Tramadol Reverses the Effects of Neuropathic Pain on Oocyte Maturation and Copulation Ratio in Mice

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ABSTRACT

Objective: Neuropathic pain (NP) is an inescapable stressor that significantly affects both the nervous and endocrine system functions. In this study, we investigated the effect of NP on female reproductive function using the number of oocytes as an index as well as the copulation rates of female mice, with and without males. We also examined whether NP symptoms stopped after injecting tramadol, an opioid analgesic.

Materials and Methods: The partial sciatic nerve was tightly ligated to produce neuropathy, and allodynia was assessed using the cold-plate test. A superovulation protocol was applied to control, sham, neuropathy, and neuropathy+tramadol groups. Each group was divided into two subgroups according to two housing conditions: female alone and female with a male. After inducing superovulation, oocytes/zygotes were isolated from the ampulla of female mice. Total number of oocytes, oocyte maturation, and copulation rates were determined.

Results: The results showed that allodynia, which is a prominent NP symptom, was detected in all neuropathic mice, but tramadol (50 mg/kg, i.p.) stopped these symptoms. The results also showed that NP decreased oocyte maturation and copulation rates of mice, and tramadol reversed all these effects.

Conclusion: In conclusion, we suggest that NP affects reproductive performance by altering the regulation of neuroendocrine mechanisms. Prospective studies that determine the levels of cortisol, fertility hormone, cytokine, and other potential endogenous substances in NP animals are needed to clarify the mechanisms.

Keywords: Neuropathic pain, oocytes, mice, tramadol, oocyte maturation

Introduction

Chronic pain is one of the most debilitating health problems worldwide as it negatively affects daily activities, reduces personal and social productivity, and generates hopelessness. Neuropathic pain (NP) is a particularly troubling subtype of chronic pain that is caused by damage to the somatosensory nervous system; its management is unsatisfactory because of the complex underlying mechanism [1]. Similar to other types of chronic pain, NP is an inescapable stressor that significantly affects the quality of life according to the changes in the physical and mental functioning of patients. NP and chronic pain lead to permanent changes in brain structure and function, which can affect brain processes not directly connected with the pain itself [2]. The endocrine system is particularly affected by NP [3] as NP activates the hypothalamic-pituitary-adrenal-thyroid-gonadal system, which controls the stress mechanism [4]. The relationships between chronic pain (and NP) and stress have been discussed in several recent reviews that have investigated the relevance of the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-gonadotropic (HPG), and corticotrophin-releasing hormone (CRH) axes [5, 6]. As a consequence of the interactions among these axes, chronic pain can affect reproductive function. Although one study on the effects of stress on fertility in dairy cows has been published [7], we found no experimental study that has analyzed the effects of NP on fertility.

In this study, we used an experimental neuropathy model to investigate whether NP affects reproductive parameters in mice by examining the number and maturity of oocytes and the copulation ratio. We also investigated whether tramadol, which has been shown to be an effective agent to treat NP in preclinical and clinical studies and produces an anti-allodynic effect in sciatic nerve-ligated mice [8, 9], could alter the effects of peripheral neuropathy on the oocyte count, oocyte maturity, and the copulation ratio of female mice.

Materials and Methods

Animals

Adult female and male BALB/c mice obtained from Çukurova University, Health Sciences Experimental Application and Research Centre, Turkey, were housed in standard cages (10 mice/cage) with *ad libitum* access to food and water. The mice were maintained in a laboratory under a controlled temperature of 2°C±1°C and a 12-h light/dark cycle; they were assigned randomly to experimental groups after a 2-week habituation period. Behavioral tests were performed during the light cycle. Mice were 4-8 weeks old at the time of the experiments. All procedures were conducted in accordance with protocols approved by the Çukurova University Institutional Laboratory Animal Care and Use Committee and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP).

Peripheral Nerve Injury Procedure

Tight ligation of the partial sciatic nerve was performed to produce neuropathy, as described previously in rats by Seltzer et al. [10]. Briefly, the mice were anaesthetized with ketamine (80 mg/kg, intramuscularly (i.m.) and xylazine (2.5 mg/kg, i.m.). The skin was prepared, and an incision was made to expose the sciatic nerve and its three terminal branches at the upper-thigh level. A tight ligature with an 8-0 silk suture was made at around one-third to one-half of the diameter of the sciatic nerve. The muscle/fascia layer and skin layer were closed separately with 4-0 silk sutures [11]. The nerve was exposed, but not ligated, in sham-operated mice. The cold-plate (CP) test was performed 2 or 4 weeks after surgery.

