Peripheral nerve injury potentiates excitatory synaptic transmission in locus coeruleus neurons

Kambiz Rohampour^{1,2}, Hossein Azizi¹, Yaghoub Fathollahi¹, Saeed Semnanian^{1,3*}

¹Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

²Neuroscience Research Center, Guilan University of Medical Sciences, Rasht, Iran

³School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), P.O. Box 1954851167, Tehran, Iran

***Corresponding author:**

Saeed Semnanian, MD, PhD

Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; School of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), P.O. Box 1954851167, Tehran, Iran

Tel: +98-21-82884520

E-mail address: ssemnan@modares.ac.ir

Abstract:

Peripheral nerve injury (PNI) is believed to cause maladaptive changes at synaptic level, leading to neuropathic pain which is difficult to treat with common analgesic drugs. Noradrenergic locus coeruleus (LC) neurons have a crucial role in neuropathic pain modulation. In this study we examined whether chronic constriction injury (CCI) could affect glutamatergic synaptic transmission in LC neurons.

CCI was performed on P10 to P12 Sprague Dawley pups. Seven days after CCI, horizontal slices of brainstem (300 μ m thick) were prepared and whole-cell patch clamp recording was performed. Evoked and spontaneous excitatory postsynaptic currents (eEPSC and sEPSC) were recorded from LC neurons at a holding potential of -70 mV, in the presence of bicuculline (20 μ M).

The sEPSCs recorded from LC neurons of neuropathic rats showed a significant increase in amplitude, but not in frequency. The eEPSC amplitude in neurons of rats under gone CCI was significantly increased compared to the control group ($P < 0.05$). The paired pulse ratio (PPR) elicited with different inter-stimulus intervals (50-250 ms) did not show any difference between neurons of CCI and control pups.

This study shows that PNI increases excitatory synaptic transmission in LC neurons 7 days after chronic constriction injury. The observed synaptic potentiation is mainly due to postsynaptic mechanisms.

Keywords:

Peripheral nerve injury; neuropathic pain; locus coeruleus; excitatory synaptic transmission

1. Introduction

Neuropathic pain is caused by damage or lesion to those parts of the peripheral and central nervous system that conduct or process somatosensory information. Neuropathic pain is a chronic medical condition that is generally resistant to nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics (Havelin, et al. 2016). Although long-term potentiation (LTP) is known as the cellular mechanism of learning and memory in the brain, many studies support the involvement of the same mechanism in chronic pain conditions such as peripheral neuropathies (Ji, et al. 2003). Noxious stimuli and painful experiences could provoke plastic changes in different spinal and supra-spinal structures. The afferent barrage provoked by primary lesions generally leads to development of hypersensitivity and neuronal hyperexcitability. The most studied site for this phenomenon is the spinal dorsal horn (SDH), where the intense activity of peripheral afferents leads to synaptic strengthening following persistent inflammatory (Ikeda, et al. 2006) or neuropathic pain (Woolf and Salter 2000, Ikeda, et al. 2003, Taves, et al. 2013) through induction of molecular changes at synaptic level, which is known as the mechanism of central sensitization (Ji, et al. 2003, Boadas-Vaello, et al. 2016). There are also several evidences indicating that the same changes in synaptic strength could develop in supra-spinal structures involved in pain modulation and perception; such as anterior cingulate cortex (ACC) (Xu, et al. 2008), insular cortex (Qiu, et al. 2013), nucleus accumbens (Xu, et al. 2015), rostral ventral medulla (RVM) (Robinson, et al. 2002) and amygdala (Nakao, et al. 2012).

