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# The role of the GABA-A receptor of the adjacent intact dorsal root ganglion neurons in rats with neuropathic pain

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We aim to investigate the changes of The γ-aminobutyric acid (GABA) signals in the adjacent intact dorsal root ganglion (DRG) and the contribution of these changes to the development and maintenance of neuropathic pain (NPP). After establishment of neuropathic pain model with the lumbar 5 spinal nerve ligation (L5 SNL), the GABA-evoked currents were recorded in the acutely dissociated L4 DRG neurons using whole-cell patch clamp. Moreover, Muscimol or Bicuculline were respectively topically injected to the L4 DRG at the time of nerve injury and post-operative 5 days (POD5). The pain thresholds of hind paw were recorded. We found that the incidence and amplitude of GABA currents significantly decreased in the large and medium L4 DRG neurons after SNL ($P<0.05$). Furthermore, in the L5 SNL rats, 20 μg Muscimol injected immediately after injury caused a long-lasting attenuation of mechanical allodynia ($P<0.05$), whereas the thermal hyperalgesia was not alleviated ($P>0.05$). 0.15 μg Bicuculline further decreased the pain thresholds ($P<0.05$) and reversed the pain-alleviating effects of Muscimol. However, at POD5, 20 μg Muscimol only exhibited transient alleviating effects ($P<0.05$). In conclusion, the decrease of inhibition signal mediated by GABA-A receptor in the adjacent intact DRG neurons may be crucial in the development of hyperalgesia in NPP.

Key words: Rat, GABA, GABA-A, dorsal root ganglion, neuropathic pain

## INTRODUCTION

Peripheral nerve injuries can lead to the development of neuropathic pain (NPP). The symptoms affect the quality of daily life and can be unresponsive to conventional treatments (Nickel et al. 2012, Gilron et al. 2013). Although there are some new targets of treatment being issued in these years, such as calcium channel antagonists, NMDA antagonist, potassium channel agonist, sodium channel modulation and vanilloid receptor antagonist, etc, there is no effective therapeutic option for clinical treatment because the poor understand of the mechanism of neuropathic pain (Nickel et al. 2012).

NPP induced by peripheral nerve injury may be the result of a long-lasting increase in synaptic transmission in the spinal dorsal horn (Nickel et al. 2012). DRG neurons typically convey nociceptive information from the peripheral nerve to spinal dorsal horn. Evidence shows GABA-A receptor expressed on DRG neurons (Xiong et al. 2010, Naik et al. 2012). The activation of GABA-A receptor results in neuron's partial depolarization, which induces the blockade of the following information transmission from DRG to the spinal cord (Enna and McCarson 2006). A previous study has shown down-regulation of the GABA-A receptor in injured DRG neurons after spinal nerve injury might play an important role in the development of increased synaptic transmission in NPP (Naik et al. 2008, 2012). Many lines of evidence have revealed that a long-lasting increases of synaptic transmission, such as the augmented responsiveness and spontaneous activity of the neurons, do not only exist in the injured DRG, but also in the intact DRG adjacent to injured one (L4 DRG in the L5 SNL rats) (Obata et al. 2003, Jang et al. 2007b). However, no report has shown the

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functional role of GABA-A receptor of these intact adjacent DRG neurons in the mechanism of NPP.

Consequently, in the present study, we investigated the changes of the GABA-evoked currents in L4 DRG neurons and the effects of exogenous GABA modulation in the L4 DRGs on the symptoms of NPP after L5 SNL in rats. Our overall goal was to devise more effective therapeutic options for patients who suffer from NPP.

## METHODS

### Animals and reagents

Sixty Sprague-Dawley adult male rats (Faculty of Laboratory Animal Science, Hubei University of medicine), each weighing between 150–200 g at the time of the surgery, were used for experiments in this study. Muscimol (Sigma-Aldrich, St. Louis, MO, USA) is a GABA-A receptor agonist. Bicuculline (Sigma-Aldrich, St. Louis, MO, USA) is a GABA-A receptor antagonist. Both pharmacological reagents were freshly prepared and diluted in a sterile saline solution (400 μg/ml and 3 μg/ml, respectively) at pH 7.4 immediately prior to their application on to the L4 DRGs.

### L5 SNL Surgery

All experimental protocols were approved by the Animal Care and Use Committee of Hubei University of medicine. All of the procedures were performed according to the guidelines of the International Association for the Study of Pain in order to minimize the animal's suffering and minimize the number of animal used. The surgery for the L5 spinal nerve ligation was performed as previously described (Kim and Chung 1992). Briefly, the animals were anesthetized with isoflurane inhalation (3% for induction and 2.5% for maintenance). After the skin incision at the left side of spine at the L4–S2 level, the L5 transverse process was removed and exposed the L5 spinal nerve. The L5 spinal nerve were carefully isolated and tightly ligated with a 6-0 silk thread before suturing of the wound. The sham rats underwent the same procedure but without nerve ligation. Behavioral tests were performed to identify the reliable and stable hyperalgia state within 3–5 PODs (postoperative days).

