Basic and Clinical NEUROSCIENCE

September, October 2021, Volume 12, Number 5

Research Paper: Evaluation of the GABAA Receptor Expression and the Effects of Muscimol on the Activity of Wide Dynamic Range Neurons Following Chronic Constriction Injury of Sciatic Nerve in Rats

Mehdi Sadeghi[1], Homa Manaheji[2,3*], Jalal Zaringhalam[2,3], Abbas Haghparast[4], Samad Nazemi[5], Zahra Bahari[6], Seyed Mohammad Noorbakhsh[7]

1. Department of Physiology, Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran.
2. Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Department of Physiology, Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
6. Department of Physiology and Medical Physics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
7. Science Beam Institute, Tehran, Iran.

Use your device to scan and read the article online

Citation: Sadeghi, M., Manaheji, H., Zaringhalam, J., Haghparast, A., Nazemi, S., & Bahari, Z., et al. (2021). Evaluation of the GABAA Receptor Expression and the Effects of Muscimol on the Activity of Wide Dynamic Range Neurons Following Chronic Constriction Injury of Sciatic Nerve in Rats. *Basic and Clinical Neuroscience, 12*(5), 651-666. http://dx.doi.org/10.32598/bcn.2021.1726.1

http://dx.doi.org/10.32598/bcn.2021.1726.1

Article info:
Received: 09 May 2019
First Revision: 05 Aug 2020
Accepted: 02 Aug 2021
Available Online: 01 Sep 2021

Keywords:
Neuropathic pain, Chronic Constriction Injury (CCI), γ-Aminobutyric acid type A (GABAA) receptor, Muscimol, Wide Dynamic Range (WDR) neurons

ABSTRACT

Introduction: The modality of γ-aminobutyric acid type a receptors (GABAA) controls dorsal horn neuronal excitability and inhibits sensory information. This study aimed to investigate the expression of the GABAA receptor and the effects of its agonist muscimol on Wide Dynamic Range (WDR) neuronal activity in the Chronic Constriction Injury (CCI) model of neuropathic pain.

Methods: Adult male Wistar rats weighing 200 to 250 g were used to induce CCI neuropathy. Fourteen days after surgery, muscimol (0.5, 1, and 2 mg/kg IP) was injected. Then, the behavioral tests were performed. After that, the animals were killed, and the lumbar segments of the spinal cords were collected for Western blot analysis of the GABAA receptor α1 subunit expression. The electrophysiological properties of WDR neurons were studied by single-unit recordings in separate groups 14 days after CCI.

Results: The outcomes indicated the development of thermal hyperalgesia and mechanical allodynia after neuropathy; nonetheless, the expression of the GABAA receptor α1 subunit did not change significantly. Moreover, the evoked responses of the WDR neurons to electrical, mechanical, and thermal stimuli increased considerably. Fourteen days after CCI, muscimol administration decreased thermal hyperalgesia, mechanical allodynia, and hyperresponsiveness of the WDR neurons in CCI rats.

Conclusion: The modulation of the spinal GABAA receptors after nerve injury can offer further insights to design new therapeutic agents to reduce neuropathic pain symptoms.

*** Corresponding Author:**
Homa Manaheji, PhD.
Address: *Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Tel: *+98 (912) 1856257*
E-mail: *manahejih@sbmu.ac.ir, hshardimanaheji@yahoo.com*

Highlights

- Behavioral symptoms of neuropathic pain and the evoked responses of WDR neurons to stimuli significantly increased after CCI.
- Muscimol reduced behavioral signs and WDR neuronal excitability in neuropathic rats.
- CCI did not affect the expression of spinal GABAA receptors.

Plain Language Summary

The present study was used to investigate the expression of GABAA receptor and the effects of its agonist muscimol on modulation of neuropathic pain and Wide Dynamic Range (WDR) neuronal activity of spinal dorsal horn neurons in an animal model of neuropathy called chronic constriction injury (CCI) model of neuropathic pain. Adult male rats were used for the induction of neuropathy. Behavioral tests including thermal hyperalgesia (sensitivity to noxious heat stimulation) and mechanical allodynia (sensitivity to nonnoxious mechanical stimuli) for pain evaluation were performed by plantar test and Von Frey filaments, respectively. Fourteen days after surgery, muscimol (0.5, 1, and 2 mg/kg i.p.) was injected. After behavioral tests, the animals were sacrificed, and the lumbar segments of the spinal cords were collected for molecular analysis of the GABAA receptor expression. The electrophysiological properties of WDR neurons were studied on the 14th day after neuropathy. The results indicated the unusual pain symptoms after neuropathy. Electrophysiological activity of dorsal horn neurons was also increased after neuropathy, but GABAA receptor expression did not change significantly; however muscimol administration alleviated behavioral signs of pain and electrophysiological hyperexcitability of WDR neurons. It seems that modulation of the spinal GABAA receptor after neuropathy can open a new area to design new therapeutic agents for reduction of neuropathic pain.

1. Introduction

Chronic neuropathic pain due to peripheral nerve injury is a clinical disorder characterized by allodynia, hyperalgesia, and spontaneous burning pain (Clark, Old, & Malcangio, 2013; Gosselin, Bebber, & Decosterd, 2010; Polgar & Todd, 2008). The mechanisms, treatment, and management of painful neuropathy are still unclear in neuroscience. Several studies propose that the loss of γ-aminobutyric acid (GABA) inhibitory effect in the superficial laminae of the spinal dorsal horn could cause neuropathic pain following peripheral nerve injury (Janssen, Truin, Van Kleef, & Joosten, 2011; Miletic & Miletic, 2008). GABAergic interneurons inhibit the nociceptive information of primary afferents via GABA type A (GABAA) and GABAB receptors in the dorsal horn of the spinal cord (Gwak & Hulsebosch, 2011; Sokal & Chapman, 2003). Furthermore, GABAA receptors are expressed on Aδ and C afferent fiber terminals within the dorsal horn (Sokal & Chapman, 2003). Their activation could induce presynaptic inhibition (Naik, Pathirathna, & Jevtovic-Todorovic, 2008) and reduce hyperalgesia after peripheral nerve injury (da Motta, Veiga, Francischi, & Tatsuo, 2004). Recent molecular studies have identified the decreased expression of mRNA and protein of the GABAA receptor α1 subunit in the spinal cord of mice following formalin-induced pain (Yang et al., 2012). However, some experiments showed no significant change in the expression of GABAA receptors in the spinal cord after nerve injury (Polgar & Todd, 2008). It has also been reported that the level of GABAA receptors does not change in rats with Chronic Constriction Injury (CCI) 4 h after the sciatic nerve ligation (Miletic & Miletic, 2008). There is also evidence that GABA can become an excitatory neurotransmitter in neuropathic pain, and excess GABAergic transmission can lead to hyperexcitability and sensitization (Coull et al., 2003). The relative contribution of all these processes to various forms of neuropathic pain remains to be elucidated. One way to verify the spinal GABA involvement in the development of neuropathic pain is to examine the changes of spinal GABAergic inhibition, which depends on GABA level and GABA receptor activity. Hence, control of spinal GABA levels could effectively treat neuropathic pain following nerve injury (Lee, Nam, Jung, Gwak, & Leem, 2015).

Spinal Wide Dynamic Range (WDR) neurons relay and encode sensory information. Besides, these neurons mediate behavioral nociceptive responses (Zheng, Chen, & Arendt-Nielsen, 2004). Dorsal horn neurons

designate high-frequency responses to thermal and mechanical stimulation of the receptive fields and show high background activity after neuropathic pain (Miki et al., 2000). Some electrophysiological reports in different animal models of neuropathic pain have demonstrated abnormal hyper-responsiveness, receptive field enlargement (Takaishi, Eisele, & Carstens, 1996), and increased spontaneous activity of WDR neurons following nerve injuries (Pertovaara, Kontinen, & Kalso, 1997). These abnormal features demonstrate prominent hyperexcitability in WDR neurons, which explains the neuronal basis for the behavioral symptoms of allodynia and hyperalgesia in neuropathy (Yakhnitsa, Linderoth, & Meyerson, 1999).

Animal studies have reported reduced responsiveness to noxious stimuli after administering the GABAA receptor agonist, muscimol. Direct involvement of GABAA receptors in persistent pain states is demonstrated by the attenuating action of muscimol on thermal hyperalgesia and mechanical allodynia in rats with Spared Nerve Injury (SNI) model of neuropathic pain (Rode, Jensen, Blackburn-Munro, & Bjerrum, 2005). It has also been reported that muscimol administration reduced mechanical allodynia in the rat tail model of peripheral neuropathy (Lee et al., 2010) and alleviated electrically evoked A and C-fiber neuronal responses in nerve-injured rats (Sokal & Chapman, 2003). In addition, a recent study indicated that in the chronic compression of the dorsal root ganglion (CCD) neuropathic model, muscimol suppressed the spinal WDR neuronal excitability in the early phase of CCD (post-CCD week 1) (Lee, Nam, Jung, Gwak, & Leem, 2015). On the other hand, it has been shown that in normal and allodynic rats, the GABAB agonist baclofen (0.1 mg/kg IP) administered 1 to 3 h before the neuronal recording suppressed the responses of WDR neurons to high-intensity mechanical pressure. Nonetheless, muscimol (1 mg/kg IP) cannot affect the reactions of WDR neurons in either normal or allodynic animals (Hao, Xu, Yu, Seiger, & Wiesenfeld-Hallin, 1992).