Locus coeruleus (LC) is the main source of noradrenaline in the CNS and its descending inhibitory projections to superficial layers of SDH cause antinociception. This system is more effective in persistent pain conditions (West, et al. 1993, Tsuruoka, et al. 2004). It has been shown that tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH) immunoreactivity in spinal cord as well as the noradrenergic innervation to SDH increases following peripheral nerve injury (PNI) (Ma and Eisenach 2003). The ascending LC projections to the medial thalamus can also exert antinociceptive effects (Voisin, et al. 2005). On the other hand, the first-line drugs recommended for the treatment of neuropathic pain include serotonin-noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) (Attal, et al. 2010). The main mechanism proposed for the analgesic action of these antidepressants is the inhibition of noradrenaline (NA) and serotonin (5HT) reuptake. Studies have revealed that the necessary component of the analgesic effect of TCAs on neuropathic pain is the reinforcement of noradrenergic system (Suzuki, et al. 2008, Arsenault and

Sawynok 2009, Bohren, et al. 2013) and as their effect appears after a chronic treatment, the possible involvement of neuronal plasticity in this system could be supposed (Kremer, et al. 2016).

Since neuropathic pain results at least in part, from maladaptive changes in synaptic transmission at SDH and regarding the involvement of LC as the main noradrenergic site in modulation of chronic pain, we examined whether peripheral nerve chronic constriction injury (CCI) could affect excitatory synaptic transmission in LC neurons as a compensation for pain modulation.

2. Materials and methods

2.1. Animals

All animal experiments were carried out in accordance with the “Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University”. Wistar rats of either sex (17-19 days old) were deeply anaesthetized by ether and decapitated. The brain was quickly removed from the skull and trimmed in ice-cold (1-4°C) low-calcium, sucrose based artificial cerebrospinal fluid (sucrose-aCSF) containing (mM): sucrose 213, KCl 2.6, CaCl₂ 0.1, MgCl₂ 2, NaHCO₃ 26, NaH₂PO₄ 1.23, L-Ascorbic Acid 0.4, D-glucose 2 (290–310 mOsmol/L, pH 7.3 - 7.4 when bubbled with 95% O₂, 5% CO₂).

2.2. Slice preparation

A block of brainstem tissue was glued to the cutting stage of a vibratome (1000 Plus, Vibratome, USA) with the dorsal side up. 2-3 horizontal slices of 300 µm thickness containing the LC nuclei were prepared at 1-4°C. The slices were first incubated in a holding chamber with standard aCSF (mM): NaCl 125, KCl 3, NaH₂PO₄ 1.25, NaHCO₃ 25, CaCl₂ 2, MgCl₂ 1.3, L-Ascorbic Acid 0.4 and D-Glucose 10, saturated with 95% O₂ and 5% CO₂, for 30-40 min at 35°C. Osmolarity was maintained between 290 and 310 mOsmol/L and pH between 7.3 and 7.4. The slices were then kept at room temperature (20-25°C) in the same chamber until the recording. The slice was transferred to a recording chamber mounted on a Zeiss Axioskop. It was fixed in the chamber under nylon strings attached to a U-shaped platinum frame and continuously perfused with the standard aCSF at a flow rate of 1-2 ml/min. Cells were visualized with an upright microscope (Axioskop2, Zeiss, Germany) using infrared differential interference contrast (IR-DIC) illumination system with a 10X or 40X water immersion objective lens (IR-1000, USA).

2.3. Whole-cell recording

Whole cell recording in LC neurons was performed as has been previously described in details (Kaeidi, et al. 2015) and is briefly reviewed here. The patch electrodes were made from borosilicate glass pipettes of 1.5 mm outer diameter (GC150F-10, Harvard Apparatus, USA) with a programmable puller (P-97, Sutter instruments, USA). The tip resistance of the electrode was 3-7 M Ω when filled with intracellular solution of the following composition (mM): CsMeSO₃ 120, NaCl 5, MgATP 4, NaGTP 0.3, EGTA 1.1, HEPES hemisodium 10, TEA-Cl 10, QX314 5 (pH 7.3 as adjusted with CsOH; osmolarity was approximately 285 mOsmol/L). The LC neurons were preselected according to the IR-DIC image. After establishment of the cell attached configuration (2-10 G Ω seal resistance), the whole cell mode was established with a brief negative pressure pulse (before rupture, holding potential was put on -70 mV). Recording was started at least 5 min after the rupture of the patch membrane to stabilize the intracellular milieu. During this time, the membrane potential was held at -70 mV. Neurons showing unstable or large (>50 pA) holding current were rejected. Access resistance of <25 M Ω was considered acceptable and was monitored periodically throughout the experiment. The experiment was terminated if the access resistance changed more than 15%. Whole-cell voltage clamp recordings were acquired with a MultiClamp 700B amplifier and pClamp 10 software (Molecular Devices, USA), with a 3-kHz low-pass Bessel filter, and digitized at 10 kHz using a Digidata 1440A data acquisition system (Molecular Device, USA). For all experiments, 20 μ M bicuculline (Sigma-Aldrich, USA) was added to eliminate GABA_A receptor-mediated inhibitory postsynaptic currents.

Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded in voltage clamp at a holding potential of -70 mV. The sEPSC of LC neurons appeared as a rapidly rising and slowly decaying inward current. Each event of sEPSCs was detected and its amplitude and frequency were measured in 120 second recordings. All detected sEPSCs were monitored and checked visually so that all analyzed events had a typical sEPSC waveform.

In order to perform eEPSC, a glass monopolar stimulating electrode filled with aCSF was placed 100–300 μ m caudal to the recording electrode and the afferents were stimulated at 0.1 Hz. Neurons were voltage clamped at -70 mV to record AMPAR-mediated evoked excitatory postsynaptic currents (AMPA-eEPSCs). The eEPSC traces were constructed by averaging 12 eEPSCs (120s). Paired-pulse ratio was elicited by evoking two eEPSCs at a holding potential of -70 mV with an interstimulus interval of 50 to 250 ms. Paired-pulse traces were constructed by averaging 10 sweeps of paired pulses (100 s).

2.4. Chronic constriction injury (CCI) model

In order to achieve a neuropathic pain model, CCI surgery was performed on 10-12 days young rats. Animals were deeply anesthetized, then sciatic nerve was exposed and four loose ligations were placed on the sciatic nerve by 6/0 chromic gut strings. Seven days after the CCI microsurgery rats were decapitated and the brain stem was cut in 300 μ m horizontal slices, for patch clamp recording.

2.5. Data analysis

Data were analyzed off-line using Clampfit software. All values are expressed as mean $\pm$ SEM. The results were compared between neurons of neuropathic rats vs. neurons of control ones, using unpaired Student's T-test, $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Passive membrane proprieties of LC neurons

Passive membrane proprieties were examined in LC neurons of both groups. Resting membrane potential was -50 ± 0.83 mV and -51 ± 1.8 mV in control and neuropathic brain slices, respectively. Membrane resistance varied from 208.6 ± 22.2 m Ω in control to 210.8 ± 17.3 m Ω in neuropathic rats. None of the measured passive membrane properties showed a significant difference among the CCI and control groups.

3.2. Effect of neuropathy on instantaneous discharge rate and excitability of LC neurons

In order to assess the effect of PNI on excitability of LC neurons, different intensities of depolarizing current were injected to LC neurons of control or neuropathic rats ($n=12$) and the instantaneous frequencies were analyzed. There was no significant difference between the spontaneous discharge rate of neuropathic and control rats (1.01 ± 0.38 Hz in untreated LC neurons vs. 1.17 ± 0.48 Hz in neurons of neuropathic rats). Injection of depolarizing currents in 50 pA steps showed an increased excitability in LC neurons of neuropathic rats compared to neurons of control ones (Fig. 1), statistical significance of the elevated discharge rate was observed at 300 and 350 pA current steps ($p < 0.05$, Student's T-test). Fig. 1A compares 2 trace sets of instantaneous firing frequency of LC neurons one week after CCI with control LC neurons. As depicted in Fig. 1B, the neurons of control rats responded to 300 pA current with 17.6 ± 1.8 spikes/sec, while the same current induced 21.4 ± 0.6 spikes/sec in neurons of injured

rats. The instantaneous frequency induced by 350 pA was 19.0 ± 1.9 and 22.7 ± 0.6 Hz in neurons of control and neuropathic rats, respectively.

