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Spinal cord processing of cardiac nociception: Are there sex differences between male and proestrous female rats?

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Abstract

Sex differences in the characteristics of cardiac pain have been reported from clinical studies. For example, women experience chest pain less frequently than men. Women describe their chest pain as sharp and stabbing, while men have chest pain that is felt as a pressure or heaviness. Pain is also referred to the back more often in women than men. The mechanisms underlying sex differences in cardiac pain are unknown. One possible mechanism for the observed differences could be related to plasma estradiol. This study investigated the actions of estradiol on the activity of T₃ spinal neurons that process cardiosomatic information in male and female rats. Extracellular potentials of T₃ spinal neurons were recorded in response to mechanical somatic stimulation and noxious chemical cardiac stimulation in pentobarbital-anesthetized male and proestrous female rats. Fifty one percent and 50% of neurons responded to intrapericardial algogenic chemicals (0.2 ml) in male and female rats, respectively. Somatic fields were located by applying brush, pressure, and pinch to the upper body. Of those neurons receiving cardiac input, 54% in female and 55% in male rats also received somatic input. In both male and female rats, 81% of neurons responding to somatic stimuli had somatic fields located on the side of the upper body, while 19% of neurons had somatic fields located on the chest. These results indicate there are no significant differences in the responses of T₃ spinal neurons to cardiosomatic stimulation between male and proestrous female rats, despite differences in estradiol levels.

Keywords

Angina pectoris; visceral pain; estradiol; rats; spinal cord; sex differences

1. Introduction

Angina pectoris is the most common symptom of ischemic heart disease (Bonica, 2001; Caldwell and Miaskowski, 2000). Although both men and women can experience angina during myocardial ischemia, clinical studies have shown sex differences in the presentation of anginal pain. Ischemic heart disease was always thought of as ‘a man’s disease’; therefore, the presentation of angina was based on descriptive characteristics, known as ‘typical’ angina, provided by men (Healy, 1991). Patients who experience typical angina mainly feel chest pain, although the pain can also radiate down the left arm and, occasionally, to the neck and jaw (Devon and Zerwic,; Herlitz et al., 1999; Lovlien et al.,

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2006). The chest pain is usually described as a pressure or heaviness. Although most men experience typical angina, many women have 'atypical' angina. Most women do not have chest pain. Instead, they have pain that is referred to the back (DeVon and Zerwic, 2002; Douglas and Ginsburg, 1996; Patel et al., 2004). Those women who have chest pain describe the pain as stabbing or burning (Douglas and Ginsburg, 1996; Methot et al., 2004), and it is usually longer in duration, more frequent, and more severe than chest pain felt by men (Cannon et al., 1992; DeVon and Zerwic, 2003; Douglas and Ginsburg, 1996; Methot et al., 2004). The mechanisms underlying sex differences in the characteristics of cardiac pain are unknown.

Is estradiol responsible for differences in the presentation of cardiac pain between women and men? Several animal studies have shown that estrogen receptors are located in the sensory areas of the central nervous system, including the dorsal horn of the cervical, thoracic, and lumbosacral spinal cord (Amandusson et al., 1995; Papka and Mowa, 2003) in males and females; therefore, estradiol may play a role in modulating nociceptive information. Evidence from the literature suggests that high levels of estradiol may be responsible for increased sensitivity to noxious visceral stimulation in female animals. Proestrous female rats have a lower threshold and higher magnitude of response to colorectal distension (CRD) compared to female rats in the other stages of the estrous cycle (Holdcroft et al., 2000; Ji et al., 2008; Sapsed-Byrne et al., 1996) and male rats (Holdcroft et al., 2000). In rats with neonatal colon irritation, responses of L₆-S₁ postsynaptic dorsal column (PSDC) neurons to CRD were greater in proestrous female rats than male rats (Wang et al., 2008). Ovariectomy reduced responses of PSDC neurons to CRD, while estradiol replacement in ovariectomized rats enhanced neuronal responses.

