending force when pressure is applied (e.g., .023–446.7 g). Animals were placed under a

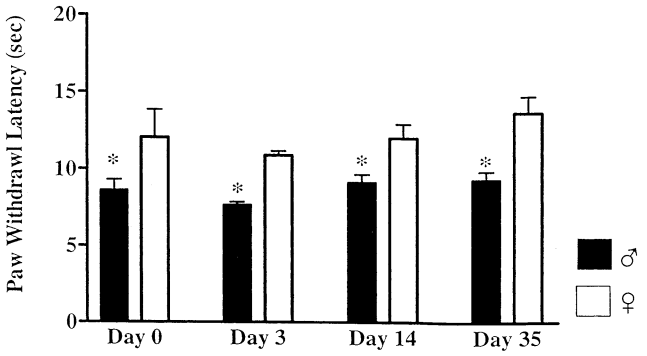

Fig. 1. Paw withdrawal latency (PWL) control values from the left hind paw of *unligated* gonadally intact male and female rats on test days 0, 3, 14 and 35. * $P \leq .05$ vs. female PWL values on the corresponding test day. Values represent the mean ± S.E.M.

transparent dome on a plastic mesh floor, and increasing filament strength was applied sequentially to the plantar surface of the ligated vs. unligated hind paw at 10-min intervals. Pre- and post-CCI PWT values were defined as the minimum gram strength eliciting two sequential responses (lifting of the paw). Gender differences in the onset and maintenance of nerve injury-induced tactile-evoked allodynia were tested on pre-CCI day 0 (control) and on post-CCI days 3, 14 and 35.

2.5. Data analysis

All data are presented as mean ± S.E.M. PWL values, difference scores and PWT values represent the dependent factors in this investigation, and each was analyzed separately by analysis of variance (ANOVA; Systat version 7.0.1, SPSS 1997). The data presented in Figs. 1 and 2 were analyzed with a two-factor ANOVA (Male/Female × Test day). The data presented in Figs. 3 and 4 were analyzed with a three-factor ANOVA (Male/Female × Intact/Gonadectomy × Test day). For all data analyses, a P value $\leq .05$ was considered statistically significant.

3. Results

Preliminary studies in this laboratory demonstrated a lack of significant effect between PWL and PWT values from the right (control) hind paw of unligated ($n = 48$ rats) vs. sham-ligated ($n = 51$ rats) control animals. Because of the lack of significant difference between these values, CCI sham surgeries were not conducted in control animals in the present study. Gonadectomy sham surgeries were performed in a separate cohort of male and female rats, and it was found that neither the PWL nor PWT values differed significantly in gonadally intact males vs. males receiving sham castration surgeries ($n = 6$) or in gonadally intact females vs. females receiving sham ovariectomy surgeries ($n = 6$).

In the absence of nerve injury or gonadectomies, PWL values were consistent for at least 35 days (Fig. 1). Unligated males (♂) responded significantly faster than unligated females (♀) to the thermal nociceptive stimulus on test days 0, 3, 14 and 35 (P=.01). No significant effect of test day or gender × day interaction was detected. Further, in the absence of sciatic nerve injury, sex-related differences in PWT responses to tactile-evoked stimuli were undetectable.

By post-CCI day 14, gonadally intact male and female rats elicited significant thermal hyperalgesic responses compared to unligated control values compiled on day 0 (Fig. 2A; $P \leq .05$). To better quantify the extent to which thermal hyperalgesia developed in the two sex cohorts, difference scores were computed for each rat by subtracting the PWL of the unligated right hind paw from the PWL of the ligated left hind paw. A negative difference score signified the development of thermal hyperalgesia. However, our results revealed a dissociation between the onset of thermal hyperalgesia and negative difference scores. For example, in the absence of detectable changes in PWL values on post-CCI day 3 in the male cohort (Fig. 2A), significant difference scores were computed (Fig. 2B). By postligation day 14, both the PWL values and difference scores of gonadally intact males and females were significantly different from day 0 controls (Fig. 2A and B).