CP Test

The CP test was used to evaluate allodynia, which is a prominent NP symptom. Allodynia and hyperalgesia are symptoms and signs for indexing pain and thus contribute to improved delineation of NP12. Allodynia refers to pain elicited by a stimulus that normally does not cause pain, according to the IASP. Mechanical (light touch) and heat (hot/cold) stimuli are usually used to measure allodynia. Clinical and animal studies have shown that a cold stimulus is more effective in measuring allodynia [12]. To measure allodynia and track NP development, the CP test was performed using a hot/CP apparatus (Ugo Basile Biological Research Apparatus, Varese, Italy). The CP temperature was kept at 5°C±0.5°C. Each mouse was placed on the metal surface of the apparatus. The latency to first withdrawal behavior (lifting, shaking, licking of one hind paw, or jumping) was measured and recorded as cold-plate latency (CPL). Each mouse was used in one experiment. A maximum cut-off time of 20 s was used to prevent tissue damage at low temperature [13].

Isolation of Oocytes: Isolation of Metaphase I (MI) and Metaphase II (MII) Oocytes

Human chorionic gonadotropin (5 IU hCG) (CG-5; Sigma, St Louis, MO, USA) was injected intraperitoneally (i.p.) into female mice 48 h after injecting pregnant mare serum gonadotropin (PMSG, 5 IU, i.p.) Oocytes were collected from the ampulla under a stereo zoom microscope (SDZ; Kyowa Optical, Nagano, Japan) 18-20 h after hCG injection. Cumulus cells were removed by hyaluronidase (75 mg/mL) treatment. The oocytes were washed three times with 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES)-buffered KSOM+bovine serum albumin (KFHM+BSA) and were classified using a phase-contrast microscope (Eclipse TE2000-U; Nikon, Tokyo, Japan). Oocytes with homogeneous cytoplasm and no polar body were classified as MI, those with homogeneous cytoplasm and one polar body were classified as MII, and those with fragmented cytoplasm were classified as degenerated.

Zygotes: Female mice induced with hCG 48 h after PMSG injection were placed in a cage with a male for copulation. The ampulla of a female mouse with positive vaginal plaque was removed 22 h after hCG injection. Cumulus cells of oocytes/zygotes were removed using hyaluronidase (75 mg/mL) and washed three times with KFHM+BSA. The oocytes were classified as described above. Oocytes containing two polar bodies and/or double pronucleus were classified as zygotes.

Experimental Groups

Female mice were divided into Control (C), Sham (Sh), Neuropathic Pain (NP) and NP+Tramadol (NP+TR) groups. Ten animals were used in each group. The C group did not undergo any surgical intervention. The Sh and NP groups were incised after anesthesia. Following the incision, the sciatic nerve was ligated, as described above, in the NP group but not in the Sh group. The superovulation protocol was performed 2 weeks after sciatic nerve ligation. Each group was divided into two subgroups: female alone and female with a male. One day after the superovulation protocol was performed, vaginal plaque control was made in the female mice caged with male mice. Subsequently, the CP test was performed in all groups. Oocytes obtained from single female mice and those caged with males were classified as MI, MII, degenerated, or zygote according to their morphology. All MII oocytes and zygotes were considered as mature. Oocytes obtained from the single mice were classified as MI, MII, or degenerated; and MII oocytes were considered as mature. The percentages of oocytes/zygotes were calculated. The CP test and superovulation protocol were performed in the groups 2 weeks after surgery to investigate the effect of NP and tramadol on oocytes. Tramadol (50 mg/kg, i.p.) was injected 30 min before the CP test. Tramadol doses was chosen according to similar previous publications generating in our laboratory in similar conditions [14]. The results by experimental group and protocol are summarized in Table 1.

Drugs and Solutions

Ketamine and xylazine were purchased from Sigma and injected i.m. for anesthesia. Tramadol hydrochloride was purchased from Sigma and injected i.p., and HEPES-buffered KFHM solution was prepared as KSOM and used to obtain oocytes and zygotes [15].