Animal studies have shown that cardiac afferent fibers transmitting nociceptive information converge with somatic afferents onto the same cells in the dorsal horn of the spinal cord (Blair et al., 1984a, 1984b; Chandler et al., 2000; Qin et al., 2001; Qin et al., 2003; Zhang et al., 1997). For example, neurons in the dorsal horn of the T₂-T₆ spinal cord receive convergent input from cardiac sympathetic afferents and from somatic afferents that innervate the chest and left shoulder in monkeys (Foreman, 1999). In rats, upper thoracic (T₂-T₄) spinal neurons responding to chemical stimulation of the heart have somatic receptive fields located on the chest (Euchner-Wamser et al., 1994), axilla, forelimb joints, or upper back (Qin et al., 2003). Convergent input from the heart and somatic structures onto upper thoracic neurons provides a mechanism for referral of anginal pain from an ischemic heart to the chest, shoulder, and back. However, studies examining responses of upper thoracic spinal neurons to cardiosomatic stimulation have only been performed in male animals. The role of estradiol in the processing of cardiosomatic input in the upper thoracic spinal cord of males and females is also unknown. The purpose of the present study was to test the hypothesis that one mechanism that might explain sex differences in cardiac pain is the actions of estradiol on the activity of T₃ spinal neurons that process cardiosomatic information. The present study was conducted to compare responses of T₃ spinal neurons to cardiosomatic stimulation between male and proestrous female rats.

2. Results

2.1. Thoracic experiments

2.1.1. Concentration of plasma estradiol—The levels of plasma estradiol were measured successfully in 7/18 male and 13/21 proestrous female rats. The concentration of estradiol was significantly greater in the female rats (71.7 ± 5.8 pg/ml) than in the male rats (6.6 ± 0.2 pg/ml, $p < 0.0001$).

2.1.2. Neuronal responses to algogenic chemical stimulation of the heart—The T₃ spinal neurons were located between 100 μ m and 1200 μ m from the surface of the cord and 1.0 to 1.5 mm from the midline. No significant differences in the depth of the T₃ spinal neurons between the male ($828.9 \pm 27.5 \mu$ m) and proestrous female ($762.7 \pm 28.9 \mu$ m) rats were observed.

Responses of T₃ spinal neurons to intrapericardial algogenic chemicals were examined and compared between male ($n = 18$) and proestrous female ($n = 21$) rats. There were 47/92 (51%) neurons in male and 52/104 (50%) neurons in female rats that responded to cardiac stimulation. Of the responsive neurons, 36 and 39 had excitatory responses in male and female rats, respectively. Two types of excitatory response patterns were observed: short-lasting excitatory (SL-E) and long-lasting excitatory (LL-E). Neurons were considered to have short-lasting responses (≤ 60 seconds) if they responded only when the algogenic chemicals were applied (Figure 1A). Neurons that continued to respond even after cardiac stimulation was removed (Figure 1B) were described as having a long-lasting response (> 60 seconds). A comparison of the number of T₃ spinal neurons that had LL-E and SL-E responses in male and proestrous female rats is shown in Figure 1C. There were a greater number of LL-E neurons (male, $n = 27$; female, $n = 31$) than SL-E neurons (male, $n = 9$; female, $n = 8$) in both groups of rats. However, no significant differences were found in the number of LL-E and SL-E neurons between the male and female rats.

Spontaneous activity was measured for both the SL-E and LL-E neurons. No significant difference was found in the spontaneous activity of the SL-E neurons between male (11.4 ± 4.2 imp/s, $n = 9$ neurons) and proestrous female (7.4 ± 2.5 imp/s, $n = 8$ neurons) rats. There was also no difference in the spontaneous activity of LL-E neurons between the two groups of rats (male, 8.2 ± 1.6 imp/s, $n = 27$; female, 10.5 ± 1.8 imp/s, $n = 31$).

Comparison of response characteristics of the SL-E and LL-E neurons to noxious chemical cardiac stimulation are shown in Figure 2. No significant differences were observed in the latency (Figure 2A), duration (Figure 2B), maximal response (Figure 2C), and total response (Figure 2D) of SL-E and LL-E neurons between the male and proestrous female rats.