### Preparation of L4 DRG neurons

Five days after the SNL or sham operation, the L4 DRG neurons from the ipsilateral side of either the sham or SNL rats were dissociated as previously described (Li et al. 2005). After the rats were anesthetized (4% isoflurane) and decapitated, the lumbar segments of the vertebrate column were dissected and longitudinally divided into 2 halves. The L4 DRGs were removed from the intervertebral foramen and transferred immediately into Dulbecco's Modified Eagle's medium (DMEM) (Sigma Chemical Co., St. Louis, MO, USA). The attached nerves and surrounding connective tissue were removed using scissors under anatomical microscope. The DRGs were subsequently minced with fine spring scissors, and the ganglion fragments were transferred into a flask containing 5 ml DMEM with 0.05 mg/ml trypsin (type III, Sigma) and 1 mg/ml collagenase (type I, Sigma) and were incubated at pH 7.4 and 35°C for 30–35 min in a

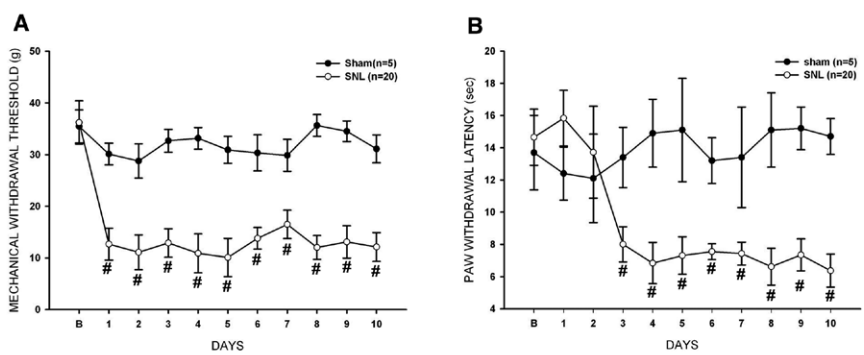


Fig. 1. Pain thresholds of the ipsilateral paw after L5 SNL before the surgery (indicated as B-baseline) and daily thereafter for up to 10 days. (A) The mechanical withdrawal threshold. (B) Paw withdrawal latency. # indicates significant differences ($P$<0.05) from the value of B (baseline sessions). Error bars = SEM.

shaking water bath. To neutralize the digestion, 1.25 mg/ml soybean trypsin inhibitor (type II-s, Sigma) was added, and the isolated neurons were placed into a 35-mm Petri dish and kept for at least 30 min before electrophysiological recording.

**Size determination**

We separated the neurons by size. Using a calibrated reticule in the light path of the microscope (Olympus CX41, Japan), the cell diameter was visually determined using an estimated average of the longest and shortest dimensions during the recording. Neurons that were 17–60 μm diameter in size were used, which represented essentially the entire size range of the neurons in the ganglia.

**Patch-clamp recordings**

Whole-cell voltage clamp recordings were performed on acute isolated DRG neurons using the EPC-10 patch clamp amplifier (HEKA Instruments, Germany). The patch pipettes were filled with an internal solution containing (mmol/L): KCl 140,