Statistical Analysis

The CPL results were analyzed using one-way analysis of variance with a *post-hoc* Bonferroni test. The oocyte classification results were analyzed using the Kruskal-Wallis and Bonferroni-corrected Mann-Whitney U multiple comparison tests. Results are expressed as mean±standard error, and $p<0.05$ was considered significant.

Table 1. Experimental groups and applied protocols

		Surgical procedure					Drug (TR)/SP**	
Group	Housing	Incision	Ligation	SO*	CP	Oocyte (O and C)	Drug	SP
SC	Single	-	-	+	+	+	-	+
MC	With male	-	-	+	+	+	-	+
SSh	Single	+	-	+	+	+	-	+
MSh	With male	+	-	+	+	+	-	+
SNP	Single	+	+	+	+	+	-	+
MNP	With male	+	+	+	+	+	-	+
MNP+TR	Single	+	+	+	+	+	+	-
MNP+TR	With male	+	+	+	+	+	+	-

SC: single female control, MC: female with male control, SSh: single female sham, MSh: female with male sham, SNP: single neuropathic female, MNP: neuropathic female housed with male, SO: superovulation, oocyte (O and C): oocyte collected and classified, TR: tramadol, SP: serum physiological
*: superovulation protocol was performed 2 or 4 weeks after the experiments to investigate the effects of NP on oocyte features and fertility (drug-free experiments), **: drug or SF application and superovulation protocol were performed only 2 weeks after the experiments that investigated the effects of tramadol.

Results

CPL of the C, Sh, NP, and NP+TR Groups

As shown in Figure 1, no differences were observed in CPLs between single females and females kept with males in any group single control (SC) vs. male control (MC), single sham (SSh) vs. male sham (MSh), single NP (SNP) vs. male NP (MNP), and single NP (SNP)+TR vs. male NP (MNP)+TR). CPLs of the NP single females and NP females kept with males were different from those of all other groups ($p<0.05$). Tramadol enhanced CPL in NP single females and NP females kept with males to nearly the same level as that in the C and Sh groups. The CPL of the SNP group was shorter than that of the SC, SSh, and SNP+TR groups (2.21±0.29, 5.53±0.55, 5.04±0.69, and 6.37±0.65, respectively, $p<0.05$). The CPL of the MNP group was shorter than that of the MC, MSh, and MNP+TR groups (2.35±0.43, 7.54±1.16, 5.90±0.60, and 5.55±1.26 respectively; $p<0.05$). These results showed that NP induced by sciatic nerve ligation was prevented by tramadol.

Classification and Number of Oocytes Recovered by Superovulation in the Experimental Groups and the Effects of Tramadol

No differences in total oocyte numbers were observed between single females and females kept with males in the C, Sh, NP, and NP+TR groups [single females: 36.33±4.34, 31.85±3.88, 30.66±5.18, and 27.85±4.70, respectively; females kept with males: 30.77±3.33, 33.25±4.81, 30.00±3.70, and 33.50±4.43, respectively (Table 2). The percentages of oocytes retrieved from all groups after superovulation are shown in Table 3 and Figure 2. The percentage of mature oocytes retrieved from the MNP group was significantly lower than that retrieved from the MC, MSh, and MNP+TR groups (30.24±13.64, 94.79±3.27, 86.67±3.75, and 75.45±6.24, respectively; $p<0.01$). No significant difference was observed in the percentage of mature oocytes among the SNP, SC, SSh, and SNP+TR groups. The copulation ratio of the MNP group was lower than that of the MC, MSh, and MNP+TR groups (44.44%, 100%, 100%, and 85.71%, respectively; $p<0.05$). The percentage of MI oocytes in the MNP group was higher than that in the MC, MSh, and MNP+TR groups (66.09±13.30, 5.28±2.61, 4.63±1.92, and 13.96±6.98, respectively; $p<0.01$). However, no significant difference was observed in the percentage of immature oocytes among the SNP, SC, SSh, and SNP+TR groups. The results according to housing conditions were as follows: the numbers of mature oocytes in the MC, MSh, and MNP+TR groups were higher than those in the SC, SSh, and SNP+TR groups, respectively; however, no difference was observed between the MNP and SNP groups (Table 3 and Figure 2). These results showed that tramadol reversed the negative effects of NP on oocyte maturation and copulation ratio in female mice.