3.3. Effects of CCI on the spontaneous excitatory postsynaptic currents (sEPSCs)

In order to assess whether neuropathy affects the spontaneous excitatory synaptic transmission in LC neurons, sEPSCs were recorded and compared in healthy and neuropathic rats. Fig. 2A shows the representative traces of sEPSCs recorded in neurons with and without sciatic nerve ligation. 20 μ M bicuculline were dissolved in the extracellular solution to block the inhibitory synaptic transmission. Although there was a slight increase in sEPSC frequency, but this increase was not significant. Meanwhile the raise in sEPSC amplitude was statistically significant ($n=11$, $P<0.05$, unpaired Student's T-test). The cumulative probability plot of amplitude and inter-event intervals of sEPSC is shown on Fig. 2B; while Fig. 2C shows the bar charts of mean sEPSC amplitude and frequency in LC neurons of neuropathic rats compared to control ones.

3.4. Effects of sciatic nerve injury on the evoked excitatory postsynaptic currents (eEPSCs) of LC neurons

Stimulating the afferents 100-300 μ m caudal to LC neurons by different current intensities (100-600 μ A) induced evoked EPSCs. Recording eEPSCs at a holding potential of -70 mV and applying bicuculline 20 μ M to block inhibitory synaptic transmission, provided AMPA current through the cell membrane, which was blocked completely by CNQX. Each stimulus intensity was applied 10 times at 0.1 Hz frequency and the average currents of 10 eEPSCs were analyzed. As Fig. 3A shows, in all applied intensities except 100 μ A, the excitatory synaptic current was significantly potentiated in LC neurons 7 days after CCI (* $P<0.05$ vs. control, unpaired T-test, $n=8$). Fig. 3B shows the representative traces of eEPSC recordings from LC neurons of control and CCI rats evoked by a 200 μ A stimulus.

3.5. Sciatic nerve injury did not affect paired pulse ratio (PPR)

Paired pulse stimuli were applied with different inter-stimulus intervals (50, 75, 100, 150, 200 and 250 ms). Although there was a slight increase in PPR in some LC neurons of neuropathic rats compared to control neurons, the alteration observed in paired pulse facilitation (PPF) was not statistically significant. Fig. 4A compares mean changes in PPF between LC neurons of neuropathic and control rats, in different interstimulus intervals. The

representative traces of paired pulse evoked EPSCs elicited by stimuli applied at 50 ms interval is shown in Fig. 4B.

4. Discussion

The results of this study showed that PNI could increase excitatory synaptic transmission and excitability in LC neurons. This finding is in accordance with other reports which revealed amplification in synaptic strength and neuronal excitability in other structures involved in pain processing; such as RVM (Robinson, et al. 2002) and anterior cingulate cortex (ACC) (Jia, et al. 2007). Covey et al, have reported that peripheral neuropathy and repetitive activation of nociceptive afferents raise TNF α release in LC (Covey, et al. 2002), which diminishes spinal noradrenaline release through α_2 -adrenoceptor redistribution (Covey, et al. 2000). It is supposed that TCAs deplete TNF α in noradrenergic area including LC (Ignatowski, et al. 1997, Bohren, et al. 2013), causing an augmentation of noradrenaline release in SDH and a stronger descending inhibition of nociception, which could explain the analgesic effect of these antidepressants in neuropathic pain conditions.

On the other hand, Ma et al, reported an augmentation of noradrenergic immunoreactivity in SDH, two weeks after CCI (Ma and Eisenach 2003), which is consistent with our results indicating that each of the applied stimulus intensities could cause a stronger eEPSCs in LC neurons of neuropathic rats in contrast to control group, one week after PNI. This could imply a gradual enhancement in noradrenergic system which may lead to an upregulation of noradrenergic innervations to SDH as an adaptive mechanism opposing chronic pain transmission.