Intrapericardial injection of algogenic chemicals in male and proestrous female rats inhibited 9/47 (19%) and 11/52 (21%) of responsive T₃ spinal neurons, respectively. The inhibitory neurons were divided into two groups, based on their duration of response to cardiac stimulation: short-lasting inhibitory (SL-I) and long-lasting inhibitory (LL-I). The response characteristics for the SL-I and LL-I neurons in male and female rats are shown in Table 1. No significant differences in the latencies, durations, maximal responses, and total responses of SL-I and LL-I neurons were observed between the two groups of rats.

Two neurons each in male and proestrous female rats had an excitatory-inhibitory (or biphasic) response to cardiac stimulation. Both of the neurons in the male had a long-lasting response, while one neuron in the female had a short-lasting response. Since recordings were made from only a few of these neurons, statistical analysis was not performed.

2.1.3. Cardiosomatic convergence—Somatic receptive field characteristics of T₃ spinal neurons that received cardiac input were compared between male and proestrous female rats. Of the 47 neurons responsive to cardiac stimulation in male rats, 26 (55%) were also responsive to mechanical somatic stimulation. This number was not significantly different from the number of neurons (28/52, 54%) in female rats that received convergent input from cardiac and somatic structures (Figure 3A).

The locations of the somatic receptive fields were determined for T₃ spinal neurons that received input from cardiac and somatic structures. Neurons had somatic fields on the chest and side of the upper thoracic area of the body (Figure 3B). Although more neurons received somatic input from the side (male, $n = 21$; female, $n = 22$) than the chest (male, $n = 5$; female, $n = 5$, location was not recorded for 1 neuron) in both male and female rats, there were no significant differences in locations of somatic fields between the groups as shown in Figure 3B.

The sizes of the somatic receptive fields of T₃ spinal neurons that responded to both mechanical somatic stimulation and noxious chemical cardiac stimulation were measured and compared before and after intrapericardial injections of algogenic chemicals in male ($n = 24$ neurons) and female ($n = 25$ neurons) rats. The fields were not mapped after chemical stimulation of the heart for 2 neurons in male and 3 neurons in female rats. No changes in the sizes of the receptive fields were observed after intrapericardial injection of chemicals in either group of rats (Figure 3C). The areas of the somatic fields were $614 \pm 100 \text{ mm}^2$ in male rats and $674 \pm 97 \text{ mm}^2$ in female rats before cardiac stimulation. After injection of chemicals into the pericardial sac, the areas of the somatic fields were $743 \pm 136 \text{ mm}^2$ and $750 \pm 92 \text{ mm}^2$ in males and proestrous female rats, respectively.

No differences were observed in the magnitude of responses of T₃ spinal neurons to somatic stimulation (brush, pressure, and pinch) between male and proestrous female rats (data not shown).

2.2. Lumbosacral experiments

The excitatory responses of L₆-S₁ spinal neurons to CRD were examined and compared at distension pressures of 20, 40, 60, and 80 mmHg between male ($n = 23$ neurons) and proestrous female ($n = 22$ neurons) rats. There were no significant differences in the rate of responses of the L₆-S₁ spinal neurons at any of the distension pressures between the two groups of rats (Figure 4).

3. Discussion

This study was performed to examine and compare the responses of T₃ spinal neurons to noxious chemical cardiac stimulation and mechanical somatic stimulation between male and proestrous female rats. Since estrogen receptors are located in the dorsal horn of the thoracic spinal cord, this study investigated whether plasma estradiol would differentially alter the activity of spinal neurons to cardiosomatic stimulation through actions on estrogen receptors between male and proestrous female rats. One of the main predictions was that high levels of plasma estradiol in proestrous female rats would increase the responses of T₃ spinal neurons to stimulation of the heart and somatic structures. Results of this study indicate that although there were the expected differences in the concentrations of plasma estradiol between the sexes, no differences were observed in the spinal neuronal processing of cardiosomatic input.