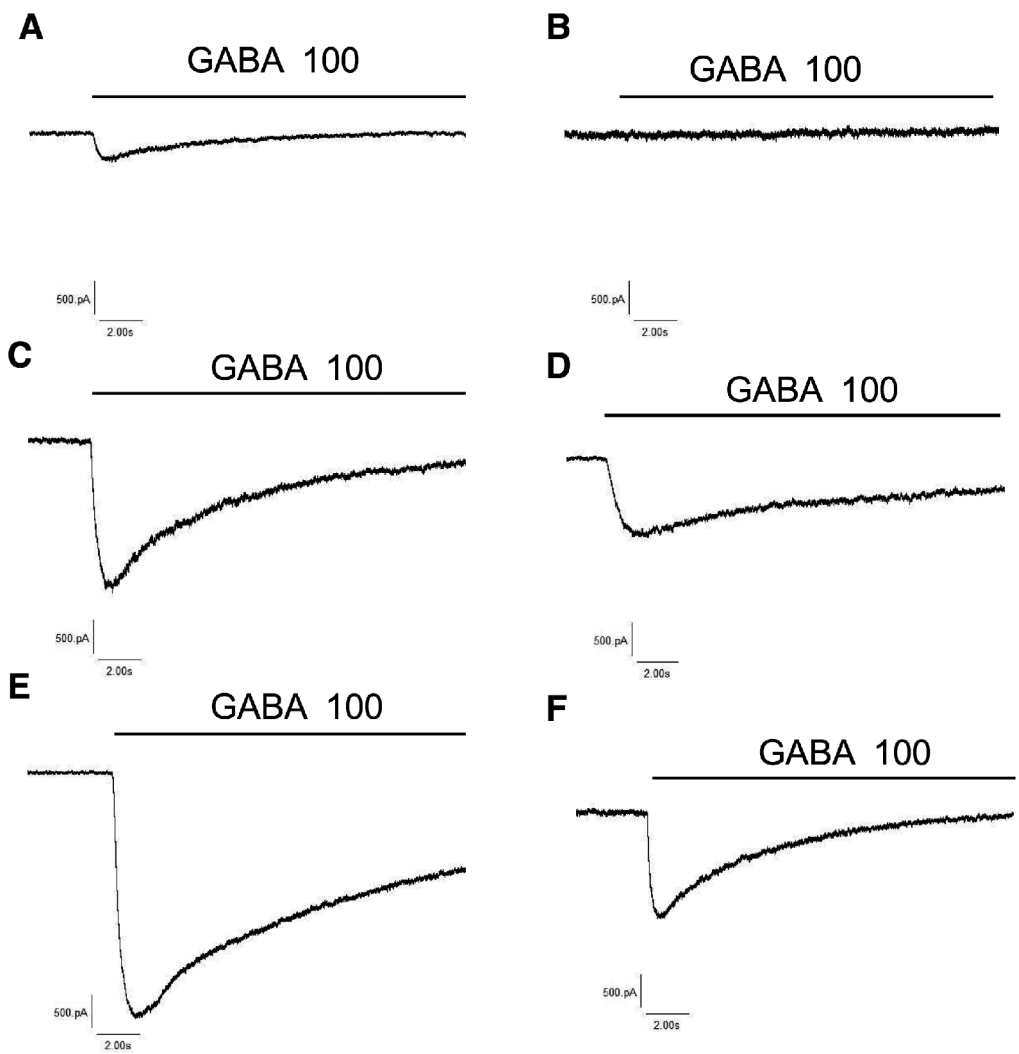


Fig. 2. GABA currents of DRG neurons. The neurons were voltage-clamped at −60 mV. Continued application of 100 μM GABA induced an inward current in neurons of the right L4 DRG of sham rats and SNL rats. This inward current reached a peak within 200 ms and declined to a plateau, despite continued application of GABA. (A) Small neuron in sham rat. (B) Small neuron in SNL rat. (C) Medium neuron in sham rat. (D) Medium neuron in SNL rat. (E) Large neuron in sham rat. (F) large neuron in SNL rat. GABA current was apparent in the large neurons and medium neurons. There was almost no current in small neurons. The amplitude of GABA current in SNL rats was lower than that in sham rats.

$CaCl_2$ 1, $MaCl_2$ 2.5, HEPES 10, and EGTA 11, at pH 7.2 (adjusted with KOH), and an osmolarity of 310 mOsmol/L (adjusted with sucrose). Neurons were bathed in an external solution containing (mmol/L): NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, D-glucose 10, at pH 7.4 (adjusted with NaOH), and an osmolarity of 330 mOsmol/L (adjusted with sucrose). The electrodes had a resistance of 2–4 MΩ. The series resistance (<15 MΩ) and capacitance were compensated prior to drug application. The GABA (Sigma-Aldrich, St. Louis, MO, USA) was extracellularly applied by gravity flow from an array of fused silica glass tubes (o.d./i.d.=500 μm/200 μm). The distance from the mouth of the tube to the cell was approximately 100 μm. The membrane potential was held at −60 mV. The GABA currents, which were filtered at 2 kHz and sampled at 100 kHz, were recorded after the 100 μmol/L GABA was applied on to the cell. The data were recorded using a Patchmaster software (HEKA, Germany).

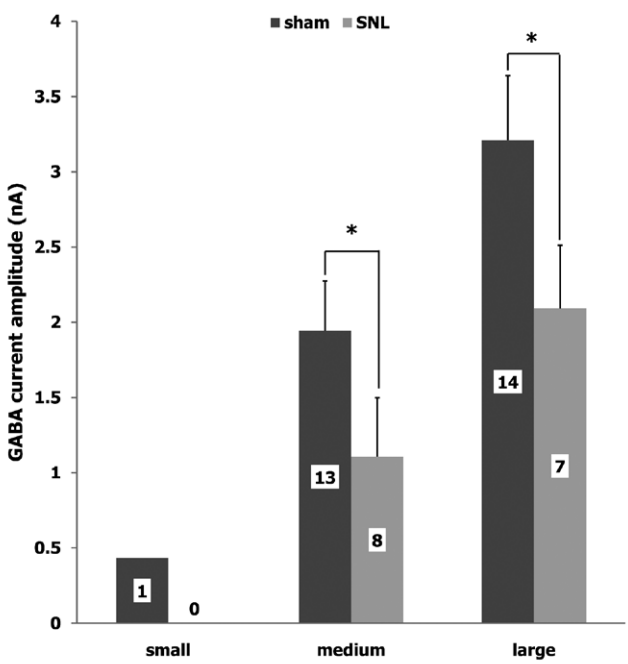


Fig. 3. The amplitude of GABA currents of different L4 DRG neurons in the L5 SNL rats and sham rats. The number on the bar shows the amount of the neurons. * indicates significant differences between the values in the SNL rats and sham rats ($P$<0.05). The apparent decline was seen in the large and medium neurons in the SNL rats. Error bars = SEM.

**Topical application to the L4 DRGs and treatment protocol**

To test the effects of GABA modulation, we applied 50 μl of muscimol (400 μg/ml) or bicucullline (3 μg/ml) directly on to the right L4 DRGs either at the time of the SNL surgery or on postoperative day 5 (POD 5).

The animals were anesthetized with isoflurane (3% for induction and 2.5% for maintenance), the skin was incised, and the right paravertebral region was exposed. The connective tissue and muscles were removed by iris scissors, thereby exposing the bony structures and the right L4 intervertebral foramen. The transverse processes and the marginal laminar rim caudal to the L4 ganglion were partially removed using a small rongeur to expose the caudal part of the ganglion. To avoid an injury to the ganglion from the needle, a PVC catheter (internal diameter 200 μm) was connected to the microsyringe needle. The tip of the catheter was subsequently placed near the ganglion through the right L4 intervertebral foramen. After 50 μl of the vehicle had been slowly injected into the L4 DRG, the catheter was left in place for at least 3 min to ensure complete delivery of the solution and to avoid potential extravasation outside of the DRG site. Moreover, 50 μl muscimol was sequentially co-administered with 50 μl bicuculline a few minutes apart.