The sEPSCs of LC neurons did not show any changes in frequency, but a significant increase in amplitude ($P<0.01$), 7 days after CCI, which could be interpreted either as a potentiation in postsynaptic AMPA receptor efficacy or as an increase in glutamate content of presynaptic vesicles. Paired pulse stimuli applied at 50 to 250 ms intervals to LC neurons showed no significant alteration in PPF after PNI. These results favor a postsynaptic component for the synaptic strengthening developed in LC after sciatic nerve injury. Whereas Li et al., showed a decline in paired pulse depression (PPD) in SDH after PNI (Li, et al. 2009). Zhao et al., have reported a diminished PPF after neuropathy in ACC and suggested a calmodulin-stimulated adenylyl cyclase (AC1 and/or AC8) mediated long-lasting enhancement in presynaptic glutamate release in the ACC after chronic pain (Zhao, et al. 2006). Xu et al., have reported both an increase in presynaptic release probability and postsynaptic AMPA receptor-mediated responses, due to upregulated phosphorylation of GluR1 subunit (Xu, et al. 2008).

Cellular responses to nerve injury are mainly attributed to microglial activation and pro-inflammatory cytokines such as TNF α and BDNF (Liu, et al. 2016). Although some studies have shown that neonatal nerve injury does not cause pain hypersensitivity before postnatal day 28 (Howard, et al. 2005), others have argued that neuropathic pain is not really absent in neonates, but is actively suppressed by anti-inflammatory cytokines such as IL-4 and IL-10 and leads to delayed onset of pain hypersensitivity (McKelvey, et al. 2015, Fitzgerald and McKelvey 2016). Such active suppression of central sensitization at SDH has been shown to be unmasked by intrathecal anti IL-10 administration (McKelvey, et al. 2015). The compensatory synaptic changes observed in LC neurons could also be caused by inflammatory cytokines, as 3 hours incubation of slices in TNF α (100 nM) elicited the same changes in synaptic parameters (i.e. increased EPSC amplitude and unchanged PPF) as PNI [unpublished data], arguing the possible involvement of TNF α as a mediator for induction of synaptic changes in LC. The role of TNF α in synaptic plasticity is shown in the hippocampus (Stellwagen and Malenka 2006), SDH (Zhang, et al. 2011), prefrontal cortex (Jia, et al. 2007) and LC (Ignatowski, et al. 1997). Stellwagen et al., demonstrated that TNF α leads to a rapid exocytosis of GluR2-lacking AMPA receptors in a PI3K-dependent manner in hippocampal pyramidal neurons (Stellwagen, et al. 2005). It has also been shown that peripheral neuropathy leads to TNF α expression in some structures including LC (Covey, et al. 2002). Moreover, behavioral studies have revealed that TNF α neutralization in LC could alleviate hyperalgesia and allodynia (Rohampour, et al. 2011), which further supports TNF α -mediated superficial expression of AMPA receptors, as the probable mechanism of increased glutamatergic synaptic transmission in LC, following PNI.

5. Conclusion

The results of this study showed that sciatic nerve injury could affect the excitatory synaptic transmission at supraspinal level in LC neurons which are involved in higher order pain processing and modulation. This study proposes a mainly postsynaptic mechanism for the observed synaptic potentiation, which might be elicited by a TNF α -mediated increase in surface expression of AMPA receptors. However further studies are required to precisely elucidate the underlying cellular mechanisms.