Indeed, the involvement of the GABAergic system in the development of neuropathic pain is quite controversial. Therefore, our study focused on GABAA receptors and assessed the expression of the GABAA receptor α1 subunit and the effect of its agonist muscimol on thermal hyperalgesia and mechanical allodynia in CCI-induced neuropathic rats. A limited number of electrophysiological studies have examined the impact of GABA receptor activation on the firing rate of spinal cord WDR neurons after peripheral nerve injury. To the best of our knowledge, the effects of muscimol on the hyperexcitability of WDR neurons following chronic constriction nerve injury have not been well studied before. Hence, the present study examined the effects of muscimol administration on natural and electrical evoked responses of spinal dorsal horn WDR neurons after loose ligation of the sciatic nerve.

2. Methods

Study animals

Seventy-one adult male Wistar rats (Pasture Institute, Tehran, Iran) weighing 200 to 250 g were used in this study. All animals were housed in cages under a standard 12/12 h light/dark cycle with food and water ad libitum. The entire procedure was performed according to the IASP recommendations and the ethical guideline standards for investigations of experimental pain in animals (Zimmermann, 1983). In addition, all efforts were made to use the lowest number of animals for the experiments and minimize animal suffering in this study.

Neuropathic pain induction

CCI of the sciatic nerve was performed according to the Bennett and Xie method (Bennett & Xie, 1988). Briefly, the animals were anesthetized with sodium pentobarbital (60 mg/kg IP), and their right sciatic nerves were exposed at the mid-thigh level. Thus, four 4-0 chromic gut ligatures were loosely tied around the nerve with a 1 mm interval until a fine twitch was observed in the respective hind limb. After that, the muscle and skin were sutured with 4-0 silk thread. In sham-operated controls, the right sciatic nerve was exposed and freed from the surrounding connective tissue, but it was not ligated.

Thermal hyperalgesia

Thermal hyperalgesia was assessed using a plantar test (Ugo Basile, Italy). Briefly, the rats were placed in a transparent Plexiglas container and habituated there for 30 min. After habituation, a radiant heat source (50-W halogen reflector bulbs with intensity controlled by a constant voltage source) was focused on the plantar surface of the ipsilateral hind paw. The time between heat onset and paw withdrawal was recorded as Paw Withdrawal Latency (PWL). Each hind paw was tested three times at 5 min intervals, and the average value of the withdrawal latency for the three consecutive tests was recorded. In the absence of a response, a cut-off time of 33 s was set to avoid tissue damage.

Mechanical allodynia

Mechanical allodynia was measured by the von Frey test. Before the test, the rats were positioned in a transparent Plexiglas box on a metal mesh floor, allowing access to the plantar surface of the injured hind paw. They were permitted to acclimate for 30 minutes. Mechanical sensitivity for the ipsilateral hind paw was determined by measuring Paw Withdrawal Threshold (PWT) to von Frey monofilaments (2, 8, 15, 26, and 60 g, Stoelting Co. Wood Dale, IL, USA). The calibrated monofilaments were applied from below through the mesh floor onto the plantar surface of the injured hind paw. The von Frey monofilaments were applied three times at intervals of 5 min with approximately 1 s holding for each filament. The lowest von Frey monofilament that produced a quick withdrawal or flinching two out of three applications was the paw withdrawal threshold. The cut-off point was considered 60 g to prevent tissue damage.

Electrophysiological recording

In vivo, the extracellular single-unit recording was performed 14 days after CCI, as described earlier (Nazemi et al., 2015). Briefly, all animals were anesthetized with 2.0% to 2.5% isoflurane in 66% N_2O and 33% O_2 gaseous mixture. Surgery was initiated upon the loss of corneal and cutaneous reflexes. Throughout the experimental period, rectal temperature was continuously monitored and maintained at 36.5 °C to 37.5 °C using a heating pad (Borje Sanat Co. Tehran, Iran). Rats were secured in a stereotaxic frame. A laminectomy was performed to expose the lumbar enlargement of the spinal cord for recording. The vertebral column was clamped rostral and caudal to the exposed section using rat vertebral clamps to ensure stability during electrophysiological recording. Then, the dura of the exposed spinal cord was removed. The isoflurane concentration was lowered to 1.5%–2% while performing surgery and then held at 1%–1.2% during the experiment. At the end of the investigation, the animals were killed with an overdose of isoflurane.

A parylene-coated tungsten electrode was used to make single-unit extracellular recordings of the dorsal horn WDR neurons that received afferent input from receptive fields in the toe regions and the plantar surface of the ipsilateral hind paw. The electrode was lowered directly into the spinal cord approximately 1 mm lateral to the spinal midline by using a SCAT microdrive (Digitimer, Welwyn Garden City, UK) which allowed estimating the depth of the neuron from the dorsal surface of the spinal cord. The electrode was driven in 10 µm steps into the spinal cord. Single-unit recordings were made by exploring the dorsal horn while applying mechanical stimuli onto the peripheral receptive field. Light stroking and probing the skin at the hind paw ipsilateral to the recording site (plantar surface of the ligated hind paw in CCI animals) was used as a search stimulus to identify a WDR dorsal horn neuron. A dorsal horn unit was identified as a WDR neuron based on its characteristic responses to mechanical stimuli applied to the receptive field. Only units that responded to noxious (pinch) and non-noxious or innocuous (brush) mechanical stimuli were selected for the study. The neurons of this experiment were located at depths of 300-1000 µm from the spinal cord's dorsal surface. We did not include the spontaneously active neurons with no clear receptive fields.

After isolating WDR dorsal horn neurons, their receptive fields were stimulated by electrical, mechanical, and thermal stimuli. Electrical stimulation consisted of a train of 16 transcutaneous electrical stimuli (2-ms wide pulses, 0.5 Hz), which were applied using two pins inserted into the center of the receptive field, at three times the threshold current for the C-fiber responses. A Post-Stimulus Time Histogram (PSTH) was constructed, and the reactions of different fibers were separated according to their latencies: Aß-fiber (0–20 ms), Aδ-fiber (20–90 ms), and C-fiber (90–300 ms). All of the responses that occurred after the C-fiber latency (90–300 ms) were characterized as post-discharge (300–800 ms). Wind-up was calculated as the total number of action potentials evoked at three times the C-fiber threshold after all 16 stimuli minus the baseline response (input spikes multiplied by 16). Input spikes were the number of spikes in the range of C-fiber latency elicited by the first electrical stimulus.

The peripheral neuronal receptive field was also stimulated using a range of noxious and innocuous natural stimuli, including brush, mechanical punctuate (von Frey filaments 2-60 g), pinch, and heat (35°C-50°C) for 10 s. The heat was applied with a constant water jet onto the center of the receptive field. Time intervals of 20 s and 90 s were left between mechanical and thermal stimuli, respectively, to prevent sensitization of the hind paw receptive field. The spontaneous activity of the neuron was taken into account by recording the number of action potentials for 10 s before each stimulus. This background activity was then subtracted from the response to mechanical and thermal stimuli. Furthermore, the number of spikes evoked by the 35°C water was subtracted from the number of action potentials evoked by the 40°C, 45°C, and 50°C water to detect the response of WDR neurons to heat.

The unit activity was amplified and filtered at 0.3–5 kHz (Electromodule; Science Beem, Iran) and fed directly into the data acquisition unit (Electromodule; Science Beem, Iran), stored on a Pentium 4 computer to construct the stream type of recording or plot the PSTH. The collected data were analyzed using the Plexon Offline Sorter (version 2.88, Plexon Inc, Dallas, TX, USA) and NeuroExplorer (version 4.109, Nex Inc, Madison, AL, USA).

Western blotting

After behavioral tests on Postoperative Day (POD) 14, the animals were killed under isoflurane anesthesia, and the lumbar (L5–L6) regions were rapidly removed. Tissue samples were homogenized (Brinkmann Polytron Homogenizer, 20000 rpm for 30 s at 4°C, Kinematica AG, Lucerne, Switzerland) in RIPA buffer (50 mmol/L Tris–HCl, pH 7.5, 150 mmol/L NaCl, 1 mmol/L Ethylenediaminetetraacetic Acid (EDTA), 1% NP40, 0.5% Sodium Dodecyl Sulfate (SDS), 1 mmol/L sodium orthovanadate, 2.5 µg/mL aprotinin, 2 µg/mL leupeptin, 2 µg/mL pepstatin A) and cleared by centrifugation (10000 ×g, 15 °C, 10 min). The protein concentration in the supernatant was determined using a Bradford assay. Equal amounts of samples (50 µg of protein) was heated for 8 min at 99°C in loading buffer (4% SDS, 25 mmol/L Tris–HCl, pH 6.8, 5% glycerol, 0.5% 2-mercaptoethanol, 0.01% bromophenol blue) and resolved by SDS-PAGE on 10% polyacrylamide gels (120 V for 60 min). After gel electrophoresis, the proteins were electrophoretically shifted onto Poly(vinylidene fluoride) membranes (Millipore, Bedford, MA, USA) using the Mini-PROTEAN II (Bio-Rad, Hercules, CA, USA) at 100 V for 85 min. Nonspecific binding sites were blocked by incubating the membrane in 2% blocking buffer (0.2% Aurora blocking reagent) in Tris-buffered saline with 0.1% Tween-20 for 90 min at 24ºC. After that, the membranes were incubated overnight at 4°C with mouse monoclonal primary antibody of the GABAA receptor α1 subunit (diluted 1:5000, Abcam, Cambridge, UK). After three 10-min washes in TBS and 0.1% Tween-20, the blots were incubated (1 h at room temperature) with secondary antibody in blocking buffer (anti-mouse, diluted 1:10000, Abcam) for protein detection.