Discussion

The results showed that NP affected oocyte maturation and copulation ratio of female mice and that tramadol reversed the negative effects of NP in female mice. Although no significant difference was observed in the total number of oocytes between NP mice and control mice, oocytes maturation rates decreased significantly in NP female mice housed with male mice compared with those in the control, sham, and tramadol groups.

We used the NP model to induce chronic pain in our experimental mice because of its clinical relevance, convenience of application, and reduced experimenter bias [16]. All NP models that include a manipulation are designed to produce nerve damage [16]. The most common nerve injury target is the sciatic nerve, which is readily accessible and can be easily probed because it innervates the hind limbs. We used a tight ligation of the partial sciatic nerve model because it is easy to implement and is sensitive to cold stimuli and has an appropriate time course of experimental chronic pain [17]. Our team has experience with this model, and all surgeries were performed by the same experienced individual (S.D.), thereby greatly decreasing variability. Sensitivity to cold stimuli develops more robustly in these models, and allodynia and hyperalgesia, which are NP symptoms, become more apparent under cold conditions [12]. Our preliminary results were in line with those of other studies [18] and showed that neuropathy symptoms started during the first week. In the present study, cold allodynia was detected within the first day after the sciatic nerve was ligated and persisted for 2 weeks.

In the present study, CPLs of the Sh and NP mice were lower than those of the C mice. Salo [19] showed that surgical trauma and anesthesia activated pro-inflammatory and anti-inflammatory responses. The shorter CPL in the Sh mice compared with that of the C mice may due to the effect of cytokines induced by incision and anesthesia. On the other hand, no differences were found between single females and females housed with males (C, Sh, and NP groups; Figure 1). In other words, housing female mice with or without males did not affect the degree of allodynia. These results show that ligation of the sciatic nerve induces NP in all female mice, regardless of whether they are housed with males.

The total numbers of oocytes retrieved after superovulation from of the C, Sh, NP, and NP+TR mice did not differ according to whether the females were housed with or without males (Table 2). However, the percentages of immature and mature oocytes differed in NP mice versus C, Sh, and NP+TR mice that were housed with males (MC, MSh, and MNP+TR, respectively; Table 3). The percentage of mature oocytes and the copulation rate was signifi-

Table 2. Total numbers of oocytes in the SC, SSh, SNP, SNP+TR and MC, MSh, MNP, MNP+TR groups

	Control	Sham	NP	NP+TR
Single	36.33±4.34 (n: 6)	31.85±3.88 (n: 7)	30.66±5.18 (n: 6)	27.85±4.70 (n: 7)
With male	30.77±3.33 (n: 9)	33.25±4.81 (n: 6)	30.00±3.70 (n: 6)	33.50±4.43 (n: 6)

SC: single female control; SSh: single female sham; SNP: single neuropathic female; MC: female with male control; MSh: female with male sham; MNP: neuropathic female housed with male; TR: tramadol.

Table 3. Percentage of oocytes values obtained from all groups of mice in the experiments that evaluated the effect of tramadol

	Control		Sham		NP		NP + TR	
% Values	Single	With male	Single	With male	Single	With male	Single	With male
MI	57.55±5.79	5.28±2.61[a]	57.67±8.13	4.63±1.92[a]	73.61±7.29	66.09±13.30*	67.06±7.86	13.96±6.98[a]
MII	32.76±4.31	20.12±9.67	39.93±7.46	19.45±7.35	20.95±6.22	25.28±12.70	27.64±7.74	15.95±6.11
PN	-	74.67±10.70	-	67.22±7.77	-	4.88±2.16	-	59.50±12.94
Degenerated	9.57±3.41	1.63±0.96	2.40±1.66	8.33±2.12	4.38±2.79	3.90±1.46	6.84±2.96	3.30±2.58
Mature	32.76±4.31	94.79±3.27[a]	39.93±7.46	86.67±3.75[a]	20.95±6.22	30.24±13.64*	27.64±7.74	75.45±6.24[a]
Copulation (%)	-	100	-	100	-	44.44**	-	85.71

*, Different from MC, MSh, and MNP+TR groups ($p<0.01$); **, Different from MC, MSh, and MNP+TR groups ($p<0.05$); [a], different from related single groups ($p<0.05$)
PN: pronucleus; MI: metaphase I; MII: metaphase II; MC: female with male control; MSh: female with male sham; MNP: neuropathic female housed with male; TR: tramadol.