**DRG fluorescein study**

It is possible that the drug solutions could spread to the adjacent DRGs or into areas surrounding the spinal cord. To address this issue, we randomly chose 10 rats and injected a 50 μl test solution of sodium fluorescein (Sigma-Aldrich, concentration 700 μg/100 μl) using the same protocol that was described earlier. Then, we placed back the surrounding tissue (without suturing) and kept the animal anesthetized. After 30 min, the rat was perfused *via* the aorta with 4% paraformaldehyde. The right L4 and L5 DRGs and the corresponding L4 segment of the spinal cord were harvested and quickly frozen in 2-isobutane until sectioning. Next, we analyzed the images using a fluorescence microscope (Olympus MF51, Japan) equipped with an FITC filter.

### Behavioral test

The Electronic von Frey Anesthesiometer (IITC Inc. Life Science Instrument, Woodland Hills, CA, USA) was used to measure the nociceptive response to the mechanical stimulation. Initially, the animals were placed unrestrained in the acrylic cage with a wire grid floor. The rigid tip of von Frey filament (0~90 gram range) was placed beneath the wire grid floor and upward probed the rat's hindpaw with a gradual increase in pressure. The mechanical withdrawal threshold was automatically recorded when the hindpaw was withdrawn. of the paw. Each session included five stimulus presentations at 5 minute intervals, and the mean values of the five readings were used as the mechanical threshold for this test.

We used a thermal paw stimulation system (Hargreaves's Apparatus, Ugo Basile Inc, Italy) to measure the nociceptive thermal threshold. The plastic chamber (10×20×24 cm) sat on a clear elevated glass floor. A mobile infrared heat source was positioned beneath the glass floor to deliver a thermal stimulus to the plantar side of the hind paw. When the animal felt pain and withdrew its paw, the instrument would automatically shut off the timer and record paw withdrawal latency (PWL) with a precision of ±0.1 s. To prevent thermal injury, the light beam was automatically discontinued for 20 s if the rat failed to withdraw its paw. For each experiment, the procedure was repeated five times with 5 minute intervals, and the mean value of the five readings was used for analysis.

### Statistical analysis

The data are presented as means ± standard error of the mean (SEM). The electrophysiological data were statistically compared using Student's *t* test and the χ-square test. The behavioral data were statistically analyzed with an ANOVA test, and the *post-hoc* Dunn's multiple comparison test was used to compare the means of each group. $P<0.05$ were considered to be statistically significant. All of the statistical analyses were performed using the SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA).

## RESULTS

### Nociceptive response of rats with L5 SNL

The rats with the L5 SNL ($n$=20) developed characteristic postural manifestations in the ipsilateral hind leg and also displayed cupped hindpaws. There was a significant decrease of the mechanical withdrawal thresholds (Fig. 1A) and PWLs (Fig. 1B) on the side ipsilateral to the nerve injury compared with pre-operative baseline, which was interpreted as mechanical allodynia and thermal hyperalgesia, respectively. The mechanical allodynia was observed as early as the first day after injury and lasted up to

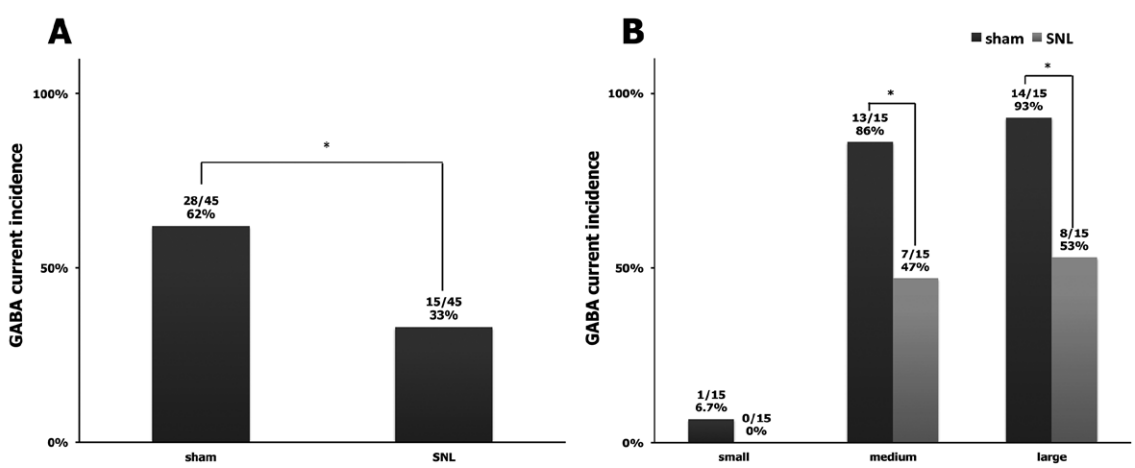


Fig. 4. The incidence of GABA currents in the L4 DRG neurons of SNL rats and sham rats. * indicates the significant difference between these groups ($P<0.05$). (A) The significant decrease of the incidence of GABA currents in total L4 DRG neurons in SNL rats. (B) The significant decrease of the incidence of GABA currents in medium and large L4 DRG neurons in SNL rats.

10 days as well (Fig. 1A). The thermal hyperalgesia was apparent on the third day after injury and lasted up to 10 days (Fig. 1B). The sham-operated rats ($n$=5) displayed no postural manifestations, hyperalgesia and allodynia after operation (Fig. 1A, B).