Following three consecutive 10-min washes in TBS and 0.1% Tween-20 and one-time wash in TBS, the immunoreactivity of the proteins on the membranes were detected with a chemiluminescence detection system (ECL, Amersham, Freiburg, Germany). The membranes were subsequently incubated in stripping buffer (100 µmol/L 2-mercaptoethanol, 2% SDS, 62.5 mmol/L Tris-HCl with pH 6.7) at 50°C for 30 min and reprobed with mouse monoclonal β-actin primary antibody (diluted 1:5000, Abcam) to confirm equivalent loading. The densities of target protein blots and β-actin immunoreactive bands were analyzed by ImageJ software (V1.41, NIH, Bethesda, MD, USA) after background subtraction. Target protein levels were normalized against β-actin levels and expressed as relative fold changes compared to the sham group.

Experimental protocol and drug administration

Rats were randomly assigned to 8 groups (five groups of behavioral experiments and three groups of electrophysiological experiments). The behavioral groups were included (i) a sham group; (ii) a group subjected to CCI and was injected with normal saline; and (iii) three groups subjected to CCI and were injected with muscimol (0.5, 1, and 2 mg/kg, dissolved in 0.9% saline, Tocris, UK, IP) on day 14 after surgery. There were eight rats in each group. Thirty minutes after drug or saline administration, the behavioral tests were applied. Then, in the sham and vehicle (saline)-treated CCI groups, lumbar segments were removed for Western blot analysis of the GABAA receptor α1 subunit expression. In the electrophysiological experiments, 31 rats were randomly divided into three groups: (i) a sham group (n=8), (ii) a group (n=12) subjected to CCI and injected with normal saline; (iii) a group (n=11) subjected to CCI and injected with muscimol (2 mg/kg, IP). Likewise, the electrophysiological recordings were performed 30 minutes after administration.

Statistical analysis

The values were expressed as Mean±Standard Error of the Mean. One-way ANOVA, followed by Bonferroni post-hoc test, was used to determine significant differences between the groups in behavioral studies. Unpaired Student t test was also used to compare the results obtained from groups in the electrophysiological and molecular experiments. P-values less than 0.05 were considered significant.

3. Results

Thermal hyperalgesia and mechanical allodynia in CCI rats after acute administration of muscimol

Ipsilateral paw withdrawal latency measured by noxious radiant heat (plantar test) and paw withdrawal threshold to non-noxious stimuli (von Frey hairs) significantly decreased in the CCI group compared to sham-operated animals on POD 14 (P<0.001) (Figure 1). Intraperitoneal

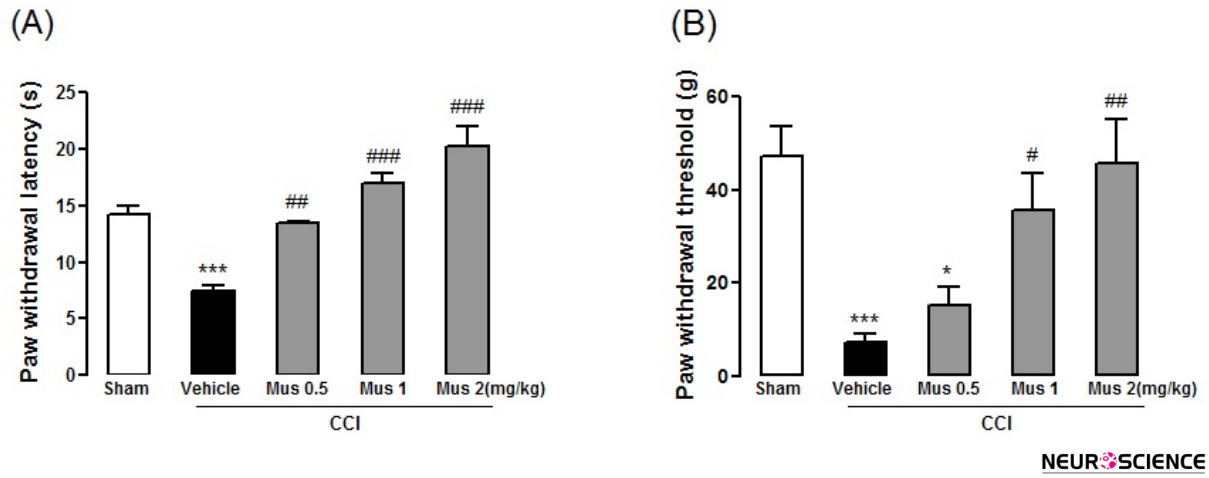


Figure 1. The effect of post-injury administration of muscimol (0.5, 1, and 2 mg/kg) on the development of

A: Thermal hyperalgesia; and B: Mechanical allodynia on POD 14.

At least 30 min after drug administration, hyperalgesia and allodynia were evaluated (n=8). Intergroup differences were assessed by 1-way ANOVA followed by Bonferroni post-hoc test.

*P<0.05, ***P<0.001, significant difference with sham values; #P<0.05, ##P<0.01, ###P<0.001, significant difference with vehicle-treated CCI values.

CCI: Chronic constriction injury; POD: Postoperative day.

muscimol administration at doses of 0.5 (P<0.01), 1, and 2 mg/kg (P<0.001) significantly attenuated thermal hyperalgesia (Figure 1-A) and also alleviated the development of mechanical allodynia in CCI rats (Figure 1-B) at doses of 1 (P<0.05) and 2 mg/kg (P<0. 01) on POD 14. Therefore, rats administered muscimol had a higher PWL and PWT than the vehicle-treated CCI group.

Alterations in electrical and naturally evoked responses of WDR neurons in CCI rats

Comparing the responses of WDR neurons in vehicle-treated CCI rats with sham-operated animals indicated a significant increase of Aδ-fiber evoked responses (P<0.001), C-fiber evoked responses (P<0.05), post-discharge (P<0.001), input spikes (P<0.001), and wind-up of spinal neurons (P<0.05) in vehicle-treated CCI rats. Nonetheless, there was no noticeable difference in the Aß-fiber evoked responses of spinal WDR neurons between the two mentioned groups (Figure 2).

When the mean neuronal activities of spinal WDR neurons were compared between two groups, it was found that the neuronal responses were evoked by innocuous mechanical stimuli, including brush (P<0.05), 2 g (P<0.01), and 8 g von Frey filaments (P<0.05) and also to noxious mechanical stimuli, including 15 g (P<0.01), 26 g (P<0.05), 60 g von Frey filaments (P<0.05), and pinch (P<0.01) which were greater in nerve ligated rats (Figure 3).

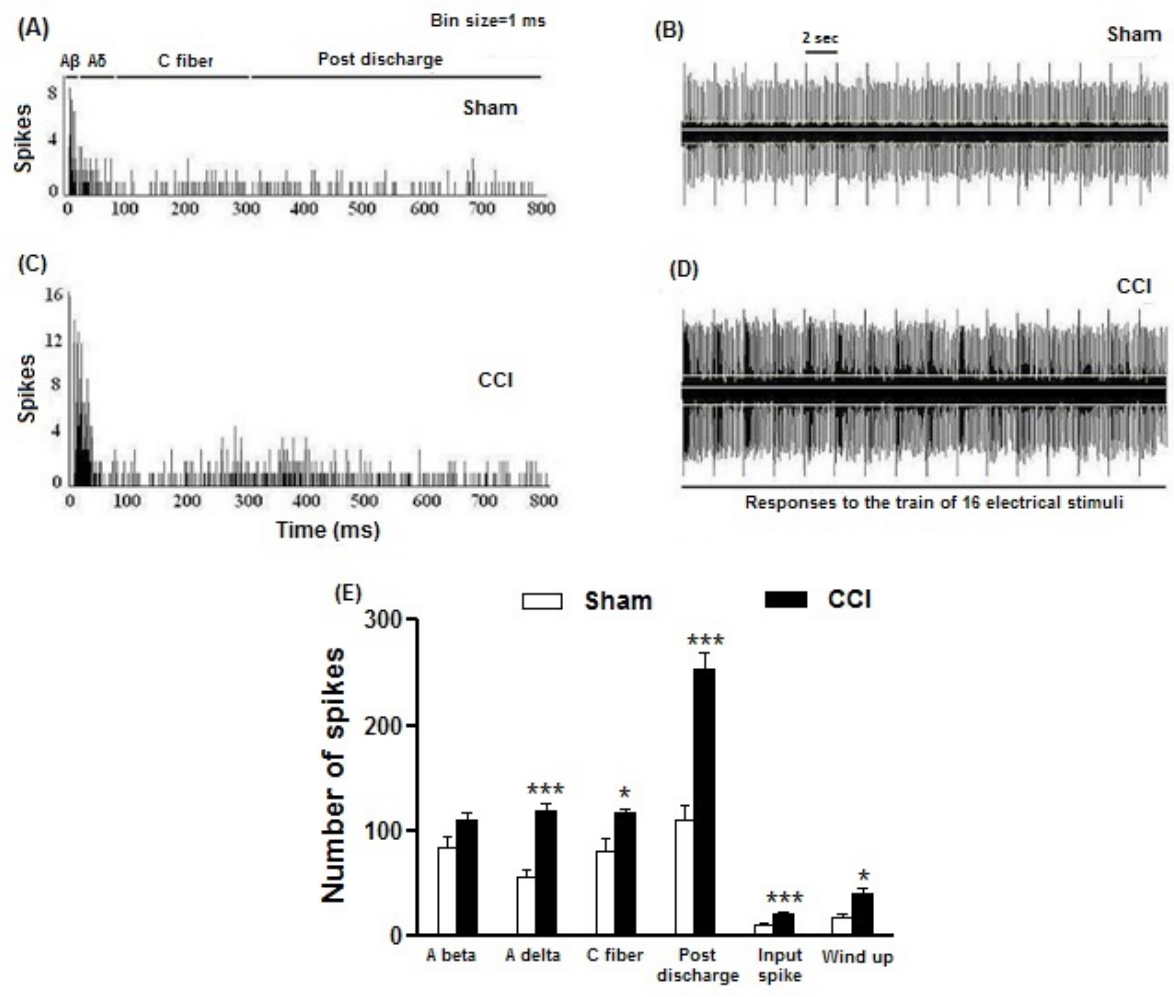
Moreover, WDR neurons evoked responses to heat in neuropathic rats, demonstrated a significant increase to noxious thermal stimuli, including 45 °C (P<0.05) and 50 °C (P<0.001) compared to sham-operated rats. However, the evoked responses to innocuous thermal stimuli (35 °C and 40 °C) were not significantly different (Figure 4).