3.1. Thoracic spinal cord

Women and men who have ischemic heart disease present different characteristics of cardiac pain. Both women and men who experience myocardial ischemia have pain that is referred to the chest; however, men experience chest pain more frequently than women (Herlitz et al., 1999; Lovlien et al., 2006). Women who experience chest pain describe it as longer in duration and more severe than the chest pain observed in men (Cannon et al., 1992; Douglas and Ginsburg, 1996). The reason for sex differences in cardiac pain is unknown. The present study examined whether there were differences in the processing of cardiac nociception in

the thoracic spinal cord between healthy male and female rats using an experimental animal model of cardiac nociception. In this animal model, algogenic chemicals injected into the pericardial sac were used to mimic myocardial ischemia. These chemicals have been shown to produce both behavioral and neuronal responses in male rats (Euchner-Wamser et al., 1994). The uniqueness of this study was to 1) examine the responses of the T₃ spinal neurons to algogenic chemical stimulation of the heart in female rats and 2) compare the responses of the T₃ spinal neurons between male and proestrous female rats. The results showed that the chemicals activated T₃ spinal neurons in both the male and female rats; however, no sex differences in the neuronal responses were observed. This suggests that in normal animals, the differing levels of plasma estradiol between the male and proestrous female rats do not influence the activity of T₃ spinal neurons differently between the sexes.

Several specific characteristic differences in the responses of T₃ spinal neurons to cardiosomatic stimulation were tested based on differences in the perception of anginal pain in human males and females. The characteristics of the responses (duration and total number of impulses) of neurons to chemical stimulation of the heart were compared between male and proestrous female rats. The majority of neurons in both groups of rats had long-lasting excitatory responses to cardiac stimulation (male, 75%; female, 79%), and the number of impulses that occurred during the durations of the responses were similar. Although estradiol was expected to enhance responses of T₃ spinal neurons in proestrous female rats compared to males, there did not appear to be any differences in the modulation of nociceptive information from the heart between the sexes. These results are consistent with another study done in the lumbosacral spinal cord (Wang et al., 2008), where responses of L₆-S₁ PSDC neurons to CRD between control male and proestrous female rats did not differ.

In the present study, the locations and sizes of somatic receptive fields of T₃ spinal neurons that responded to cardiac stimulation were determined for male and proestrous female rats. Most of the neurons in the two groups of rats had receptive fields located on the side of the upper body (male, 81%; female, 81%), and no differences were observed in the sizes of the somatic fields. Previous studies that examined the responses of neurons in the lumbosacral spinal cord to CRD between male and female rats did not examine or compare the somatic receptive field characteristics of those neurons between the sexes (Wang et al., 2008).

The experiments for the present studies were performed in pentobarbital-anesthetized rats. However, there is no indication that the anesthetic would cause differences in the effects between male and proestrous female rats.

3.2. Lumbosacral spinal cord

In the present study, responses of T₃ spinal neurons to cardiosomatic stimulation and L₆-S₁ spinal neurons to colorectal distension were examined in experimental animal models in healthy male and proestrous female rats. Results for both sets of experiments showed that there were no differences in the neuronal processing of noxious visceral input between the sexes. These results were consistent with another study that examined the responses of L₆-S₁ PSDC neurons to CRD in male and female rats. Wang et al. (2008) performed experiments in a normal (control) animal model and a chronic pain (CI) model. No differences were found in the responses of L₆-S₁ PSDC neurons to colorectal distension between control male and proestrous female rats. However, responses of the PSDC neurons in CI proestrous female rats were significantly larger than the responses in CI male rats. The responses of the PSDC neurons were also larger in CI male and female rats compared to control rats of the same sex. The results of the Wang et al. study suggest that chronic pain may be responsible for both the sex differences in visceral pain and the increased sensitivity to colorectal distension in the CI model. Several other studies have also compared the responses of

lumbosacral (Lin and Al-Chaer, 2005) and thoracolumbar (Traub et al., 2008) spinal neurons to colorectal distension in a normal (control) animal model and a chronic pain model in male rats. Both of the studies showed that the responses of the spinal neurons were significantly greater in the chronic pain rats than the control rats. Although these two studies did not use female rats, the results were consistent with the Wang et al. study (2008), in which neurons in rats with chronic pain showed larger responses to the distension pressures than neurons in control rats.