### GABA currents on the DRG neurons

Dissociated neurons obtained from five sham rats ($n$=45) and six SNL rats ($n$=45) were divided into three groups according to cell diameter: small DRG neurons (24.6 ± 4.5 μm, $n$=30), medium DRG neurons (33.4 ± 3.7 μm, $n$=30) and large DRG neurons (45.7 ± 4.9 μm, $n$=30). When 100 μM GABA was applied to L4 DRG neurons that were voltage clamped at −60 mV, some of the large and medium neurons in both groups displayed an inward current with a characteristic rapid desensitization (Fig. 2C–F). There is almost no current activation in small neurons (Fig. 2A,B). Furthermore, compared with the sham rats, the decline in the amplitude of the GABA current was found in the L4 DRG neurons after L5 SNL. This decline was apparent in the large and medium neurons ($P$<0.05) but not in the small L4 DRG neurons (Fig. 3).

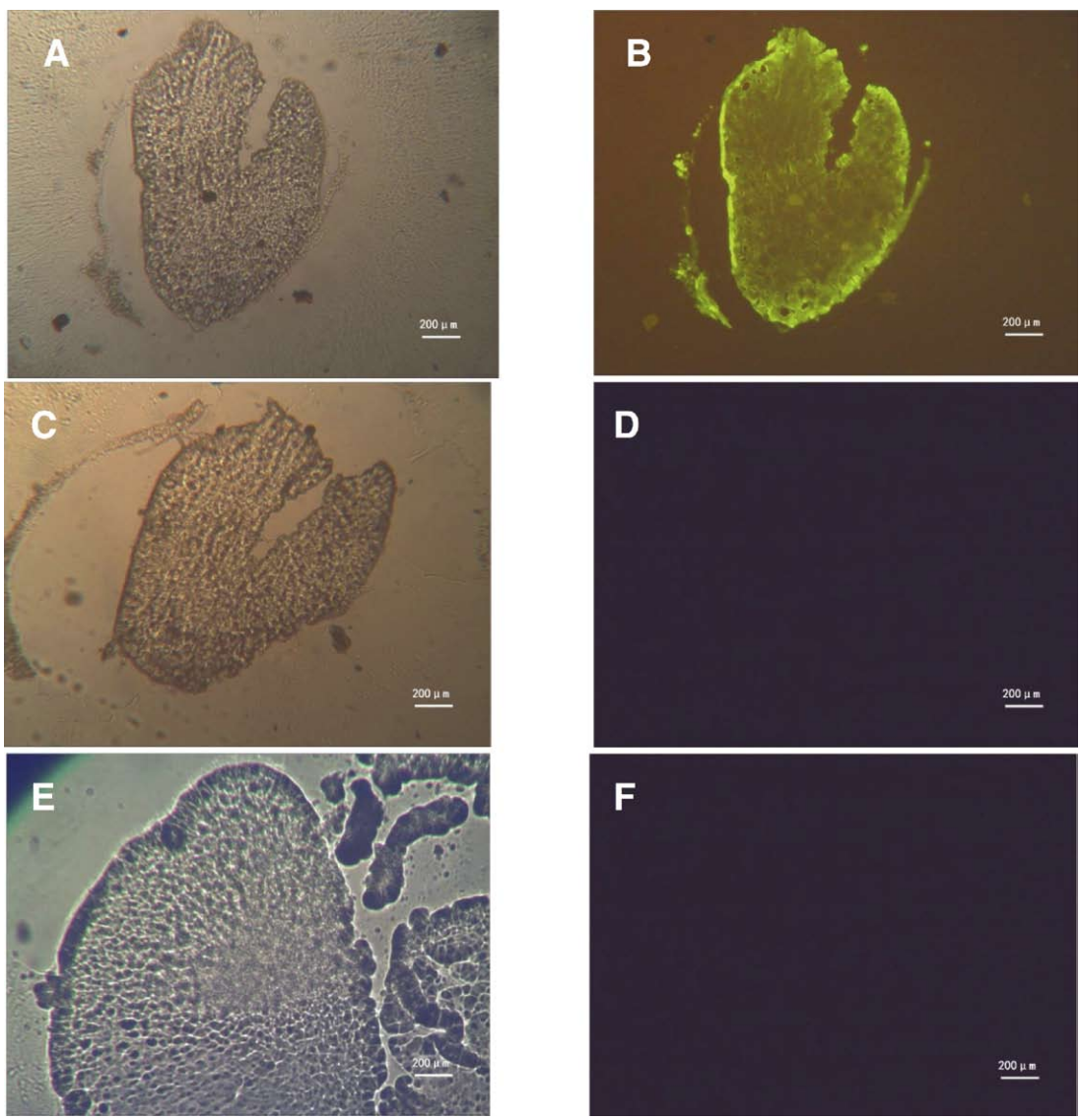


Fig. 5. The image of DRG and dorsal horn at 30min after sodium fluorescein topically injected into L4 DRG. (A) Bright-field image of the right L4 DRG. (B) Fluorescence image of the right L4 DRG. (C) Bright-field image of the right L5 DRG. (D) Fluorescence image of the right L5 DRG. (E) Bright-field image of the right L4 dorsal horn. (F) Fluorescence image of the right L4 dorsal horn.