The effect of acute administration of muscimol on electrical and natural evoked responses of WDR neurons

Administration of muscimol (2 mg/kg) inhibited Aδ (P<0.01), C-fiber neuronal responses (P<0.05), post-discharge (P<0.001), input spikes (P<0.001), and wind-up of WDR spinal neurons (P<0.05) in CCI rats. Nevertheless, muscimol did not significantly affect the evoked Aß-fiber neuronal responses (Figure 5).

Moreover, muscimol made a significant inhibitory effect on the innocuous brush (P<0.05), 2 g and 8 g (P<0.05) von Frey evoked responses, and also decreased hyperexcitability in WDR neurons to noxious 15 g (P<0.01), 26 g (P<0.05), 60 g (P<0.05) von Frey monofilaments, and pinch (P<0.001) compared to vehicle-treated CCI rats (Figure 6).

WDR neuronal responses to 45°C (P<0.05) and 50°C (P<0.001) noxious thermal stimuli were markedly decreased by muscimol compared to vehicle-treated CCI



NEUROSCIENCE

Figure 2. The responses of WDR neurons to electrical stimulation in CCI and sham operated groups

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR cell to electrical stimulation in the sham group; C: An example of PSTH; and D: A typical recording from a WDR neuron to electrical stimulation in the vehicle-treated CCI group; E: Comparison of the neuronal responses evoked by electrical stimulation between CCI group

(n=12) and sham-operated rats (n=8) on POD 14.

Electrical stimulation consisted of a train of 16 transcutaneous electrical stimuli (0.5 Hz, duration 2 ms at an amplitude three times the C-fiber threshold) were applied transcutaneously using two pins inserted into the hind paw receptive field. The

responses evoked by the different fibers were separated and quantified based on their latencies (Aß-fiber 0–20 ms; Aδ-fiber 20–90 ms and C-fiber 90–300 ms).

*P<0.05, ***P<0.001, significant difference with the sham group.

PSTH: Post-stimulus time histogram; WDR: Wide dynamic range; CCI: Chronic constriction injury; POD: Postoperative day.

rats, but the effect of muscimol on innocuous (35°C and 40°C) thermal stimuli evoked neuronal responses was not significant (Figure 7).

Chronic constriction nerve injury not modifying the level of spinal cord gabaa receptor α1 subunit expression on POD 14

We compared the level of GABAA receptor α1 subunit between sham-operated and vehicle-treated CCI groups on POD 14. Protein band densitometry revealed that loose ligation of the sciatic nerve did not significantly

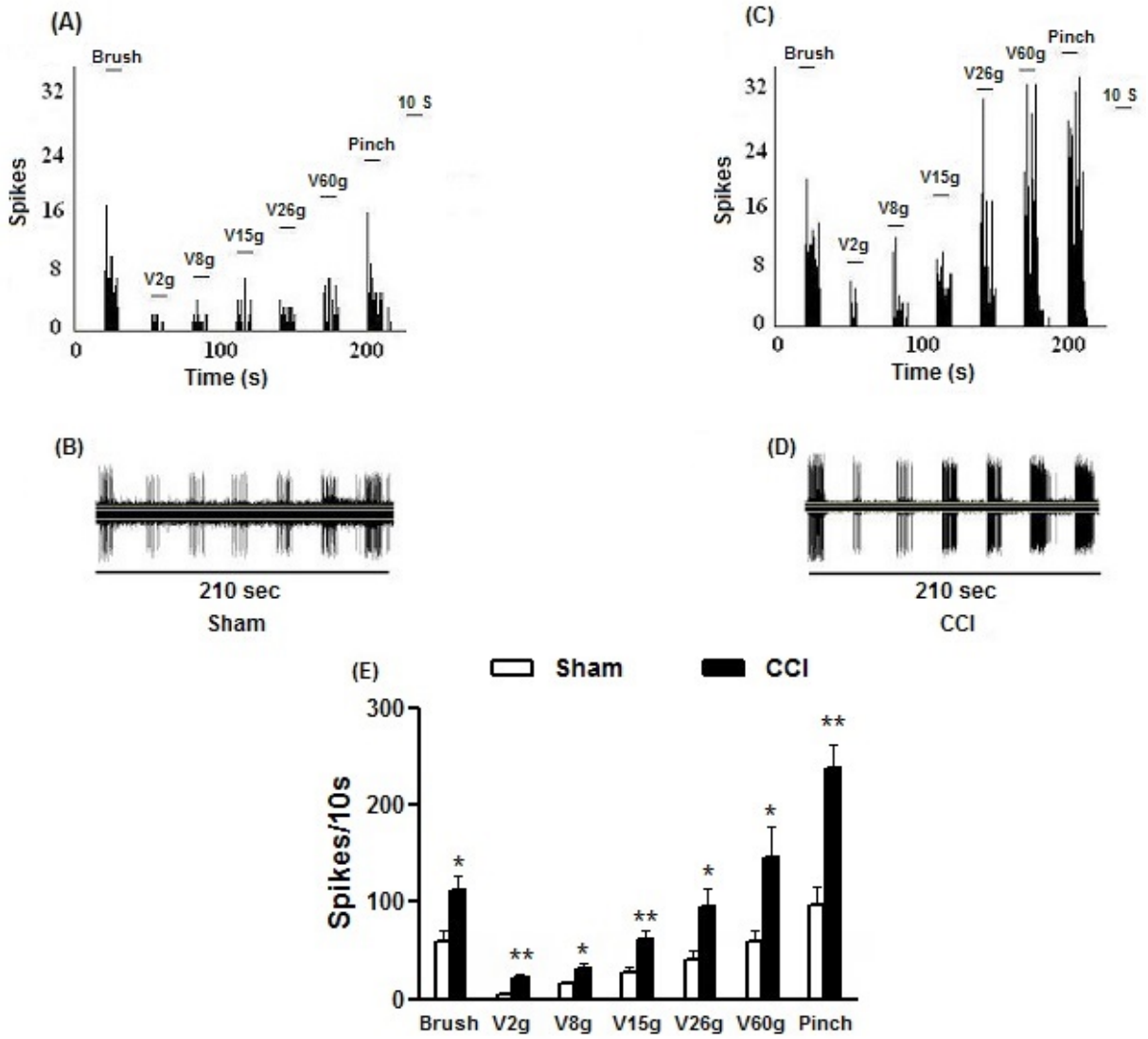


Figure 3. The responses of WDR neurons to mechanical stimulation in CCI and sham operated groups NEUROSCIENCE

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR neuron to mechanical stimulation in the sham group; C: An example of PSTH; and D: A typical recording from a spinal cord WDR neuron to mechanical stimulation in the vehicle-treated CCI group; E: Comparison of the neuronal responses evoked by mechanical stimulation between the CCI group (n=12) and the sham-operated rats (n=8) on POD 14.

Each mechanical stimulus (brush, v2 g, v8 g, v15 g, v26 g, v60 g, and pinch) was applied for 10 s at the most sensitive point of the WDR neuron receptive field.

*P<0.05, **P<0.01, significant difference with the sham group.

PSTH: Post-Stimulus Time Histogram; WDR: Wide Dynamic Range; CCI: Chronic Constriction Injury; POD: Postoperative Day; g: Gram.

alter the expression of the GABAA receptor α1 subunit compared to the sham condition (Figure 8).