Upon further examination of the development of thermal hyperalgesia across the four treatment groups (gonadally

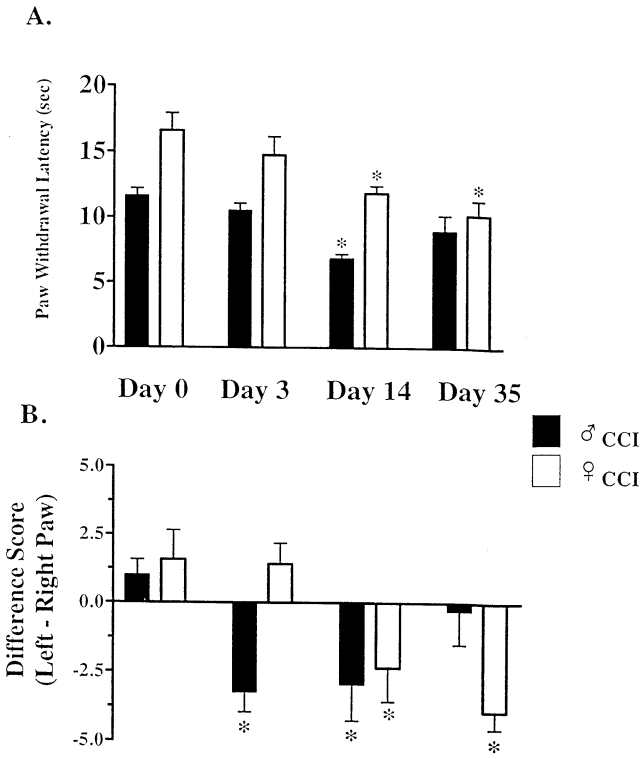

Fig. 2. PWL values from the left hind paw (A) and difference scores (B) in gonadally intact male and female rats on pre-CCI day 0 and post-CCI days 3, 14 and 35. * $P \leq .05$ vs. sex-matched presurgical control values on day 0. Values represent the mean ± S.E.M.

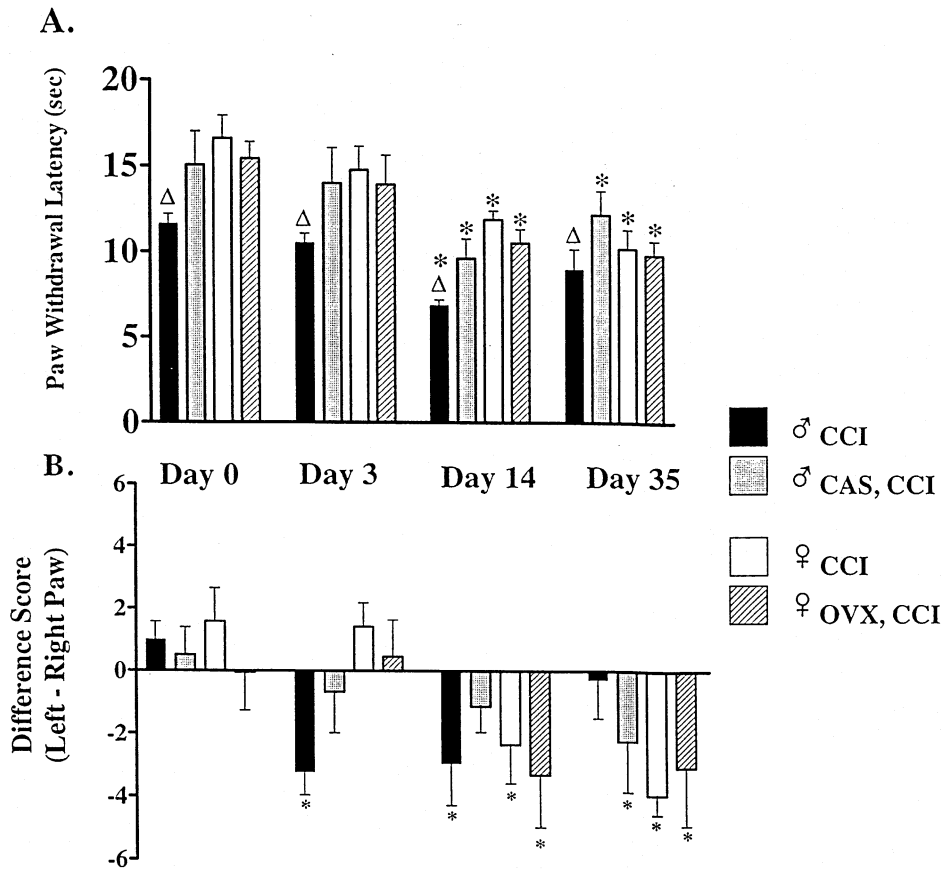

Fig. 3. PWL values from the left hind paw (A) and difference scores (B) in gonadally intact vs. gonadectomized male and female rats on pre-CCI day 0 and post-CCI days 3, 14 and 35. * $P \leq .05$ vs. sex-matched PWL control values on day 0. $^{\triangle}$ $P \leq .05$ vs. PWL for castrated males on the corresponding test day. Values represent the mean ± S.E.M.