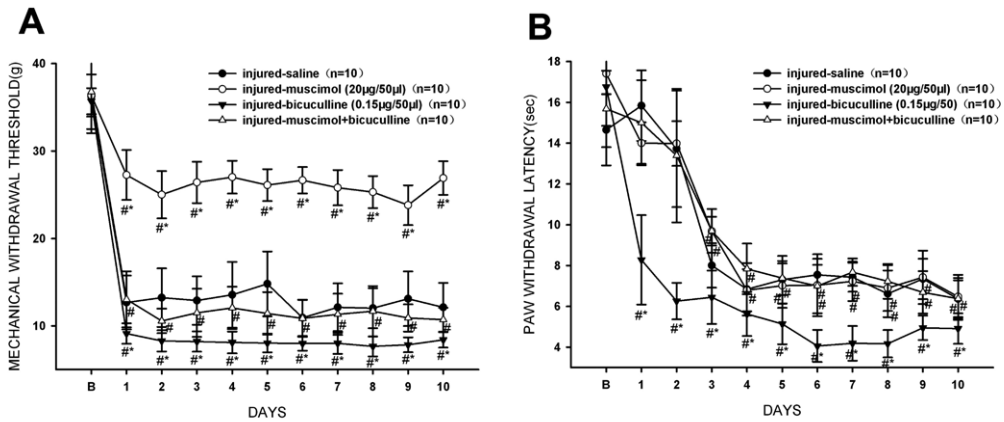


Fig. 6. The effects of GABAergic regulation in the L4 DRG at the time of lumbar 5 nerve injury on thermal and mechanical nociception. (A) The mechanical withdrawal threshold. (B) The paw withdrawal latency. * indicates significant differences ($P$<0.05) from the values in the SNL rats treated with saline. # indicates significant differences ($P$<0.05) from the value of B (baseline sessions). Error bars = SEM.

### Incidence of GABA currents on the DRG neurons of SNL rats

The GABA current was seen in 15/45, or 33% of the neurons of the SNL and in 28/45, or 62% of the neurons from the sham. The neurons obtained from the SNL rats showed a much lower incidence of GABA currents than those obtained from the sham rats ($P$<0.05) (Fig. 4A). In the sham and SNL rats, the higher incidence of GABA current was apparent in the large neurons and medium neurons. The incidence of the GABA current in the medium and large neurons was significantly decreased in the SNL rats compared with the sham rats ($P$<0.05) (Fig. 4B).

### DRG fluorescein study

We observed a high uptake of fluorescein into the right L4 DRG in all 10 rats but none in the ipsilateral L4 dorsal horn and L5 DRG (Fig. 5).

### Nociceptive responses in the SNL rats after GABA-A receptor modulation on the L4 DRG at the time of injury

In comparison with the rats that received saline injection ($n$=10), the rats that received 20 μg muscimol ($n$=10) displayed a significant increase in the mechanical withdrawal thresholds at 10 days post surgery

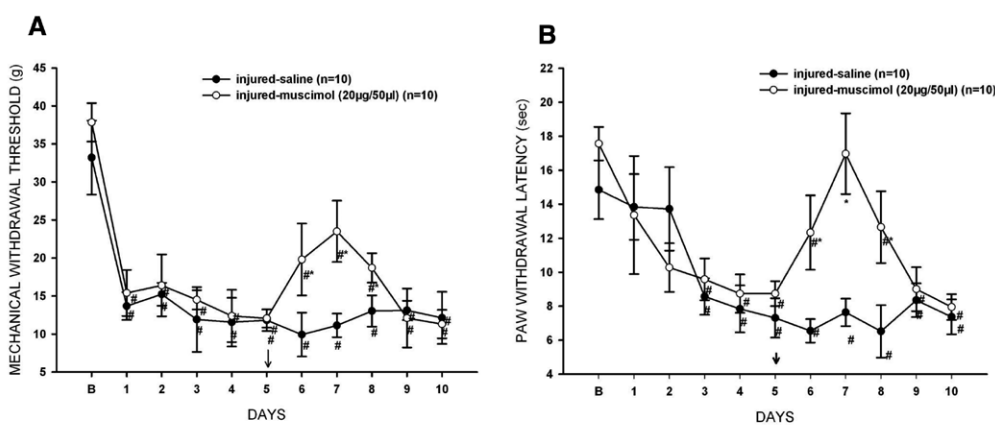


Fig. 7. The effects of Muscimol in the L4 DRG at POD 5 on thermal and mechanical nociception. (A) The mechanical withdrawal threshold. (B) The paw withdrawal latency. The arrow indicates the time of the injection. * indicates significant differences ($P$<0.05) from the values in the SNL rats treated with saline. # indicates significant differences ($P$<0.05) from the value of B (baseline sessions). Error bars = SEM.

($P$<0.05). The muscimol injected rats still exhibited a significant decrease in the mechanical threshold at 10 days post-surgery compared with the pre-surgical baseline value ($P$<0.05) (Fig. 6A). Acute muscimol injection into the DRG had no effect on the PWLs in the SNL rats, even at 10 days post surgery (Fig. 6B). In contrast, an acute L4 DRG administration of 0.15 μg bicuculline ($n$=10) produced a significant decrease in the mechanical withdrawal thresholds (Fig. 6A) and PWLs (Fig. 6B) in the L5 SNL rats at 10 days post surgery ($P$<0.05). Furthermore, compared with the pre-surgical baseline value, a significant decrease in the PWLs was observed on the first postoperative day in the bicuculline-treated rats ($P$<0.05) (Fig. 6B). The co-administration of muscimol with bicuculline ($n$=10) produced no significant effect on the mechanical withdrawal thresholds (Fig. 6A) and PWLs (Fig. 6B) ($P$>0.05).

