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RESEARCH ARTICLE

# Inhibitory Effects of *Scolopendra* Pharmacopuncture on the Development and Maintenance of Neuropathic Pain in Rats: Possible Involvement of Spinal Glial Cells

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**Abstract**
*Scolopendra* extracts were used for pharmacopuncture at the Kidney 1 acupoint to investigate the role of *Scolopendra* pharmacopuncture (SPP) in both the development and maintenance of neuropathic pain induced by L5 spinal nerve ligation in rats and the contribution of spinal glial cells. A single treatment and five once-daily treatments with SPP were given to evaluate its effects on the development and maintenance stages of neuropathic pain, respectively, which was followed by behavioral tests. Immunohistochemistry and Western blotting tests were also carried out. A single treatment of SPP delayed spinal nerve ligation-induced mechanical allodynia and thermal hyperalgesia and induced a profound decrease in the expression of ionized calcium binding adaptor protein in the lumbar spinal cord. Repeated SPP treatments reliably suppressed mechanical allodynia and thermal hyperalgesia at later time points, and these results correlated mainly with decreases in glial fibrillary acidic protein. Intriguingly, ionized calcium binding adaptor protein expression was also reduced after repeated SPP. These results illustrate that neuropathic pain in the development and

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maintenance stages is alleviated by SPP treatment, which may be ascribed principally to deactivations of microglia and astroglia, respectively. Additionally, microglial inactivation seems to be partially involved in preventing neuropathic pain in the maintenance stage.

## 1. Introduction

Peripheral nerve injury is frequently accompanied by neuropathic pain, which is characterized by spontaneous burning pain, hyperalgesia, and allodynia [1—3]. Intensive research has led to major progress in the understanding of the basic mechanisms of the development and the maintenance of neuropathic pain. Convincing evidence indicates that the activations of spinal microglia and astrocytes, as evaluated by overexpression of glial activation markers such ionized calcium binding adaptor protein (Iba-1) and glial fibrillary acidic protein (GFAP), are observable following L5 spinal nerve ligation (SNL) [4—7]. However, neuropathic pain is often poorly managed when treated by using currently available medications owing to the presence of many side effects, including motor dysfunction, sedation, and tolerance [8—12]. Thus, the issue of neuropathic pain has recently become more visible and has been recognized as one of the most intractable challenges to modern medicine [13,14].

Alternatively, acupuncture has been traditionally utilized to treat peripheral neuropathy in Korean medicine [15—18]. Laboratory studies have demonstrated that electroacupuncture alleviates neuropathic pain via suppression of the activation of spinal glial cells [19]. Furthermore, the acupoint Kidney 1 (KI1) has been shown to be involved in alleviating diabetic peripheral neuropathy, contributing to treatment with alliance combination of electroacupuncture and acupoint injection [20]. More recently, pharmacopuncture with the herbal drug Sciatica 5, which is emerging as a promising treatment, has been reported to facilitate functional recovery after peripheral nerve injury [21]. The centipede *Scolopendra subspinipes mutilans* L. Koch has a reputation in traditional East Asian medicine for being effective in treating a variety of disorders, such as joint problems, tetanus, stroke, convulsions, neoplasm, Alzheimer's disease, and so forth [22,23]. In addition, water extracts of *Scolopendra* have analgesic properties, as evidenced by the increased pain threshold in mice during hot-plate tests [24].

As many ideas are based more on speculation than on fact, the question as to whether injection of *Scolopendra* extracts into KI1 is capable of suppressing the allodynic and the hyperalgesic responses to mechanical and thermal stimuli comparable to the peripheral neuropathic condition induced by using L5 SNL [25], the signs of which closely resemble certain clinical symptoms of human patients with causalgia and reflex sympathetic dystrophy [26], is still appropriate. If the answer is yes, then deciphering whether the analgesic effect of *Scolopendra* pharmacopuncture (SPP) is correlated with the suppression of the activation of spinal glial cells in the initiation and maintenance of neuropathic pain would make more sense.

## 2. Materials and methods

### 2.1. SPP and control group treatment protocols

A pharmacopuncture recipe for *Scolopendra* extract was obtained from the Korean Pharmacopuncture Institute (Seoul, Korea). Pharmacopuncture therapy was performed at the KI1 acupoint, which is located at the depression in the middle of the metatarsal area on the plantar side of the back, using a 1 mL disposable insulin injection syringe with a 29-gauge needle to slowly inject 200 μL of *Scolopendra* extract (1 mg). The depth of needle insertion was about 5—15 mm. When the needle was inserted into the skin, a sharp and painful sensation was induced, and a spreading, lumpish sensation around the injection site was reported. With regard to a control intervention, because *Scolopendra* extract and normal saline (SAL) are identical in appearance, normal saline was utilized, instead of *Scolopendra* extract, to stimulate the acupoint identically. As additional controls, the selected acupoint, the needle used, the depth of needle penetration, the dose injected, and the treatment schedule were identical throughout this research.