intact vs. gonadectomized male and female rats), the data revealed that the *onset* of the behavioral manifestations of ligating the left sciatic nerve did not differ as a function of sex or hormonal status (Fig. 3). Gonadally intact and gonadectomized male and female rats developed thermal hyperalgesia within 14 days post-CCI. However, the *duration* of CCI-induced hyperalgesia differed in males and females. As depicted in Fig. 3, PWL values of gonadally intact males returned to baseline control values after postligation day 14, while the other three sex cohorts elicited robust thermal hyperalgesic symptoms throughout the 35-day duration of the experiment ($P \leq .05$).

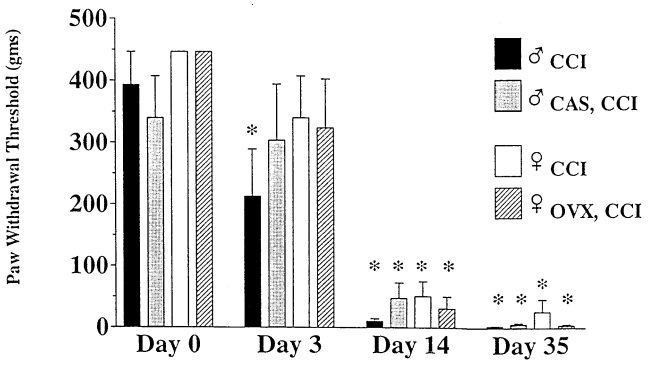

Fig. 4. PWT values from the left hind paw of gonadally intact vs. gonadectomized male and female rats on pre-CCI day 0 and post-CCI days 3, 14 and 35. * $P \leq .05$ vs. sex-matched PWT control values on day 0. Values represent the mean ± S.E.M.

Analyses of the tactile-evoked allodynia data revealed an analogous behavioral pattern across the four different sex cohorts (Fig. 4). By day 3 post-CCI, gonadally intact males developed an allodynic response to peripheral nerve injury. Gonadally intact and gonadectomized animals of both genders developed significant allodynic responses to the CCI by postligation day 14 ($P \leq .05$). All sex cohorts continued to manifest touch-evoked allodynia throughout the 35-day duration of the experiments (no gender-related differences in the duration of effect were observed).

4. Discussion

We investigated the extent to which neuropathic pain-induced thermal hyperalgesia and tactile-evoked allodynia differ as a function of gender and hormonal status. In the absence of nerve injury, hyperalgesic and allodynic responses were not elicited by gonadally intact male or female rats. However, unligated males responded signifi-

cantly faster to the thermal stimulus than the unligated females did, implying inherent gender differences in thermal nociceptive processing. These data support previous findings of sex-related differences in basal nociceptive threshold in rodents (Bodnar et al., 1988) and humans (Berkley, 1997).

It is well established that the inflammatory and associated immune responses elicited by unilateral ligation of the sciatic nerve are crucial for the development of neuropathic pain (Clatworthy et al., 1995; Maves et al., 1993). Although it has been reported that estrogen enhances, while testosterone reduces, the severity of inflammatory disease in animals (DaSilva et al., 1993), we were unable to detect significant differences in the *onset* of thermal hyperalgesia using the CCI model. Gonadally intact, as well as gonadectomized, male and female rats developed thermal hyperalgesia to a thermal nociceptive stimulus within 2 weeks following unilateral ligation of the left sciatic nerve. By post-CCI day 35, however, PWL values and difference scores from the gonadally intact male cohort returned to baseline values. It is interesting to note that this "recovery" from nerve pain was not detected in intact or ovariectomized female rats, nor was it noted in castrated males. Findings such as these suggest that testosterone may reduce the severity of nerve pain by inhibiting the inflammatory component (DaSilva et al., 1993). Previous studies have shown that testosterone reverses the immune response to experimentally induced inflammatory reactions (Gaillard and Spinedi, 1998). Further, in gonadectomized male and female mice, the enhanced immune response to bacterial lipopolysaccharide-induced inflammation was completely reversed by testosterone administration (Gaillard and Spinedi, 1998). This protective action of the androgens against inflammation has been reported by numerous laboratories (Gaillard and Spinedi, 1998; Lahita, 1996; Steward and Bayley, 1992). Moreover, the use of androgen replacement therapy in patients suffering from rheumatoid arthritis has been beneficial (Bijlsma, 1999; Cutolo et al., 1991).