4. Discussion

The results of this study indicated that nerve injury produced robust thermal hyperalgesia and mechanical allodynia and also increased WDR neuronal activity to electrical, mechanical, and thermal stimuli. Nevertheless, we did not observe any change in the spinal GABAA receptor α1 subunit expression, despite the development of neuropathic pain symptoms after CCI. The outcomes also showed that administration of muscimol on POD 14 reduced thermal hyperalgesia, mechanical allodynia,

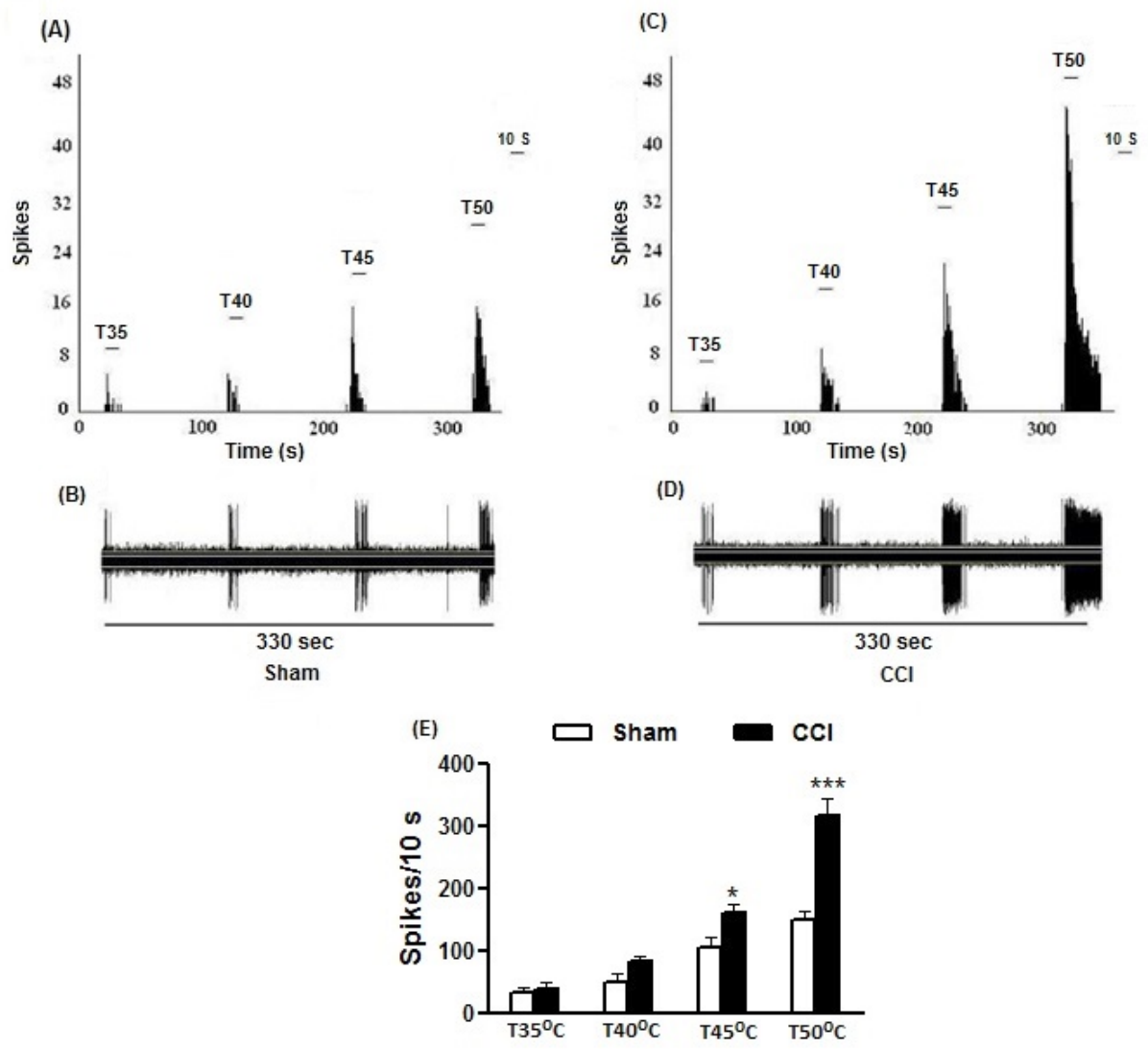


Figure 4. The responses of WDR neurons to thermal stimulation in CCI and sham operated groups NEUROSCIENCE

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR cell to thermal stimulation in the sham group; C: An example of PSTH; and D: A typical recording from a WDR neuron of the spinal cord to thermal stimulation in the vehicle-treated cci group; E: Comparison of the evoked WDR neuronal responses to thermal stimulation between the CCI group (n=12) and the sham-operated rats (n=8) on POD 14.

Each thermal stimulus (35°C, 40°C, 45°C, and 50°C) was applied for 10 s at the most sensitive point of the WDR neuron receptive field.

*P<0.05, ***P<0.001, significant difference with the sham group.

PSTH: Post-Stimulus Time Histogram; WDR: Wide Dynamic Range; CCI: Chronic Constriction Injury; POD: Postoperative Day; T: Temperature.

and the responsiveness of WDR neurons to electrical and natural stimuli in neuropathic rats. Based on our results, muscimol in a dose-dependent manner could reduce thermal hyperalgesia and mechanical allodynia in CCI rats on POD 14. It was also indicated that all doses of muscimol (0.5, 1, and 2 mg/kg) significantly alleviated thermal hyperalgesia; however, the lowest dose (0.5 mg/kg) had no significant effect on mechanical allodynia. It seems that thermal hyperalgesia is more sensitive to muscimol so that the antihyperalgesic effect of this drug is more potent than its antiallodynic effect. These findings appear to be mediated through the stimulation of the GABAA receptors by muscimol. The loss of the GABAergic inhibitory system, as well as the develop-

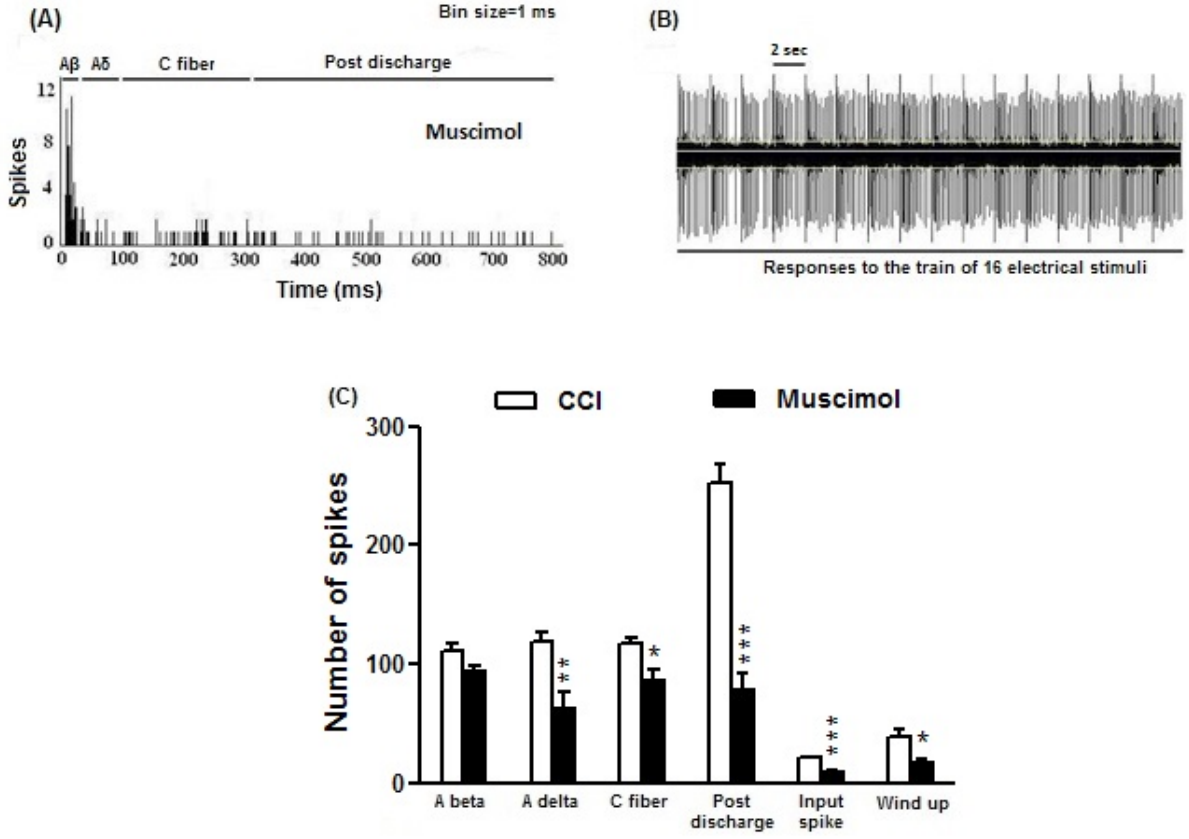


Figure 5. The responses of WDR neurons to electrical stimulation in the muscimol-treated CCI rats.

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR cell to electrical stimulation in the muscimol-treated CCI rats; C: Comparison of the neuronal responses evoked by electrical stimulation between the muscimol group (N=11) and the vehicle-treated CCI rats (n=12) on POD 14.

*P<0.05, **P<0.01, ***P<0.001, significant difference with vehicle-treated CCI group.

PSTH: Post-Stimulus Time Histogram; WDR: Wide Dynamic Range; CCI: Chronic Constriction Injury; POD: Postoperative Day.

ment of neuropathic pain symptoms, may be the result of an injury-induced reduction in the GABAA receptor activity and expression. This may decrease chloride concentration in neurons, thus leading to enhanced neuronal excitability because of depolarization and the development of neuropathic pain. It is likely that increased chloride ion conductance due to stimulation of the GABAA receptor by muscimol drives the membrane potential towards hyperpolarization, which inhibits the firing of new action potentials to alleviate neuropathic pain behavioral symptoms and hyper-responsiveness of WDR neurons. In support of our data, it has been demonstrated that systemic administration of muscimol reduces both thermal hyperalgesia and mechanical allodynia in SNI-induced neuropathic pain (Rode, Jensen, Blackburn-Munro, & Bjerrum, 2005). Similarly, dorsal root ganglion application of muscimol abolished the development of thermal hyperalgesia and improved myelin stability in rats subjected to sciatic nerve crush injury (Naik, Latham, Obradovic, & Jevtovic-Todorovic, 2012). In addition, muscimol could reduce pain-related behavior in arthritic rats (Simjee, Pleuvry, & Coulthard, 2004) and incisional pain (Reichl, Augustin, Zahn, & Pogatzki-Zahn, 2012). According to other reports, muscimol attenuates mechanical allodynia following spinal Cord Injury (SCI) model of neuropathic pain (Gwak et al., 2006) and partial injury of tail-innervating nerves (Lee et al., 2010). Previous studies have also demonstrated that spinal GABA receptor activation and inactivation could influence mechanical sensitivity and neuronal excitability after chronic compression of dorsal root ganglion (CCD), thus suggesting the involvement of spinal GABAergic function in CCD-induced neuropathic pain (Lee, Nam, Jung, Gwak, & Leem, 2015).