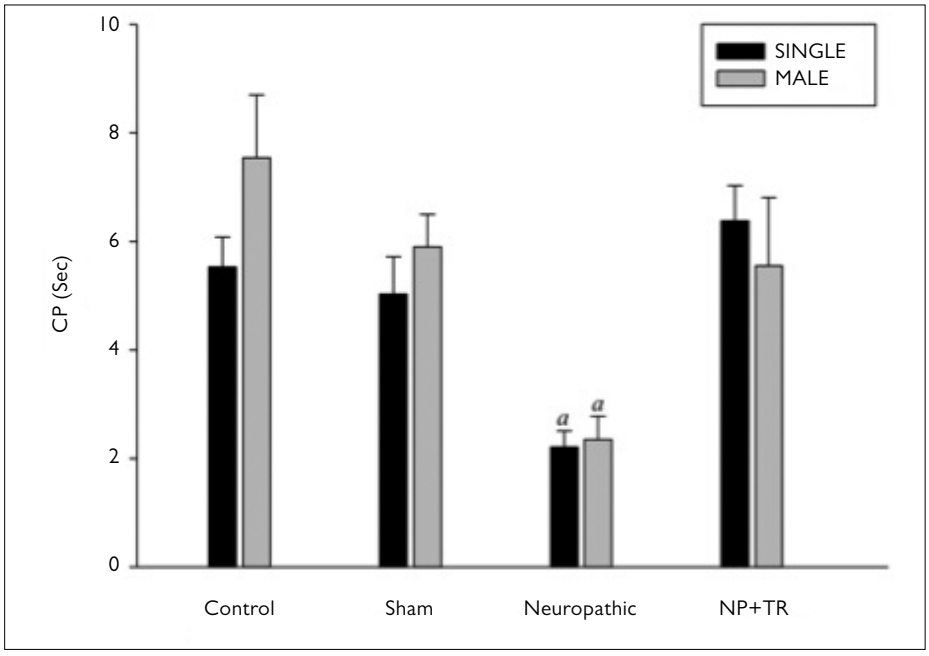

Figure 1. Mean CPLs of the control, sham, neuropathic (NP), and Neuropathic+Tramadol (NP+TR) groups. a, different from the control (C), sham (Sh), and NP+TR groups, p<0.05.