Acknowledgements

This work is supported by the Iran National Science Foundation (INSF) and the Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

References

- Arsenault, A. and J. Sawynok (2009). "Perisurgical amitriptyline produces a preventive effect on afferent hypersensitivity following spared nerve injury." Pain **146**(3): 308-314.
- Attal, N., et al. (2010). "EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision." Eur J Neurol **17**(9): 1113-e1188.
- Boadas-Vaello, P., et al. (2016). "Neuroplasticity of ascending and descending pathways after somatosensory system injury: reviewing knowledge to identify neuropathic pain therapeutic targets." Spinal Cord **54**(5): 330-340.
- Bohren, Y., et al. (2013). "Antidepressants suppress neuropathic pain by a peripheral β_2 -adrenoceptor mediated anti-TNF α mechanism." Neurobiol Dis **60**: 39-50.
- Covey, W. C., et al. (2000). "Brain-derived TNF α : involvement in neuroplastic changes implicated in the conscious perception of persistent pain." Brain Res **859**(1): 113-122.
- Covey, W. C., et al. (2002). "Expression of neuron-associated tumor necrosis factor alpha in the brain is increased during persistent pain." Reg Anesth Pain Med **27**(4): 357-366.
- Fitzgerald, M. and R. McKelvey (2016). "Nerve injury and neuropathic pain - A question of age." Exp Neurol **275 Pt 2**: 296-302.
- Havelin, J., et al. (2016). "Central Sensitization and Neuropathic Features of Ongoing Pain in a Rat Model of Advanced Osteoarthritis." J Pain **17**(3): 374-382.
- Howard, R. F., et al. (2005). "The ontogeny of neuropathic pain: postnatal onset of mechanical allodynia in rat spared nerve injury (SNI) and chronic constriction injury (CCI) models." Pain **115**(3): 382-389.
- Ignatowski, T. A., et al. (1997). "Neuronal-associated tumor necrosis factor (TNF α): its role in noradrenergic functioning and modification of its expression following antidepressant drug administration." J Neuroimmunol **79**(1): 84-90.
- Ikeda, H., et al. (2003). "Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia." Science **299**(5610): 1237-1240.
- Ikeda, H., et al. (2006). "Synaptic amplifier of inflammatory pain in the spinal dorsal horn." Science **312**(5780): 1659-1662.
- Ji, R. R., et al. (2003). "Central sensitization and LTP: do pain and memory share similar mechanisms?" Trends Neurosci **26**(12): 696-705.
- Jia, D., et al. (2007). "TNF- α involves in altered prefrontal synaptic transmission in mice with persistent inflammatory pain." Neurosci Lett **415**(1): 1-5.
- Kaeidi, A., et al. (2015). "Direct Facilitatory Role of Paragigantocellularis Neurons in Opiate Withdrawal-Induced Hyperactivity of Rat Locus Coeruleus Neurons: An In Vitro Study." PLoS One **10**(7): e0134873.
- Kremer, M., et al. (2016). "Antidepressants and gabapentinoids in neuropathic pain: Mechanistic insights." Neuroscience.
- Li, J., et al. (2009). "Peripheral nerve injury sensitizes neonatal dorsal horn neurons to tumor necrosis factor- α ." Mol Pain **5**: 10.

Ma, W. and J. C. Eisenach (2003). "Chronic constriction injury of sciatic nerve induces the up-regulation of descending inhibitory noradrenergic innervation to the lumbar dorsal horn of mice." Brain Res **970**(1-2): 110-118.

McKelvey, R., et al (2015). "Neuropathic pain is constitutively suppressed in early life by anti-inflammatory neuroimmune regulation." J Neurosci **35**(2): 457-466.

Nakao, A., et al. (2012). "Role of capsaicin-sensitive C-fiber afferents in neuropathic pain-induced synaptic potentiation in the nociceptive amygdala." Mol Pain **8**: 51.

Qiu, S., et al. (2013). "An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain." Sci Signal **6**(275): ra34.

Robinson, D., et al. (2002). "Long-lasting changes in rostral ventral medulla neuronal activity after inflammation." J Pain **3**(4): 292-300.

Rohampour, K., et al. (2011). "Microinjection of TNF- α and its antibody into locus coeruleus modifies nerve injury induced thermal hyperalgesia and mechanical allodynia." Physiology and Pharmacology **14**(4): 397-405.

Stellwagen, D., et al. (2005). "Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor- α ." J Neurosci **25**(12): 3219-3228.