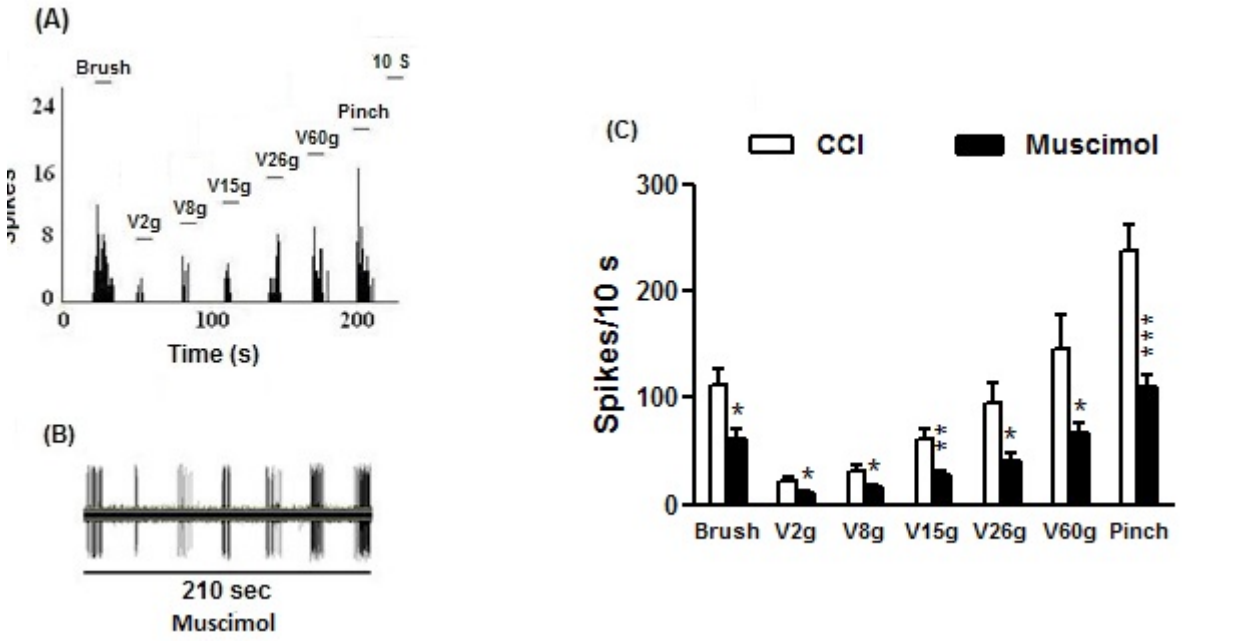


Figure 6. The responses of WDR neurons to mechanical stimulation in the muscimol-treated CCI rats

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR neuron to mechanical stimulation in the muscimol-treated CCI rats; C: Comparison of the neuronal responses evoked by mechanical stimulation between the muscimol group (n=11) and the vehicle-treated CCI rats (n=12) on POD 14.

*P<0.05, **P<0.01, ***P<0.001, significant difference with vehicle-treated CCI group.

PSTH: Post-Stimulus Time Histogram; WDR: Wide Dynamic Range; CCI: Chronic Constriction Injury; POD: Postoperative day; G: Gram.

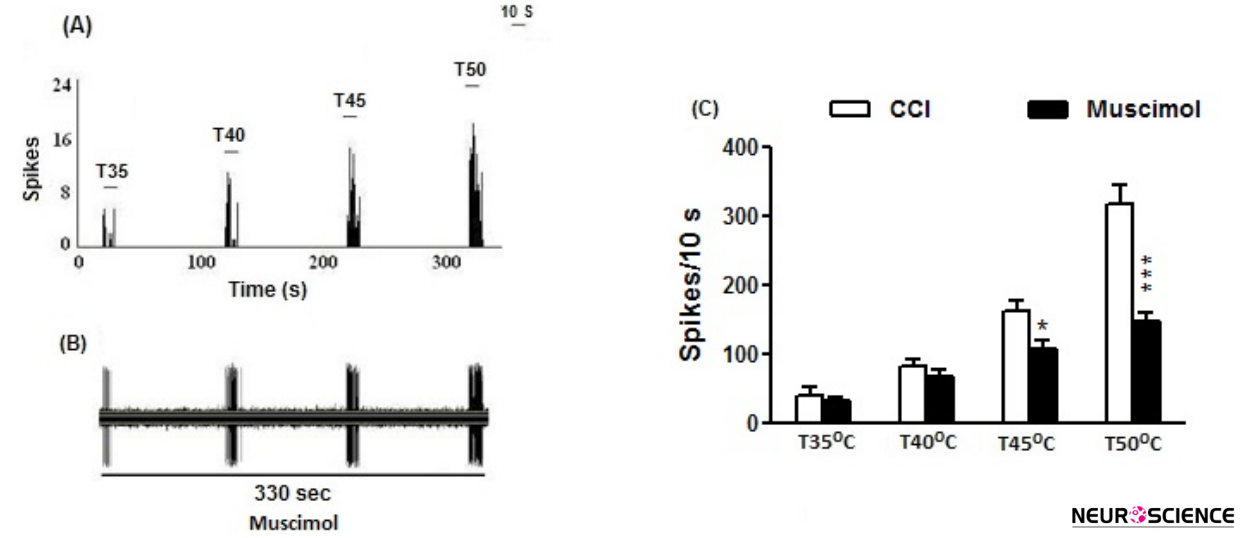


Figure 7. The responses of WDR neurons to thermal stimulation in the muscimol-treated CCI rats.

A: An example of PSTH; and B: A typical recording from a cell characterized as a WDR cell to thermal stimulation in the muscimol-treated CCI rats; C: Comparison of the evoked WDR neuronal responses to thermal stimulation between the muscimol group (N=11) and the vehicle-treated CCI rats (n=12) on POD 14.

*P<0.05, ***P<0.001, significant difference with the vehicle-treated CCI group.

PSTH: Post-Stimulus Time Histogram; WDR: Wide Dynamic Range; CCI: Chronic Constriction Injury; POD: Postoperative Day; T: Temperature.

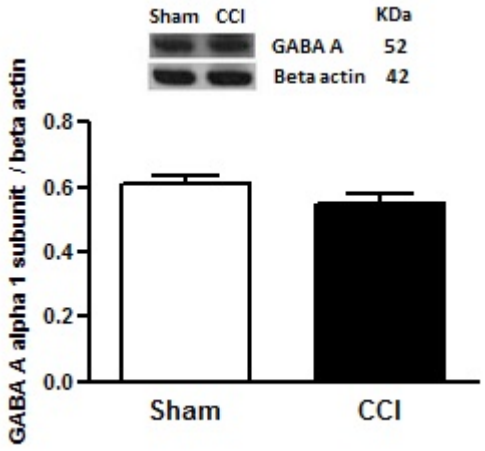


Figure 8. Comparison of the GABAA receptor α1 subunit expression between the sham and CCI groups (n=8) on POD 14

The expression of the GABAA receptor α1 subunit was assessed by immunoblot analysis. An equal amount (50 µg/lane) of each sample was loaded on an SDS-PAGE gel. The results were normalized to β-actin, and statistical significance was assessed by Student's t-test. Note that there is no significant difference in the GABAA receptor content between the sham and CCI rats.

Representative immunoblots are shown above the graph.

GABAA: γ-Aminobutyric Acid Type A receptor; CCI: Chronic Constriction Injury; POD: Postoperative Day.

In molecular experiments, there was no noticeable change in the spinal GABAA receptor α1 subunit expression, despite the development of neuropathic pain symptoms after CCI. Likewise, previous reports suggested that the expression of the GABAA receptor did not change in the spinal cord four hours after loose ligation of the sciatic nerve (Miletic & Miletic, 2008) and in the rat tail model of neuropathic pain (Lee et al., 2010). Since muscimol can enhance GABA inhibitory tone through activation of the GABAA receptors and could alleviate thermal hyperalgesia and mechanical allodynia in CCI rats, the loss of GABA-mediated inhibition in this model of neuropathic pain may not be due to GABAA receptors down-regulation in the superficial dorsal horn. The loss of spinal GABAergic inhibition in neuropathic states may be related to the changes in other GABAergic elements, such as apoptosis following nerve injury and a decrease in the GABA release from axon terminals of inhibitory interneurons or loss of its synthesizing enzyme glutamate decarboxylase (Moore et al., 2002). Another possible mechanism for reduced GABAergic inhibitory tone after nerve injury may be the changes in the expression of GABA transporters. However, we did not investigate the level of other GABAergic elements, such as GABA interneurons, GABAB receptors, and its synthesizing enzyme in CCI-induced neuropathic pain. The outcomes of the present study are different from the results of Yang et al. (2012), who demonstrated the reduced expression of the mRNA and GABAA receptor α1 subunit protein in the spinal cord of mice in formalin-induced pain. This conflict may be related to the difference in the induction of the pain model.