Difference scores have been used in past studies as a means to quantify the extent to which peripheral nerve injury induces thermal hyperalgesia. Difference scores are computed by subtracting the PWL of the unligated hind paw from the PWL of the ligated hind paw, and a negative difference score signifies the development of thermal hyperalgesia in the injured paw. However, our data revealed dissociations between the onset of thermal hyperalgesia and the computation of negative difference scores. The utilization of difference scores in previous studies was predicated on the supposition that the hind paw contralateral to the nerve lesion exhibited no significant change in thermal response latency. With this particular qualifier, the contralateral side serves as a control for comparison to the side ipsilateral to nerve injury. However, this approach has its limitations (Clatworthy et al., 1995). In the presence of nerve injury, no significant contralateral changes were detected in the female cohorts (φ_{CCI}, $\varphi_{OVX,\ CCI}$), whereas contralateral effects were detected in gonadally intact (σ_{CCI}) and castrated male rats ($\sigma_{CAS,\ CCI}$). This divergence is depicted in Figs. 2 and 3, where the post-CCI PWL mean of gonadally intact males on day 3 was not significantly different from the day 0 control mean, while the difference score for this particular cohort signified the onset of thermal hyperalgesia. It is possible that some of the rats attempted to guard the injured hind paw by holding it close to their torso, which may have inadvertently increased the PWL response of the weight-bearing contralateral hind paw. Thus, the use of difference scores may not be the most appropriate parameter for comparison when using the CCI model of peripheral neuropathy. Further study is warranted to investigate potential neuroplastic changes in the spinal cord following peripheral nerve injury, as well as the consequences of these changes on ipsilateral and contralateral withdrawal latencies to thermal nociceptive stimuli (Koltzenburg et al., 1999).

Comparable allodynic responses were elicited by male and female rats in the present study. These data differ somewhat from what has been reported in earlier studies (e.g., female rats were more inclined than males to develop tactile-evoked allodynia; Coyle et al., 1995, 1996), and this apparent disparity is most likely attributable to the employment of different animal models in the two studies. The present finding that male and female rats exhibit discordant thermal hyperalgesic response patterns, while the expression of tactile-evoked allodynia is less variable across genders suggests that nociceptive input is differentially modulated. It has been reported that tactile allodynia is mediated via large Aβ afferent fibers while thermal hyperalgesia is C-fiber-mediated (Lekan et al., 1996; Ossipov et al., 1999; Yaksh, 1989). Estrogen receptors are located in the dorsal horn of the spinal cord (Amandusson et al., 1995), and the localization of the sex hormone receptors in the vicinity of spinal nociceptive nerve terminals provides the means for direct modification of noxious input by the gonadal hormones. Thus, the present finding that thermal hyperalgesia and tactile-evoked allodynia are differentially affected by the gonadal hormones supports the hypothesis that these signs of peripheral nerve injury are mechanistically distinct.

The current investigation utilized randomly cycling female rats, and experiments are currently underway to ascertain how fluctuations in the estrous cycle (Frye et al., 1993; Kayser et al., 1996; Martinez-Gomez et al., 1994) and alterations in gonadal hormone concentrations (Gintzler and Bohan, 1990) affect chronic nociceptive processing. To our knowledge, this is the first report of gender-related differences in the expression of neuropathic hyperalgesia using the sciatic nerve ligation model (Bennett and Xie, 1988). Further studies assessing the potential modulatory roles of estrogen and testosterone in nociceptive processing will contribute to important therapeutic advances for the treatment of chronic pain of neuropathic origin.

References

Amandusson Å, Hermanson O, Blomqvist A. Estrogen receptor-like immunoreactivity in the medullary and spinal dorsal horn of the female rat. Neurosci Lett 1995;196:25–8.

Bendelow G. Pain perceptions, emotions and gender. Sociol Health Illness 1993;15:273–94.

Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33:87–107.