3.3. Conclusion

The present study shows that differences in plasma estradiol levels between male and proestrous female rats does not lead to sex differences in the responses of T₃ spinal neurons to cardiosomatic stimulation in a normal experimental animal model.

4. Experimental Procedures

4.1. Animals

Adult male and female Sprague-Dawley rats weighing 300 to 400 grams were acquired from Charles Rivers Laboratories, Inc. Same sex animals housed two to a cage were kept on a 12 hour light/12 hour dark cycle. Animals had access to food and water ad libitum.

Experiments were performed in the thoracic spinal cord to determine if there were differences in the responses of T₃ spinal neurons to noxious chemical cardiac stimulation and mechanical somatic stimulation between male ($n = 18$) and proestrous female ($n = 21$) rats. Experiments were also done in the lumbosacral spinal cord of male ($n = 9$) and proestrous female ($n = 7$) rats to obtain results in a region of the spinal cord where other studies have examined sex differences in neuronal processing of visceral input. A pool of data was collected and analyzed for comparison. All experiments were approved by the Institutional Animal Care and Use Committee (Protocol 07-134H) at the University of Oklahoma Health Sciences Center and adhered to the guidelines for experimental pain in animals published by the International Association for the Study of Pain.

4.2. Vaginal cytology

Vaginal samples were obtained two hours after lights went on in the animal facility (lights went on at 6:00 a.m.). To make sure that each of the rats had a normal estrous cycle, samples were collected for at least two cycles (8 to 10 days) before experiments were performed. The stage of the estrous cycle (metestrus, diestrus, proestrus, or estrus) was determined by what types of cells (leukocytes, nucleated, and cornified squamous) were present.

4.3. Electrophysiological experiments

4.3.1. Surgical procedures

General surgery: Male and female rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The left jugular vein was catheterized to administer a continuous infusion of sodium pentobarbital (15–25 mg/kg/hr). Another catheter containing 0.1 ml heparin sodium (1000 USP units/ml) in 20 ml saline was inserted into the right carotid artery to monitor blood pressure during the experiments. A cannula was inserted into the trachea and the tube was attached to a positive pressure pump to ventilate the animals artificially (55–60 strokes/min, 3.0–4.0 ml stroke volume). The body temperature was maintained at $37 \pm 1^\circ\text{C}$ using a thermostatically controlled heating pad and thermocouple feedback sensor.

Thoracotomy: A midline thoracotomy was performed to the left of the sternum through the third rib to expose the thymus gland and heart. A 2 cm length of silicone catheter (I.D. =

0.76 mm, O.D. = 1.65 mm, 11 cm long) with three small holes at the end was inserted through the thymus and into the pericardial sac. The catheter was held in place by suturing together the thymus and overlying muscle. A 1 ml tuberculin syringe was used to inject chemicals into the pericardial sac via the catheter.

Laminectomy: A two-inch midline incision was made in the skin and fascia between the shoulder blades. A laminectomy was performed to expose the T₃ segment of the spinal cord. A midline cut was made in the dura and the T₃ spinal cord was covered with warm agar (37°C, 3–4% in saline) for stability during neuronal recordings. Rats were mounted in a stereotaxic headholder and clamps were placed onto the T₂ and T₅ spinous processes to stabilize the animal.

4.3.2. Somatic and cardiac stimulation—To determine somatic responses of each neuron located in the dorsal horn of the T₃ spinal cord, mechanical somatic stimulation (brush and pressure, innocuous; pinch, noxious) was applied to the upper thoracic area of the body (side and chest). All three types of somatic stimuli were applied to locate the area of the body and the size of the somatic receptive field that activated the neuron. Those stimuli that produced a change in the activity of the spinal neuron were each applied for 10 seconds (with a 30 second rest period between the stimuli). The response of the neuron to mechanical somatic stimulation was recorded 4 minutes before injecting algogenic chemicals (0.2 ml) into the pericardial sac. If the neuron responded to chemical stimulation of the heart, the response of the neuron to somatic stimulation was recorded 10 minutes after intrapericardial injection of chemicals. The somatic receptive fields for each neuron that responded to both somatic and cardiac stimulation were mapped out and measured (in mm²) using NIH ImageJ software. The locations of the somatic receptive fields and the number of neurons receiving convergent input (from cardiac and somatic afferent neurons) were each compared using Fisher's exact test. The sizes of the somatic receptive fields were compared before and after intrapericardial chemical injections using repeated measures two-way analysis of variance. For each of the tests used, $p < 0.05$ was considered significant.