### 2.2. Animals

Experiments were performed on young adult male Sprague—Dawley rats (200—250 g; Hyochang Science, Daegu, Korea). The rats were randomly allocated to five groups as follows: Group A (naïve, $n = 3$); Group B (1 time injection of SAL at Day 0, SNL + SAL, $n = 8$); Group C (1 time injection of SPP at Day 0, SNL + SPP, $n = 8$); Group D (repeated injection of SAL, SNL + SAL, $n = 8$); and Group E (repeated injection of SPP, SNL + SPP, $n = 8$). All rats underwent behavioral tests, as well as immunohistochemistry and Western blot analysis. During the experimental period, all animals were housed in groups of three in plastic cages with soft bedding and a 12/12-hour reversed light—dark cycle in a temperature (22—25°C)-controlled environment with *ad libitum* access to food and water. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 2008 (Korea) and complied with the recommendations of the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee on Animal Research at Pusan National University, Yangsan Gyeongnam, Korea (PNU-2012-0090).

### 2.3. Neuropathic surgery

Rats were anesthetized with isoflurane (3% induction, 2% maintenance) in a mixture of $N_2O/O_2$, and a midline skin incision was made on the back at the lower lumbar region.

The paraspinal muscles were retracted, and the right transverse process of the L6 vertebra was removed under a dissection microscope. Then, the right L5 spinal nerve was identified and tightly ligated, as described in a previous report [25].

### 2.4. Evaluation of neuropathic pain sensitivity

Mechanical allodynia was assessed by using a dynamic plantar anesthesiometer (Ugo Basile, Varese, Italy) to measure the threshold to withdraw the hind paw from a graded force applied to the paw's surface with a Von Frey filament. The cutoff force was set at 50 g. Thermal hyperalgesia was assessed by using a commercial plantar test apparatus (Ugo Basile) to measure the latency to withdraw the hind paw from a focused beam of radiant heat (infrared intensity was 70) applied to the plantar surface (Hargreave's method). The cut-off latency was set at 30 seconds.

### 2.5. Immunohistochemistry

The L5—6 segments of the spinal cords were dissected by performing a laminectomy on the 4$^{th}$ day and the 11$^{th}$ day after SNL and were fixed in 4% paraformaldehyde for 6—8 hours, after which they were immersed in 30% sucrose for 48 hours at 4°C. Frozen tissues were sectioned at a thickness of 30 μm on a freezing microtome (CM3050S; Leica Biosystems, Wetzlar, Germany). The tissue sections were processed for immunostaining using the Avidin-Biotin Complex method (Vector Laboratories, Burlingame, CA, USA). In more detail, the sections were washed with 0.05M phosphate-buffered saline (PBS) and incubated in 3% hydrogen peroxide for 10 minutes. After rinses, the sections were blocked with CAS-block (Invitrogen-Molecular Probes, Inc., Camorillo, CA, USA) for 10 minutes at room temperature, followed by incubation overnight in PBS at 4°C with primary antibodies: mouse anti-GFAP (1:400, #MAB360; Millipore, Temecula, CA, USA) and mouse anti-Iba-1 (1:200, #ab15691; Abcam, Cambridge, UK). Sections were sequentially rinsed with PBS containing Tween-20 (PBST), followed by incubation with the biotinylated secondary antibody goat anti-mouse (1:100, #BA9200; Vector Laboratories) for 2 hours at room temperature. Several rats per group were chosen, and approximately five nonoverlapping optical sections were randomly selected for each rat and utilized to quantify GFAP or Iba-1 positive glial cells.

### 2.6. Western blot analysis

The ipsilateral half of the L5-6 segments of the spinal cords on the nerve-ligated side was excised on the 4$^{th}$ day and the 11$^{th}$ day after SNL. Equal amounts of protein lysate were separated by 8—12% sodium dodecyl sulfate—polyacrylamide gel electrophoresis, after which the resolved proteins were transferred to nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Primary antibodies used were mouse anti-GFAP (1:1000, #MAB360; Millipore), rabbit anti-Iba-1 (1:250, #ab48004; Abcam), and rabbit anti-actin (1:200, #A2066; Sigma-Aldrich, St Louis, MO, USA). Horseradish peroxidase-conjugated secondary antibodies used were goat anti-mouse (1:2,000, #sc2005; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and goat anti-rabbit (1:5,000, #sc2004; Santa Cruz Biotechnology, Inc.). The membrane reacted to the enhanced chemiluminescent substrate (#34087; Thermo Fisher Scientific Inc., Rockford, IL, USA) and was analyzed using a luminescent image analyzer (ImageQuant LAS 4000; GE Healthcare).