### Nociceptive responses in the SNL rats after GABA-A receptor modulation on the L4 DRG after development of NPP

On POD 5, when the thermal hyperalgesia and mechanical allodynia were stable, 20 SNL rats were randomly assigned to receive direct administration of either saline or 20 μg muscimol on to the L4 DRG. As shown in Figure 7, the patterns of development of mechanical and thermal hypersensitivity during the first 5 days post surgery were similar among rats that were treated with saline. Both the mechanical withdrawal thresholds (Fig. 7A) and PWLs (Fig. 7B) in the animals treated with muscimol had significantly increased on PODs 6 and 7 compared with that of the animals treated with saline ($P$<0.05). By POD 8, the mechanical withdrawal thresholds (Fig. 7A) and PWLs (Fig. 7B) began to decrease. By POD 9, the mechanical withdrawal thresholds (Fig. 7A) and PWLs (Fig. 7B) were indistinguishable from that found in saline-treated rats ($P$>0.05) (Fig. 7).

## DISCUSSION

A loss of synaptic inhibiton (i.e. disinhibition) has been increasingly recognized as an important process in the development and maintenance of neuropathic pain (Nickel et al. 2012). It has been established that GABA can bind with the GABA-A receptor on DRG neurons, and open the $Cl^-$ ion channel, leads to the inward currents (Nishi et al. 1974). By causing sustained depolarization of the sensory neurons, GABA-A receptor may affect the ability of the sensory neurons to fire when stimulated (Price et al. 2009, Zeilhofer et al. 2009). The present results also found this kind of inward currents. Furthermore, this GABA-activated current mostly presents in the large and medium DRG neurons. Small neurons rarely showed the GABA-activated current.

In our study, we found the incidence of GABA currents decreased in large and medium L4 DRG neurons in SNL rat. It indicates that some medium and large neurons with GABA-A receptor may lose in the corresponding DRG. Furthermore, as for a single neuron, the amplitude of GABA currents on the medium or large L4 DRG neurons also decreased, which indicates a decrease in GABA-A receptor density on a single neuron. The GABA currents' decreases either in whole DRG or in single DRG neurons means a decrease of GABAergic transmission on the spine pre-synaptic inhibition, which may lead to the increased activity in corresponding DRG. Evidences have shown that the afferent stimulation through the uninjured DRG has the crucial role in the development of mechanical allodynia in NPP (Jang et al. 2007a, Ji et al. 2007, Shehab et al. 2008). The augmented activity in intact adjacent DRG neurons may result in more stimuli transmitted from the afferent nerve to spine, and result in the hypersensitivity existing in the NPP. This may be the mechanism of the neuropathic pain. Of course, besides the quantitative alteration of GABA-A receptor, the characteristic alteration of GABA-A receptor, such as conductance properties, may also result in the amplitude decrease of GABA-A currents under whole-cell patch-clamp recording. The identification of the reason may depend on the further study with single-channel patch-clamp techniques.

Additionally, we intended to topically inject GABA-A receptor modulators into the corresponding DRG. As to the focused therapy methods, we have manifested the high uptake of fluorescein in the right L4 DRG, but almost none in the ipsilateral L4 dorsal horn and L5 DRG, indicating that the agents can be successfully constrained in the L4 DRG with topically injection. Indeed, application of agents into the DRG has been used in many studies (Puljak et al. 2009).

This approach has the advantage that therapy targeting the DRG specifically avoids systemic and CNS toxicity during treatment.

The previous study has established that spinal administration of GABA-A receptor agonist only partially alleviates neuropathic hypersensitivity, and they believe that the GABA-A receptor in the spinal cord is not the crucial factor in the development of NPP (Munro et al. 2008). In fact, many findings suggest a close complex communication between DRG and the spinal dorsal horn, which may be crucial in controlling GABA availability (Labrakakis et al. 2003, Liu et al. 2004). The modulation of GABA-A receptor in the DRG may prevent the biochemical or biophysical changes in the corresponding spinal dorsal horns, which, then, cause the prevention and alleviation of NPP. In this study, we did find that Muscimol applied to the L4 DRG at the time of ligation caused an obvious alleviation of mechanical allodynia in rat of NPP. This effect was blocked by Bicuculline. This may be the obvious evidence to show the important role of GABA-A receptor of intact DRG in development of NPP. But, when Muscimol was applied to the same DRG with the same dose after NPP was fully developed, its pain-alleviating effects were short lived. This means the minor role of GABA-A receptor in the maintenance of NPP. It can be explained through the possible down-regulation or losses of GABA-A receptors in the DRG neurons at later stage of SNL injury, which may lead to the decrease of the effects of GABA-A receptor agonist. The other reason may be that the GABA has been down-regulated in the stage of NPP. The exogenous GABA can only transiently compensate this lack. The exact reason may need us to advanced study.