Berkley KJ. Sex differences in pain. Behav Brain Sci 1997;20(3):371–80.

Bijlsma JWJ. Can we use steroid hormones to immunomodulate rheumatic diseases? Ann NY Acad Sci 1999;876:366–77.

Bodnar RJ, Romero M-T, Kramer E. Organismic variables and pain inhibition: roles of gender and aging. Brain Res Bull 1988;21:947–53.

Clatworthy AL, Illich PA, Castro GA, Walters ET. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. Neurosci Lett 1995;184: 5–8.

Coyle DE, Sehlhorst CS, Mascari C. Female rats are more susceptible to the development of neuropathic pain using the partial sciatic nerve ligation (PSNL) model. Neurosci Lett 1995;186:35–138.

Coyle DE, Sehlhorst CS, Behbehani MM. Intact female rats are more susceptible to the development of tactile allodynia than ovariectomized female rats following partial sciatic nerve ligation (PSNL). Neurosci Lett 1996;203:37–40.

Cutolo M, Balleari E, Giusti M, Intra E, Accardo S. Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1–5.

DaSilva JAP, Peers SH, Perretti M, Willoughby DA. Sex steroids affect glucocorticoid response to chronic inflammation and to interleukin-1. J Endocrinol 1993;136:389–97.

Eggen AE. The Tromso study: frequency and predicting factors of analgesic drug use in a free-living population (12–56 years). J Clin Epidemiol 1993;46:1297–304.

Ektor-Andersen J, Janzon L, Sjolund B. Chronic pain and the socio-demographic environment: results from the pain clinic at Malmo General Hospital in Sweden. Clin J Pain 1993;9:183–8.

Frye CA, Cuevas CA, Kanarek RB. Diet and estrous cycle influence pain sensitivity in rats. Pharmacol Biochem Behav 1993;45:255–60.

Gaillard RC, Spinedi E. Sex- and stress-steroids interactions and the immune response: evidence for a neuroendocrine-immunological sexual dimorphism. Domest Anim Endocrinol 1998;15(5):345–52.

Gintzler AR, Bohan MC. Pain thresholds are elevated during pseudopregnancy. Brain Res 1990;507:312–6.

Green PG, Dahlqvist SR, Isenberg WM, Strausbaugh HJ, Miao FJ-P, Levine JD. Sex steroid regulation of the inflammatory response: sympathoadrenal dependence in the female rat. J Neurosci 1999; 19(10):4082–9.

Kayser V, Berkley KJ, Keita H, Gautron M, Guilbaud G. Estrous and sex variations in vocalization thresholds to hind paw and tail pressure stimulation in the rat. Brain Res 1996;742:352–4.

Koltzenburg M, Wall PD, McMahon SB. Does the right side know what the left is doing? Trends Neurosci 1999;22:122–7.

Lahita RG. The connective tissue diseases and the overall influence of gender. Int J Fertil Menopausal Stud 1996;41:156–65.

Lekan HA, Carlton SM, Coggeshall RE. Sprouting of A beta fibers into lamina II of the rat dorsal horn in peripheral neuropathy. Neurosci Lett 1996;208:147–50.

Martinez-Gomez M, Cruz Y, Salas M, Hudson R, Pacheco P. Assessing pain threshold in the rat: changes with estrus and time of day. Physiol Behav 1994;55:651–7.

Maves TJ, Pechman PS, Gebhart GF, Meller ST. Possible chemical contribution from chromic gut sutures produces disorders of pain sensation like those seen in man. Pain 1993;54:57–69.

McCaffery M, Ferrell BR. Does the gender gap affect our pain-control decisions? Nursing 1992;22:48–51.

Ossipov MH, Bian D, Malan TP, Lai J, Porreca F. Lack of involvement of capsaicin-sensitive primary afferents in nerve-ligation injury induced tactile allodynia in rats. Pain 1999;79:127–33.

Spinedi E, Salas M, Chisari A, Perone M, Carino M, Gaillard RC. Sex differences in the hypothalamo-pituitary-adrenal axis response to inflammatory and neuroendocrine stressors. Neuroendocrinology 1994;60:609–17.

Steward A, Bayley DL. Effects of androgens in models of rheumatoid arthritis. Agents Actions 1992;35:268–72.

Unruh AM. Gender variations in clinical pain experience. Pain 1996; 65:123–67.

Yaksh TL. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. Pain 1989; 37:111–23.