To determine the responses of T₃ spinal neurons to noxious chemical stimulation of the heart, a mixture of algogenic chemicals (0.2 ml) containing bradykinin, adenosine, serotonin, prostaglandin E₂, and histamine was injected into the pericardial sac and withdrawn after 60 seconds. The mixture contained equal concentrations (10^{-5} M) of each of the chemicals except for adenosine which was dissolved to a concentration of 10^{-3} M (all drugs were dissolved in saline) (Euchner-Wamser et al., 1994). Subsequently, the pericardial sac was flushed three times with saline (0.2 ml each) to remove any remaining chemicals. A 20 minute rest period was allowed between intrapericardial injections before testing the response of another cell.

4.3.3. Lumbosacral experiments—The general surgery methods were followed as described under the surgical procedures section.

An 8 cm dorsal midline incision was made in the skin starting from the lower ribs (rib 12) and extending caudally. A laminectomy was performed to expose the L₆ and S₁ segments of the spinal cord. A midline cut was made in the dura and warm agar (37°C, 3–4% in saline) was placed on the L₆–S₁ spinal cord to keep the spinal cord stable during the experiments. The rats were mounted in a stereotaxic frame and clamps were placed on the T₁₃ and L₄ spinous processes to stabilize the animal.

A latex balloon 5 cm in length was tied to the end of a catheter (PE-240, I.D. = 1.97 mm, O.D. = 2.42 mm, length = 35 cm) and inserted into the rectum and descending colon. The catheter was held in place by taping it to the tail. CRD was produced by inflation of the

balloon with air using a sphygmomanometer. The balloon was inflated to both innocuous (20 mmHg) and noxious (40, 60, and 80 mmHg) pressures (Ness et al., 1991). Each distension pressure was applied for 20 seconds with a two minute rest period between distensions. Each cell that was found was tested for its response to CRD.

4.3.4. Neuronal recordings—Rats were paralyzed with an i.p. injection of 0.4 mg/kg pancuronium bromide 10 minutes before the start of the experiment. Supplemental injections (0.2 mg/kg, i.p.) were given as needed to maintain muscle relaxation throughout the course of the experiment.

A glass microelectrode containing a carbon filament was moved into the dorsal horn of the T₃ or L₆-S₁ spinal cord in step-wise increments (5 μ m) using a micropositioner (model 662; Kopf Instruments, Tujunga, CA). Cells were located by the presence of spontaneous activity. Extracellular action potentials of single spinal neurons were recorded in the left dorsal horn 0.5 to 2.0 mm from the midline and up to a depth of 1.2 mm from the surface of the cord. Cell activity was amplified and displayed onto an oscilloscope. A window discriminator was used to isolate single-unit activity and the cell activity was digitized and recorded with a data acquisition system (CED 1401 and Spike2 software; Cambridge Electronics Design, Cambridge, UK). In several cases, the activity of two or three spinal neurons was recorded simultaneously. The Spike2 software program was used to separate the cells so that the responses of each cell could be determined.

4.3.5. Data analysis—Cell activity was evaluated using the Spike2 software program. A cell in the dorsal horn of the T₃ or L₆-S₁ spinal cord was considered to be responsive when the stimulus caused a change in activity that was $\geq 20\%$ of spontaneous activity.