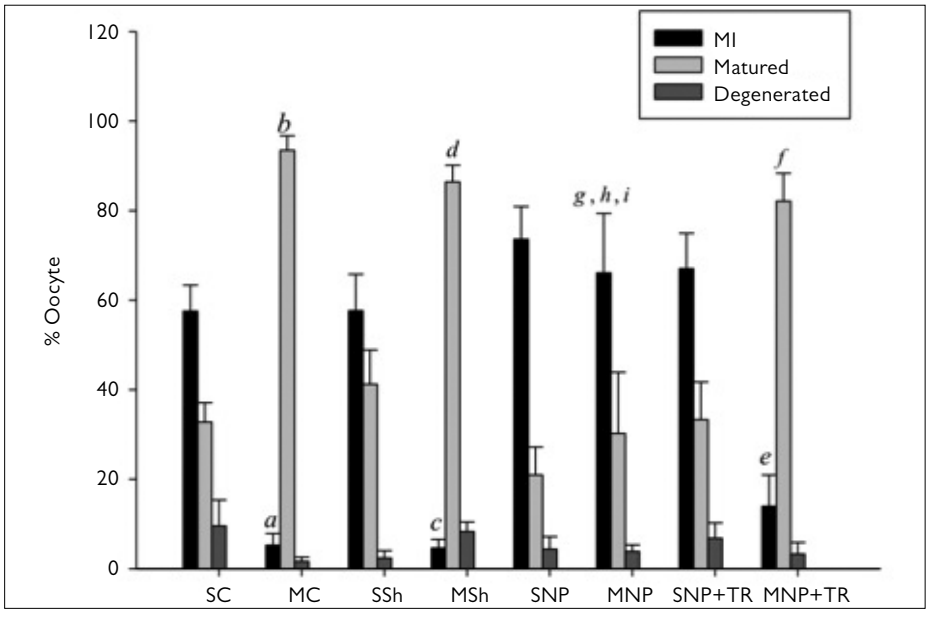

Figure 2. Effect of tramadol (TR) on oogenic processes (percentages metaphase I [MI], mature, and degenerated oocyte)
a, different from single female control (SC) MI, p<0.01; b, different from SC Mature, p<0.01; c, different from single female sham (SSh) MI, p<0.01; d, different from SSh Mature, p<0.05; e, different from single neuropathic female (SNP)+TR MI, p<0.05; f, different from SNP+TR Mature, p<0.05; g, different from MC Mature, p<0.01; h, different from MSh Mature, p<0.01; i, different from neuropathic female housed with male (MNP)+TR Mature, p<0.01).

cantly lower in the MNP group than in the MC, MSh, and MNP+TR groups but not different among the SC, SSh, SNP, and SNP+TR groups. The percentage of immature oocytes in the MNP group was higher than that in the MC, MSh, and MNP+TR groups, but the difference was not significant among the SC, SSh, and SNP groups. We hypothesized that chronic pain negatively affects oocyte maturation by generating stress-induced changes. Interactions between chronic pain and stress have been previously reviewed, and it was shown that stress affects pain and vice versa; however, the mechanisms underlying the effects remain unclear [5, 6]. Continuous pain decreases the quality of life and limits physical and sexual activity [20]; furthermore, chronic pain can be a source of increased stress and tension. On the other hand, stress increases the severity of persistent pain. Blackburn-Munro5 discussed the link among chronic pain, chronic stress, and depression on the basis of experimental and clinical evidence and suggested that these conditions are linked via chronic stress-induced HPA dysfunction. Nerve injury increases spinal glucocorticoid (GC) receptors [6, 21]. Thus, the potential of stress-induced GCs to exert effects on spinal cord neurons or glia is enhanced by nerve injury. Stress and GCs may exacerbate NP by modulating neuroplasticity [22].

Additional underlying mechanisms have been suggested with respect to the relationship between stress and pain. Bravo et al. [23] proposed a locus coeruleus-related mechanism in the chronic pain/depression relationship. They suggested that mild stress triggers several modifications in locus coeruleus-noradrenergic transmission that are exacerbated by co-morbid chronic pain. Several clinical and preclinical studies have shown that chronic pain and stress affect each other via the HPA axis and several other complex neuroendocrine mechanisms. Chapman et al. [24] reported that physical injury or wounding generates a complex stress response that extends beyond the nervous system and contributes to the experience of pain. Chronic pain can lead to permanent changes in brain structures and functions, which in turn can affect brain processes not directly connected with the pain itself [2]. It has been suggested that chronic pain alters immune and endocrine functions [21-25].

On the other hand, stress-induced changes, for example with respect to endocrine, immune, and mechanical factors, reduce fertility, whereas psychological factors, such as depression, anxiety, and stress, affect fertility in women [26]. All of these factors are related to the HPA axis, which is an important mediator of infertility involved in the secretion of CRH, adrenocorticotrophic hormone (ACTH), and cortisol. Changes in diurnal cortisol secretion patterns accompany mental stress and mediate the downregulation of the HPG axis. This effect could involve inhibitory mechanisms at the pituitary level through a reduction in the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) caused by gonadotropin-releasing hormone (GnRH). Furthermore, the effect of cortisol on the HPG axis is dependent on the endocrine status of the ovary at different stages within the menstrual cycle. It has also been suggested that stress alters cortisol secretion patterns during the menstrual cycle, which would ultimately affect the hormonal profile during critical stages of fertilization. The number of oocytes retrieved from patients undergoing IVF treatment who have a high stress score is lower than that retrieved from those with a low stress score [26]. Stress-related factors induced by pain negatively affect the HPA axis and decrease GnRH and FSH/LH release [27]. Severe pain has serious physiological effects on the endocrine system caused by the stimulation of the HPA system, which in turn results in elevated serum ACTH, cortisol, and pregnenolone levels. If pain persists for too long, the hormonal system is unable to tolerate the stress of pain, and hormone production may decrease, thereby causing serum hormone levels to drop below normal [3]. We

argue that in our study, NP persisting for 2 weeks caused severe stress, such that hormone levels and oocyte maturation rates decreased in the mice.