In this study, we also found the different effects of GABA agonist on mechanical allodynia and thermal hyperlgesia. Muscimol, applied to the L4 DRG at the time of injury, only caused transient alleviation of thermal hyperalgesia, whereas can alleviate the mechanical threshold for up to 10 days. It can be explained by the absence of GABA currents in most of small DRG neurons, which was found in the previous electrophysiological study. This absence suggested that the presynaptic inhibitory effects mediated by the GABA-A receptors' activation are less in the small DRG neurons. According to the past study, small DRG neurons largely receive the signals from the C afferent fibers (Harper and Lawson 1985). The afferent fibers responsive to heat stimuli were all C-fibers, and the sensitization of uninjured C nerve contributes to the neuropathic pain (Shim et al. 2005). Consequently, the alleviation of thermal hyperalgesia mediated by the GABA-A receptor modulation may be less and transient.

## CONCLUSION

In conclusion, an early intervention aimed at the GABA-A receptor in the adjacent intact DRG neurons can induce long-lasting alleviation of mechanical hypersensitivity, which infers that the restoration of the GABA-A inhibition system in the adjacent intact DRG may be a potential approach to be used in the management of NPP.

## ACKNOWLEDGMENTS

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## REFERENCES

Enna SJ, McCarson KE (2006) The role of GABA in the mediation and perception of pain. Adv Pharmacol 54: 1–27.

Gilron I, Jensen TS, Dickenson AH (2013) Combination pharmacotherapy for management of chronic pain: from bench to bedside. Lancet Neurol 12: 1084–1095.

Harper AA, Lawson SN (1985) Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurons. J Physiol (Lond) 359: 31–46.

Jang JH, Kim KH, Nam TS, Lee WT, Park KA, Kim D-W, Leem JW (2007a) The role of uninjured C-afferents and injured afferents in the generation of mechanical hypersensitivity after partial peripheral nerve injury in the rat. Experimental Neurology 204: 288–298.

Jang JH, Kim KH, Nam TS, Lee WT, Park KA, Kim DW, Leem JW (2007b) The role of uninjured C-afferents and injured afferents in the generation of mechanical hypersensitivity after partial peripheral nerve injury in the rat. Exp Neurol 204: 288–298.

Ji G, Zhou S, Kochukov MY, Westlund KN, Carlton SM (2007) Plasticity in intact A[delta]- and C-fibers contributes to cold hypersensitivity in neuropathic rats. Neuroscience 150: 182–193.

Kim SH, Chung JM (1992) An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50: 355–363.

Labrakakis C, Tong CK, Weissman T, Torsney C, MacDermott AB (2003) Localization and function of ATP and GABAA receptors expressed by nociceptors and other postnatal sensory neurons in rat. J Physiol 549: 131–142.

Li GH, Guan BC, Li ZW (2005) Effects of dopamine, SKF-38393 and R(-)-NPA on ATP-activated currents in rat DRG neurons. Can J Physiol Pharmacol 83: 267–277.

Liu J, Wolfe D, Hao S, Huang S, Glorioso JC, Mata M, Fink DJ (2004) Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain. Mol Ther 10: 57–66.

Munro G, Lopez-Garcia JA, Rivera-Arconada I, Erichsen HK, Nielsen EO, Larsen JS, Ahring PK, Mirza NR (2008) Comparison of the novel subtype-selective GABAA receptor-positive allosteric modulator NS11394 [3'-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitr ile] with diazepam, zolpidem, bretazenil, and gaboxadol in rat models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 327: 969–981.

Naik AK, Pathirathna S, Jevtovic-Todorovic V (2008) GABAA receptor modulation in dorsal root ganglia in vivo affects chronic pain after nerve injury. Neuroscience 154: 1539–1553.

Naik AK, Latham JR, 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. Anesth Analg 114: 674–682.

Nickel FT, Seifert F, Lanz S, Maihöfner C (2012) Mechanisms of neuropathic pain. Eur Neuropsychopharmacol 22: 81–91.

Nishi S, Minota S, Karczmar AG (1974) Primary afferent neurones: The ionic mechanism of GABA-mediated depolarization. Neuropharmacology 13: 215–219.

Obata K, Yamanaka H, Fukuoka T, Yi D, Tokunaga A, Hashimoto N, Yoshikawa H, Noguchi K (2003) Contribution of injured and uninjured dorsal root ganglion neurons to pain behavior and the changes in gene expression following chronic constriction injury of the sciatic nerve in rats. Pain 101: 65–77.

Price TJ, Cervero F, Gold MS, Hammond DL, Prescott SA (2009) Chloride regulation in the pain pathway. Brain Res Rev 60: 149–170.

Puljak L, Kojundzic SL, Hogan QH, Sapunar D (2009) Targeted delivery of pharmacological agents into rat dorsal root ganglion. J Neurosci Methods 177: 397–402.

Shehab SAS, Al-Marashda K, Al-Zahmi A, Abdul-Kareem A, Al-Sultan MAH (2008) Unmyelinated primary afferents from adjacent spinal nerves intermingle in the spinal dorsal horn: A possible mechanism contributing to neuropathic pain. Brain Res 1208: 111–119.

Shim B, Kim DW, Kim BH, Nam TS, Leem JW, Chung JM (2005) Mechanical and heat sensitization of cutaneous nociceptors in rats with experimental peripheral neuropathy. Neuroscience 132: 193–201.

Xiong YC, Li XM, Wang XJ, Liu YQ, Qiu F, Wu D, Gan YB, Wang BH, Hu WP (2010) Prokineticin 2 suppresses GABA-activated current in rat primary sensory neurons. Neuropharmacology 59: 589–594.

Zeilhofer HU, Möhler H, Di Lio A (2009) GABAergic analgesia: new insights from mutant mice and subtype-selective agonists. Trends Pharmacol Sci 30: 397–402.