Four quantitative measurements were made to characterize the responses of T₃ spinal neurons to cardiac stimulation. 1) Excitatory or inhibitory changes in neuronal activity were calculated by subtracting the spontaneous activity (impulses/second) that occurred during the 10 seconds before the stimulation from the 10 seconds of maximal activity (impulses/second) that occurred during the stimulation. 2) The total responses (impulses) were the number of impulses that occurred during the duration of the response minus the number of impulses that occurred during a control period of the same duration. 3) Latency of responses (seconds) was measured from the time the stimulus was applied to the time that the activity began to increase. 4) Duration of responses (seconds) was measured from the time the activity began to increase to the time the activity returned to control. Excitatory responses of L₆-S₁ spinal neurons to colorectal distension at each distension pressure were calculated by subtracting the spontaneous activity (impulses/second) that occurred for 20 seconds before the CRD was applied from the neuronal activity (impulses/second) that occurred during the 20 seconds the distension pressure was applied. Statistical analysis of the data was done using unpaired t-tests or repeated measures two-way ANOVA and $p < 0.05$ was considered to be significant. The data are expressed as mean $\pm$ SEM.

4.4. Estradiol assay

One to 1.5 ml of blood was obtained from the carotid artery at the end of each experiment to determine the plasma estradiol concentrations in male and female rats using non-species specific ELISA test kits (Neogen Corporation, Lexington KY). The blood was collected in centrifuge tubes and centrifuged at 12,000 rpm for three minutes to separate blood cells from plasma. The plasma was removed from the blood cells and stored at -20°C until the assays were performed.

Highlights

1. We examined responses of T₃ spinal neurons to cardiosomatic stimulation in male and proestrous female rats.
2. Sex differences in plasma estradiol levels were observed between the groups.
3. No sex differences in spinal neuronal processing of cardiosomatic input.

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Fig. 1. Typical (A) short-lasting excitatory (SL-E) and (B) long-lasting excitatory (LL-E) response patterns of T₃ spinal neurons to saline (control) and noxious cardiac stimulation (algogenic chemicals, AC) observed in male and proestrous female rats. C) Number of T₃ spinal neurons with LL-E and SL-E responses in male and proestrous female rats. Data were analyzed using Fisher's exact test.

Fig. 2. Comparison of short-lasting excitatory (SL-E) and long-lasting excitatory (LL-E) T₃ spinal neurons that responded to noxious chemical cardiac stimulation between male and proestrous female rats. A) Latency B) Duration C) Maximal response and D) Total response. Data were analyzed using unpaired t-test and are presented as mean $\pm$ SEM.

Fig. 3. Characteristics of somatic receptive fields of T₃ spinal neurons that responded to noxious chemical cardiac stimulation in male and proestrous female rats. A) Comparison of the number of T₃ spinal neurons that received both cardiac and somatic input (convergence) and the neurons with only cardiac or somatic input (no convergence) between male and proestrous female rats. B) Comparison of the number of neurons that had somatic fields on the side and chest. Cartoons illustrate the locations of somatic fields (left cartoon, side; right cartoon, chest). C) Comparison of the sizes of somatic fields before and after intrapericardial injection of algogenic chemicals (AC).

Fig. 4.

The maximal excitatory responses of L₆-S₁ spinal neurons to colorectal distension (CRD) were compared at each distension pressure (20, 40, 60, and 80 mmHg) between male, proestrous female, and ovariectomized (OVX) rats. Data were analyzed using two-way repeated measures ANOVA and are presented as mean $\pm$ SEM.

Table 1

Comparison of the characteristics of short-lasting inhibitory (SL-I) and long-lasting inhibitory (LL-I) T₃ spinal neurons that responded to noxious chemical cardiac stimulation between male and proestrous female rats.

	<i>n</i>	Latency (s)	Duration (s)	Maximal response (imp/s)	Total response (imp)
SL-I Male	6	6.0 ± 1.2	12.7 ± 5.1	8.7 ± 1.7	128.2 ± 60.9
SL-I Female	3	9.8 ± 2.8	36.6 ± 16.2	5.4 ± 0.8	141.3 ± 54.3
LL-I Male	3	6.1 ± 0.8	134.9 ± 32.0	8.7 ± 1.9	946.3 ± 162.4
LL-I Female	8	13.5 ± 2.6	113.9 ± 9.4	5.9 ± 1.2	562.5 ± 134.7

Data were analyzed using Student's unpaired t-test and are presented as mean ± SEM. *n* = number of neurons.