In the present scientific study, Aδ and C-fiber spikes, post-discharge, input spikes, and wind-up spikes increased in CCI animals compared to the sham group; nevertheless, Aß-fiber spikes did not change. Furthermore, WDR neuronal responses to innocuous and noxious mechanical stimuli, as well as to noxious thermal stimuli, increased significantly. Several studies have reported the changes in the responsiveness of WDR neurons after neuropathy. C-fiber evoked responses, post-discharge, wind-up, and spontaneous activity of WDR neurons were higher than those of sham-operated rats after SCI (Zhang, Xie, & Xie, 2005). Similarly, a significant increase has been reported in the responses of WDR neurons to noxious mechanical and thermal stimuli after CCI (Song et al., 2008). Liu et al. (2011) have also reported increased spontaneous activity and enlarged receptive field sizes of spinal neurons after the Spinal Nerve Ligation (SNL) neuropathy model. In parallel with the above studies, our electrophysiological results showed that WDR responses increased after neuropathy, thus explaining the neuronal basis for the development of allodynia and hyperalgesia after CCI.

The main causes of WDR neuronal hyperexcitability in painful neuropathy have not been fully understood. Some studies have reported that hyperexcitability of spinal dorsal horn neurons and the manifestation of neuropathic pain may be due to a decrease in GABAergic

activity and disinhibition (Drew, Siddall, & Duggan, 2004). Takahashi, Takeda, and Matsumoto (2014) have demonstrated that inhibition of GABAergic interneurons enhanced dorsal horn neuronal activity. It has also been reported that intraspinal transplantation of GABAergic progenitors attenuates WDR neuronal hyperexcitability after neuropathic pain (Jergova, Hentall, Gajavelli, Varghese, & Sagen, 2012).

The findings indicated that the WDR neuronal responses to electrical and natural stimuli significantly decreased after muscimol administration. As was mentioned, muscimol treatment can prevent the loss of GABA inhibitory tone through GABAA receptor stimulation and ultimately can attenuate WDR neuronal hyperexcitability in neuropathic rats. Miletic, Draganic, Pankratz, and Miletic (2003) reported that long-lasting potentiation of dorsal horn field potentials is prevented by muscimol after sciatic nerve ligation. Furthermore, spinal administration of muscimol alleviated dorsal horn neuronal responses in SNL rats (Sokal & Chapman, 2003). Our results are consistent with previous studies, which confirm the importance of GABA inhibitory action for the processing of sensory information in the dorsal horn of the spinal cord. This finding implies that the GABAergic inhibitory system plays a pivotal role in suppressing WDR neuronal hyperexcitability.

In the present experiment, the effect of muscimol on Aß-fiber evoked neuronal responses was relatively minor. One reason may be that Aß-fiber spikes did not change in CCI rats compared to sham-operated rats. As a result, muscimol could not modulate Aß-fiber inputs. Similarly, no significant difference was found in Aß-fiber spikes of SNL and sham-operated rats (Suzuki, Chapman, & Dickenson, 1999). On the other hand, Aδ and C-fiber spikes clearly decreased after muscimol administration. It has also been indicated that the loss of GABAergic inhibitory tone after neuropathy may produce the facilitation of Aδ and C fibers (Lu, Zheng, Xiong, Zimmermann, & Yang, 2008) that may result in enhanced nociceptive transmission in second-order neurons. These results suggest that Aδ and C-fibers evoked responses are probably under the GABAergic inhibitory system so that muscimol can reduce Aδ and C-fiber spikes by stimulating the GABAA receptors expressed on these fiber terminals.

The present data also showed that post-discharge, input spikes, and wind-up of spinal WDR neurons decreased after administration of muscimol in sciatic ligated rats. A relationship between disinhibition and the rate of post-discharge in the dorsal horn has been demonstrated (Drew, Siddall, & Duggan, 2004). GABA signaling is thought to modulate wind-up, which is used to evaluate the sensitization of dorsal horn neurons (Jergova, Hentall, Gajavelli, Varghese, & Sagen, 2012). Wind-up also reflects the alleviation of GABAergic inhibitory tone (Navarro, Vivó, & Valero-Cabré, 2007). Therefore, the reduced post-discharge and wind-up after muscimol administration in the current study could be due to the enhanced GABA inhibitory action of muscimol. Input spikes are the number of spikes elicited before the generation of wind-up. They demonstrate presynaptic events or mechanisms before the production of post-discharge and wind-up, which are predominantly post-synaptic events leading to neuronal hyperexcitability (Flatters, Fox, & Dickenson, 2003). It seems that GABA-mediated inhibition in the dorsal horn may act both presynaptically and postsynaptically. So, the inhibition of neuronal responses in muscimol-treated CCI rats suggests that muscimol may reduce hyperexcitability of WDR neurons both presynaptically and postsynaptically following nerve injury.

We also indicated that muscimol considerably decreased evoked responses of WDR neurons to noxious mechanical and thermal stimuli, thus implying that enhancement of GABA inhibitory tone after administration of muscimol can effectively reduce WDR neuronal hyper-responsiveness to noxious stimuli in sciatic ligated animals. This reduction agrees with our behavioral data that muscimol could attenuate thermal hyperalgesia. Moreover, muscimol could reduce the responses of WDR neurons to innocuous mechanical stimuli in neuropathic rats. This modification is also concurrent with the alleviation of mechanical allodynia in the behavioral part of this study. However, neuronal responses to innocuous thermal stimuli were unaffected after muscimol administration in nerve-injured rats, probably because the responses of WDR neurons to innocuous thermal stimuli were not significantly different between CCI and sham groups.

In conclusion, this study showed that muscimol could reduce behavioral signs and WDR neuronal hyper-responsiveness in CCI rats by enhancing GABA inhibitory action. This finding supports the idea of the loss of GABAergic inhibitory tone in neuropathic states. In addition, CCI did not affect the expression of spinal GABAA receptors. It seems that the loss of GABA inhibitory action in this model of neuropathy and then the manifestation of neuropathic pain symptoms may be due to the changes in other GABAergic elements. This study provides further insights regarding pain processing to design new therapeutic agents to reduce neuropathic pain symptoms.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

Funding

This work was financially supported by the Iran National Science Foundation (INSF) and Neuroscience Research Center of Shahid Beheshti University of Medical Sciences.

Authors' contributions

Conceptualization, study design and writing of the manuscript: Homa Manaheji and Mehdi Sadeghi; Experimental studies and data analysis: Mehdi Sadeghi, Samad Nazemi, and Zahra Bahari; Advisor of the molecular part of the study: Jalal Zaringhalam; Advisors of the electrophysiological part of the study: Abbas Haghparast and Seyed Mohammad Noorbakhsh

Conflict of interest

The authors declared no conflict of interest.

References

Bennett, G. J., & Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain, 33*(1), 87-107. [DOI:10.1016/0304-3959(88)90209-6]

Clark, A. K., Old, E. A., & Malcangio, M. (2013). Neuropathic pain and cytokines: Current perspectives. *Journal of Pain Research, 6*, 803-14. [DOI:10.2147/JPR.S53660]

Coull, J. A. M., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., & Sik, A., et al. (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature, 424*(6951), 938-42. [DOI:10.1038/nature01868]

da Motta, P. G., Veiga, A. P. C., Francischi, J. N., & Tatsuo, M. A. K. F. (2004). Evidence for participation of GABAA receptors in a rat model of secondary hyperalgesia. *European Journal of Pharmacology, 483*(2-3), 233-9. [DOI:10.1016/j.ejphar.2003.10.015]

Drew, G. M., Siddall, P. J., & Duggan, A. W. (2004). Mechanical allodynia following contusion injury of the rat spinal cord is associated with loss of GABAergic inhibition in the dorsal horn. *Pain, 109*(3), 379-88. [DOI:10.1016/j.pain.2004.02.007]

Flatters, S. J. L., Fox, A. J., & Dickenson, A. H. (2003). Spinal interleukin-6 (IL-6) inhibits nociceptive transmission following neuropathy. Brain Research, 984(1-2), 54-62. [DOI:10.1016/S0006-8993(03)03092-0]

Gosselin, R. D., Bebber, D., & Decosterd, I. (2010). Upregulation of the GABA transporter GAT-1 in the gracile nucleus in the spared nerve injury model of neuropathic pain. *Neuroscience Letters, 480*(2), 132-7. [DOI:10.1016/j.neulet.2010.06.023]

Gwak, Y. S., & Hulsebosch, C. E. (2011). GABA and central neuropathic pain following spinal cord injury. *Neuropharmacology, 60*(5), 799-808. [DOI:10.1016/j.neuropharm.2010.12.030]

Gwak, Y. S., Tan, H. Y., Nam, T. S., Paik, K. S., Hulsebosch, C. E., & Leem, J. W. (2006). Activation of spinal GABA receptors attenuates chronic central neuropathic pain after spinal cord injury. *Journal of Neurotrauma, 23*(7), 1111-24. [DOI:10.1089/neu.2006.23.1111]