Decreases in the copulation rates and oocyte maturation in female mice housed with male mice may be explained by the pheromone hypothesis. Pheromones are chemicals released by an animal that change the behavior of another animal of the same species [28]. These substances are secreted to trigger many types of behaviors, including sexual arousal. Pheromones released by female mice and perceived by male mice enhance libido and cause the male to exhibit mating behavior. In the present study, discrepancies between the C, Sh, and NP subgroups of female mice housed with male mice may have been due to the negative effects of stress on pheromone secretion. Female mice stressed because of NP may not have been able to release pheromones, which may explain why the copulation rates of our MNP mice were lower than those of the MC and MSh mice. A simpler explanation is that the declined copulation rate may be because of the aggressive behavior of female mice due to inflicted pain.

In our study, tramadol reversed the negative effects of NP on oocyte maturation and copulation ratio in female mice. Tramadol is an effective treatment for NP that exerts analgesic activity by inhibiting neuronal uptake of norepinephrine and serotonin and activating opioid receptors [29]. Another possible mechanism underlying tramadol-induced analgesia is the attenuation of thermal and mechanical hyperalgesia and allodynia through its effect on pro-inflammatory cytokines. Nerve injury increases the expression and secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-1b, IL-6, and interferon (INF)-γ, all of which are required for pain hypersensitivity. Pro-inflammatory cytokine levels are high in patients with NP, whereas levels of anti-inflammatory cytokines, such as IL-10, are low. Tramadol decreases the release of pro-inflammatory cytokines during NP [30]. In addition, some cytokines affect female fertility. Women with reproductive failure have increased IL-10 and INF-γ levels. IL-12 is positively correlated with fertilization rate [31]. In our study, we could not investigate the possible mechanisms underlying our results. Further research is needed to elucidate the mechanisms of the effects of NP and tramadol on oocyte maturation and copulation ratio in female mice.

In conclusion, we suggest that NP affects reproductive performance by altering various endogenous mechanisms, such as the regulation of the HPA axis and noradrenergic/serotonergic or cytokine-related pathways. Prospective studies that determine the levels of cortisol, fertility hormones, cytokines, and other potential endogenous substances in neuropathic animals are needed to clarify the roles of possible mechanisms in reproduction.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Çukurova University Institutional Laboratory Animal Care and Use Committee.

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Conflict of Interest: Authors have no conflicts of interest to declare.