Stellwagen, D. and R. C. Malenka (2006). "Synaptic scaling mediated by glial TNF- α ." Nature **440**(7087): 1054-1059.

Suzuki, T., et al. (2008). "Antiallodynic and antihyperalgesic effect of milnacipran in mice with spinal nerve ligation." Anesth Analg **106**(4): 1309-1315, table of contents.

Taves, S., et al. (2013). "Microglia and spinal cord synaptic plasticity in persistent pain." Neural Plast **2013**: 753656.

Tsuruoka, M., et al. (2004). "Persistent hindpaw inflammation produces coeruleospinal antinociception in the non-inflamed forepaw of rats." Neurosci Lett **367**(1): 66-70.

Voisin, D. L., et al. (2005). "Nociceptive stimulation activates locus coeruleus neurones projecting to the somatosensory thalamus in the rat." J Physiol **566**(Pt 3): 929-937.

West, W. L., et al. (1993). "The function of noradrenergic neurons in mediating antinociception induced by electrical stimulation of the locus coeruleus in two different sources of Sprague-Dawley rats." Brain Res **626**(1-2): 127-135.

Woolf, C. J. and M. W. Salter (2000). "Neuronal plasticity: increasing the gain in pain." Science **288**(5472): 1765-1769.

Xu, D., et al. (2015). "Persistent neuropathic pain increases synaptic GluA1 subunit levels in core and shell subregions of the nucleus accumbens." Neurosci Lett **609**: 176-181.

Xu, H., et al. (2008). "Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex." J Neurosci **28**(29): 7445-7453.

Zhang, L., et al. (2011). "TNF- α contributes to spinal cord synaptic plasticity and inflammatory pain: distinct role of TNF receptor subtypes 1 and 2." Pain **152**(2): 419-427.

Zhao, M. G., et al. (2006). "Enhanced presynaptic neurotransmitter release in the anterior cingulate cortex of mice with chronic pain." J Neurosci **26**(35): 8923-8930.

Figure Legends

Fig. 1. Effect of CCI on excitability of LC neurons. (A) Representative current clamp traces recorded during the 100 and 200 pA current steps applied to LC neurons of control slices (Cntl) and LC neurons of neuropathic rats (CCI) one week after injury. (B) Instantaneous frequency-current relation in control neurons (solid line) and in neurons of neuropathic rats (dashed line). Data presented as mean $\pm$ SEM *P<0.05 (unpaired t-test CCI vs. control, n=12).

Fig. 2. Effect of CCI on the amplitudes and frequency of sEPSCs. (A) Excitatory current traces of a representative healthy LC neuron (Cntl) and representative trace of sEPSC recorded from a LC neuron of neuropathic rats (CCI). (B) Cumulative probability plot of amplitude (B1) and inter-event intervals (B2) of sEPSC recorded from a given control/CCI neuron. (C) Quantification chart bars showing the effect of PNI on the amplitude and frequency of sEPSCs. Data are presented as mean $\pm$ SEM (n=11, **P<0.01, unpaired t-test).

Fig. 3. Effect of CCI on the eEPSCs. (A) eEPSCs elicited by different stimulus intensities (100-600 μ A) applied 100-300 μ m caudal to LC neurons. The evoked current is greater in CCI neurons in all stimulus intensities except 100 μ A. Data are presented as mean $\pm$ SEM (*P<0.05 vs. control, n=8, unpaired t-test). (B) Comparison of representative traces of eEPSCs recorded from LC neurons of control and neuropathic rats at a stimulus intensity of 200 μ A.

Fig. 4. Effect of CCI on PPR in LC neurons. (A) Comparison of paired pulse ratio between LC neurons of control and neuropathic rats in different inter-stimulus intervals (50-250 ms) did not cause any significant alteration in PPR. Data are presented as mean $\pm$ SEM (P>0.05, CCI vs. control, n=8, unpaired t-test). (B) A sample recoding trace of paired pulse stimuli applied with 50 ms intervals in control (solid line) and neuropathic LC neurons (dotted line).