Hao, J. X., Xu, X. J., Yu, Y. X., Seiger, A., & Wiesenfeld-Hallin, Z. (1992). Baclofen reverses the hypersensitivity of dorsal horn wide dynamic range neurons to mechanical stimulation after transient spinal cord ischemia; implications for a tonic GABAergic inhibitory control of myelinated fiber input. *Journal of Neurophysiology, 68*(2), 392-6. [DOI:10.1152/jn.1992.68.2.392]

Janssen, S. P., Truin, M., Van Kleef, M., & Joosten, E. A. (2011). Differential GABAergic disinhibition during the development of painful peripheral neuropathy. *Neuroscience, 184*, 183-94. [DOI:10.1016/j.neuroscience.2011.03.060]

Jergova, S., Hentall, I. D., Gajavelli, Sh., Varghese, M. S., & Sagen, J. (2012). Intraspinal transplantation of GABAergic neural progenitors attenuates neuropathic pain in rats: A pharmacologic and neurophysiological evaluation. *Experimental Neurology, 234*(1), 39-49. [DOI:10.1016/j.expneurol.2011.12.005]

Lee, J., Back, S. K., Lim, E. J., Cho, G. C., Kim, M. A., & Kim, H. J., et al. (2010). Are spinal GABAergic elements related to the manifestation of neuropathic pain in rat? *The Korean Journal of Physiology & Pharmacology, 14*(2), 59-69. [DOI:10.4196/kjpp.2010.14.2.59]

Lee, M. C., Nam, T. S., Jung, S. J., Gwak, Y. S., & Leem, J. W. (2015). Modulation of spinal GABAergic inhibition and mechanical hypersensitivity following chronic compression of dorsal root ganglion in the rat. *Neural Plasticity, 2015*, 924728. [DOI:10.1155/2015/924728]

Liu, F. Y., Qu, X. X., Cai, J., Wang, F. T., Xing, G. G., & Wan, Y. (2011). Electrophysiological properties of spinal wide dynamic range neurons in neuropathic pain rats following spinal nerve ligation. *Neuroscience Bulletin, 27*(1), 1-8. [DOI:10.1007/s12264-011-1039-z]

Lu, Y., Zheng, J., Xiong, L., Zimmermann, M., & Yang, J. (2008). Spinal cord injury-induced attenuation of GABAergic inhibition in spinal dorsal horn circuits is associated with down-regulation of the chloride transporter KCC2 in rat. *The Journal of Physiology, 586*(23), 5701-15. [DOI:10.1113/jphysiol.2008.152348]

Miki, K., Iwata, K., Tsuboi, Y., Morimoto, T., Kondo, E., & Dai, Y., et al. (2000). Dorsal column-thalamic pathway is involved in thalamic hyperexcitability following peripheral nerve injury: A lesion study in rats with experimental mononeuropathy. *Pain, 85*(1), 263-71. [DOI:10.1016/S0304-3959(99)00279-1]

Miletic, G., & Miletic, V. (2008). Loose ligation of the sciatic nerve is associated with TrkB receptor-dependent decreases

in KCC2 protein levels in the ipsilateral spinal dorsal horn. *Pain, 137*(3), 532-9. [DOI:10.1016/j.pain.2007.10.016]

Miletic, G., Draganic, P., Pankratz, M. T., & Miletic, V. (2003). Muscimol prevents long-lasting potentiation of dorsal horn field potentials in rats with chronic constriction injury exhibiting decreased levels of the GABA transporter GAT-1. *Pain, 105*(1), 347-53. [DOI:10.1016/S0304-3959(03)00250-1]

Moore, K. A., Kohno, T., Karchewski, L. A., Scholz, J., Baba, H., & Woolf, C. J. (2002). Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *Journal of Neuroscience, 22*(15), 6724-31. [DOI:10.1523/JNEUROSCI.22-15-06724.2002]

Naik, A. K., Latham, J. R., Obradovic, A., & Jevtovic-Todorovic, V. (2012). Dorsal root ganglion application of muscimol prevents hyperalgesia and stimulates myelin protein expression after sciatic nerve injury in rats. *Anesthesia & Analgesia, 114*(3), 674-82. [DOI:10.1213/ANE.0b013e31823fad7e]

Naik, A. K., Pathirathna, S., & Jevtovic-Todorovic, V. (2008). GABAA receptor modulation in dorsal root ganglia in vivo affects chronic pain after nerve injury. *Neuroscience, 154*(4), 1539-53. [DOI:10.1016/j.neuroscience.2008.04.061]

Navarro, X., Vivó, M., & Valero-Cabré, A. (2007). Neural plasticity after peripheral nerve injury and regeneration. *Progress in Neurobiology, 82*(4), 163-201. [DOI:10.1016/j.pneurobio.2007.06.005]

Nazemi, S., Manaheji, H., Noorbakhsh, S. M., Zaringhalam, J., Sadeghi, M., & Mohammad-Zadeh, M., et al. (2015). Inhibition of microglial activity alters spinal wide dynamic range neuron discharge and reduces microglial Toll-like receptor 4 expression in neuropathic rats. *Clinical and Experimental Pharmacology and Physiology, 42*(7), 772-9. [DOI:10.1111/1440-1681.12414]

Pertovaara, A., Kontinen, V. K., & Kalso, E. A. (1997). Chronic spinal nerve ligation induces changes in response characteristics of nociceptive spinal dorsal horn neurons and in their descending regulation originating in the periaqueductal gray in the rat. *Experimental Neurology, 147*(2), 428-36. [DOI:10.1006/exnr.1997.6555]

Polgár, E., & Todd, A. J. (2008). Tactile allodynia can occur in the spared nerve injury model in the rat without selective loss of GABA or GABAA receptors from synapses in laminae I-II of the ipsilateral spinal dorsal horn. *Neuroscience, 156*(1), 193-202. [DOI:10.1016/j.neuroscience.2008.07.009]

Reichl, S., Augustin, M., Zahn, P. K., & Pogatzki-Zahn, E. M. (2012). Peripheral and spinal GABAergic regulation of incisional pain in rats. *Pain, 153*(1), 129-41. [DOI:10.1016/j.pain.2011.09.028]

Rode, F., Jensen, D. G., Blackburn-Munro, G., & Bjerrum O. J. (2005). Centrally-mediated antinociceptive actions of GABAA receptor agonists in the rat spared nerve injury model of neuropathic pain. *European Journal of Pharmacology, 516*(2), 131-8. [DOI:10.1016/j.ejphar.2005.04.034]

Simjee, S. U., Pleuvry, B. J., & Coulthard, P. (2004). Modulation of the gait deficit in arthritic rats by infusions of muscimol and bicuculline. *Pain, 109*(3), 453-60. [DOI:10.1016/j.pain.2004.02.022]

Sokal, D. M., & Chapman, V. (2003). Effects of spinal administration of muscimol on C- and A-fibre evoked neuronal responses of spinal dorsal horn neurones in control and nerve injured rats. *Brain Research, 962*(1-2), 213-20. [DOI:10.1016/S0006-8993(02)04057-X]

Song, X. J., Zheng, J. H., Cao, J. L., Liu, W. T., Song, X. S., & Huang, Z. J. (2008). EphrinB-EphB receptor signaling contributes to neuropathic pain by regulating neural excitability and spinal synaptic plasticity in rats. *Pain, 139*(1), 168-80. [DOI:10.1016/j.pain.2008.03.019]

Suzuki, R., Chapman, V., & Dickenson, A. H. (1999). The effectiveness of spinal and systemic morphine on rat dorsal horn neuronal responses in the spinal nerve ligation model of neuropathic pain. *Pain, 80*(1), 215-28. [DOI:10.1016/S0304-3959(98)00208-5]

Takahashi, M., Takeda, M., & Matsumoto, Sh. (2014). Somatostatin enhances tooth-pulp-evoked cervical dorsal horn neuronal activity in the rat via inhibition of GABAergic interneurons. *Brain Research Bulletin, 100*, 76-83. [DOI:10.1016/j.brainresbull.2013.11.008]

Takaishi, K., Eisele Jr, J. H., & Carstens, E. (1996). Behavioral and electrophysiological assessment of hyperalgesia and changes in dorsal horn responses following partial sciatic nerve ligation in rats. *Pain, 66*(2), 297-306. [DOI:10.1016/0304-3959(96)03023-0]

Yakhnitsa, V., Linderoth, B., & Meyerson, B. A. (1999). Spinal cord stimulation attenuates dorsal horn neuronal hyperexcitability in a rat model of mononeuropathy. *Pain, 79*(2), 223-33. [DOI:10.1016/S0304-3959(98)00169-9]

Yang, G., Gao, J., Jia, Y., Yan, L., Yu, J., & Jiang, Y. (2012). Oxysophoridine through intrathecal injection induces antinociception and increases the expression of the GABAAα1 receptor in the spinal cord of mice. *Planta Medica, 78*(09), 874-80. [DOI:10.1055/s-0031-1298471]

Zhang, H., Xie, W., & Xie, Y. (2005). Spinal cord injury triggers sensitization of wide dynamic range dorsal horn neurons in segments rostral to the injury. *Brain Research, 1055*(1-2), 103-10. [DOI:10.1016/j.brainres.2005.06.072]

Zheng, J. H., Chen, J., & Arendt-Nielsen, L. (2004). Complexity of tissue injury-induced nociceptive discharge of dorsal horn wide dynamic range neurons in the rat, correlation with the effect of systemic morphine. *Brain Research, 1001*(1-2), 143-9. [DOI:10.1016/j.brainres.2003.12.005]

Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain, 16*(2), 109-10. [DOI:10.1016/0304-3959(83)90201-4]

This Page Intentionally Left Blank