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References

1. O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am J Med 2009; 122: 22-32. [CrossRef]
2. Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. J Neurosci 2008; 28: 1398-403. [CrossRef]
3. Tennant F. The physiologic effects of pain on the endocrine system. Pain Ther 2013; 2: 75-86. [CrossRef]
4. Akil H, Shiomi H, Mathews J. Induction of the intermediate pituitary by stress: synthesis and release of a non-opioid form of beta-endorphin. Science 1985; 227: 424-8. [CrossRef]
5. Blackburn-Munro G, Blackburn-Munro RE. Chronic pain, chronic stress and depression: coincidence or consequence? J Neuroendocrinol 2001; 13: 1009-23. [CrossRef]
6. Alexander JK, DeVries AC, Kigerl KA, Dahlman JM, Popovich PG. Stress exacerbates neuropathic pain via glucocorticoid and NMDA receptor activation. Brain Behav Immun 2009; 23: 851-60. [CrossRef]
7. Dobson H, Smith RF. What is stress, and how does it affect reproduction? Anim Reprod Sci 2000; 60-1: 743-52. [CrossRef]
8. Caspani O, Reitz MC, Ceci A, Kremer A, Treede RD. Tramadol reduces anxiety-related and depression-associated behaviors presumably induced by pain in the chronic constriction injury model of neuropathic pain in rats. Pharmacol Biochem Behav 2014; 124: 290-6. [CrossRef]
9. Sakakiyama M, Maeda S, Isami K, et al. Preventive and alleviative effect of tramadol on neuropathic pain in rats: roles of α_2-adrenoceptors and spinal astrocytes. J Pharmacol Sci 2014; 124: 244-57. [CrossRef]
10. Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 1990; 43: 205-18. [CrossRef]
11. Malmberg AB, Basbaum AI. Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. Pain 1998; 76: 215-22. [CrossRef]
12. Jensen TS, Finnerup NB. Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms. Lancet Neurol 2014; 13: 924-35. [CrossRef]
13. Jasmin L, Kohan L, Franssen M, Janni G, Goff JR. The cold plate as a test of nociceptive behaviors: description and application to the study of chronic neuropathic and inflammatory pain models. Pain 1998; 75: 367-82. [CrossRef]
14. Mete S, Aksu F. The role of nitrergic system on the effects of tramadol and agmatine in an experimental neuropathic pain model. Çukurova Med J 2015; 40: 42-54. [CrossRef]
15. Biggers JD. Reflection on the culture of the preimplantation embryo. Int J Dev Biol 1998; 42: 879-4.
16. King T, Porreca F. Preclinical Assessment of Pain: Improving Models in Discovery Research. BK Taylor, D Finn, editors. Behavioral Neurobiology of Chronic Pain. Berlin: Springer; 2014.p.101-20.
17. Kim KJ, Yoon YW, Chung JM. Comparison of three rodent neuropathic pain models. Exp Brain Res 1997; 113: 200-6. [CrossRef]
18. Walczak JS, Beaulieu P. Comparison of three models of neuropathic pain in mice using a new method to assess cold allodynia: the double plate technique. Neurosci Lett 2006; 399: 240-4. [CrossRef]
19. Salo M. Effects of anaesthesia and surgery on the immune response. Acta Anaesthesiol Scand 1992; 36: 201-20. [CrossRef]
20. Stewart WF, Ricci JA, Chee E, Morganstein D, Lipton R. Lost productive time and cost due to common pain conditions in the US workforce. JAMA 2003; 290: 2443-54. [CrossRef]
21. Wang S, Lim G, Zeng Q, Sung B, Yang L, Mao J. Central glucocorticoid receptors modulate the expression and function of spinal NMDA receptors after peripheral nerve injury. J Neurosci 2005; 25: 488-95. [CrossRef]
22. Joëls M, Krugers HJ. LTP after stress: up or down? Neural Plast 2007; 93202. [CrossRef]
23. Bravo L, Alba-Delgado C, Torres-Sanchez S, Mico JA, Neto FL, Berrocoso E. Social stress exacerbates the aversion to painful experiences in rats exposed to chronic pain: the role of the locus coeruleus. Pain 2013; 154: 2014-023. [CrossRef]
24. Chapman CR, Tuckett RP, Song CW. Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. J Pain 2008; 9: 122-45. [CrossRef]
25. Anderson RU, Orenberg EK, Morey A, Chavez N, Chan CA. Stress induced hypothalamus-pituitary-adrenal axis responses and disturbances in psychological profiles in men with chronic prostatitis/chronic pelvic pain syndrome. J Urol 2009; 182: 2319-24. [CrossRef]
26. Ebbesen SM, Zachariae R, Mehlsen MY, et al. Stressful life events are associated with a poor in-vitro fertilization (IVF) outcome: a prospective study. Hum Reprod 2009; 24: 2173-82. [CrossRef]
27. Törnhage CJ. Salivary cortisol for assessment of hypothalamic-pituitary-adrenal axis function. Neuroimmunomodulation 2009; 16: 284-89. [CrossRef]
28. Ozgunen KT, Erdogan S, Mazmanoglu N, Pamuk I, Logoglu G, Ozgunen T. Effect of gonadotrophin dose on oocyte retrieval in superovulated balb/c mice. Theriogenology 2001; 56: 435-45. [CrossRef]
29. Berrocoso E, De Benito MD, Mico JA. Role of serotonin 5-HT1A and opioid receptors in the antiallodynic effect of tramadol in the chronic constriction injury model of neuropathic pain in rats. Psychopharmacology (Berl) 2007; 193: 97-105. [CrossRef]
30. Kraychete DC, Sakata RK, Issy AM, Bacellar O, Jesus RS, Carvalho EM. Proinflammatory cytokines in patients with neuropathic pain treated with tramadol. Rev Bras Anestesiol 2009; 59: 297-303. [CrossRef]
31. Sarapik A, Velthut A, Haller-Kikkatalo K, et al. Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clin. Develop. Immunol 2012; 606459. [CrossRef]