Quantification of the immunoreactivity was performed by using a densitometric analysis with Image J version 1.47v (Wayne Rasband, National Institutes of Health, USA). Each representative band selected was calculated three times, and the relative densitometry unit of Iba-1 or GFAP expression normalized to internal actin expression was employed.

### 2.7. Statistical analyses

All values are presented as mean ± standard error of the mean. The paired *t*-test was used to determine whether significant differences in behavioral scores between prior to and after SNL were present, and a one-way repeated measure analysis of variance (ANOVA), followed by Bonferroni's *t*-test, was utilized to examine whether significant differences in behavioral scores between prior to and after SPP treatment emerged. Moreover, the two-sample *t*-test was performed between the naïve and the SAL + SNL groups, as well as between the SAL- and the SPP-treated groups. All statistical analyses were carried out using SigmaStat version 11.0 (Systat Software, San Jose, CA, USA). A $p$ value $< 0.05$ was considered statistically significant.

## 3. Results

To evaluate the effect of SPP on early stage neuropathic pain, a single injection of SPP (1 mg/200 μL) or SAL (200 μL) was administered immediately after SNL, followed by assessment of mechanical allodynia on postoperative Day (POD) 0, POD 1, POD 3, and POD 5 and assessment of thermal hyperalgesia on POD 0, POD 2, POD 4, and POD 6, respectively, in both the SNL+SAL ($n = 7$) and the SNL+SPP ($n = 7$) groups. As revealed by the behavioral results presented in Fig. 1, SPP treatment delayed mechanical allodynia and thermal hyperalgesia. The two sample *t*-test showed a statistically significant difference between the SNL rats administered SAL and SPP ($p < 0.05$, $n = 7$ rats/group), which further elucidates that SPP treatment produces relief of early-phase neuropathic pain.

As shown in Fig. 2A, a profound decrease of Iba-1 positive cells in SPP-treated SNL rats in comparison with SAL-treated ones, was confirmed, which was analyzed quantitatively as described in Fig. 2B, where a statistically significant reduction by 50.75% was revealed for one treatment with SPP ($p < 0.05$, *t*-test). In addition, one treatment with SPP resulted in diminished translational levels of Iba-1, which was also monitored using Western blot analysis (Fig. 2C). In Fig. 2D, which presents data for the levels of Iba-1 normalized to internal actin (Iba-1/actin) as measured by using a densitometric analysis and expressed as standardized ratios, a statistically significant reduction by 59.4% was identified for one treatment with SPP ($p < 0.001$, *t*-test). Strikingly, an analogous tendency

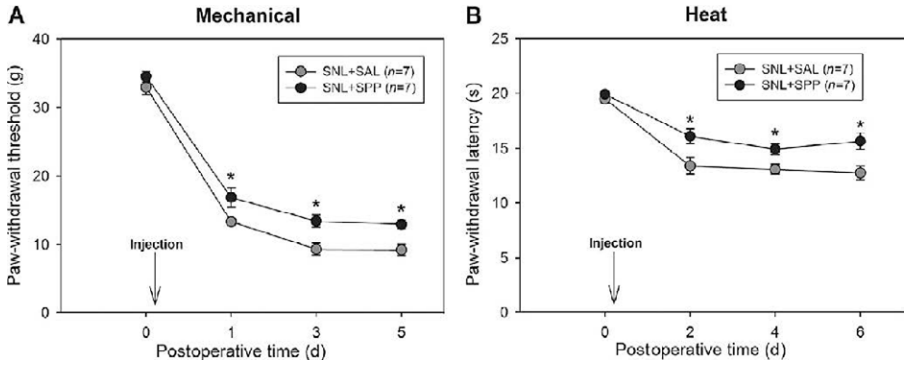


**Figure 1** Delayed (A) mechanical allodynia and (B) thermal hyperalgesia induced by spinal nerve ligation in rats treated with *Scolopendra* pharmacopuncture only once postoperatively. $*p < 0.05$ *vs*. spinal nerve ligation + saline group. Arrows indicate saline or *Scolopendra* pharmacopuncture injection time points.

following SPP treatment was also found in GFAP-positive astrocytes by using immunohistochemical analyses (Fig. 3A and B). However, the decrease in the number of activated astrocytes by 27.3% for one SPP treatment exhibited no statistically significant difference in comparison with SAL-administered SNL rats ($p > 0.05$, *t*-test; Fig. 3A and B), which was supported by the unchanged GFAP protein levels seen in the Western blot analysis (Fig. 3C and

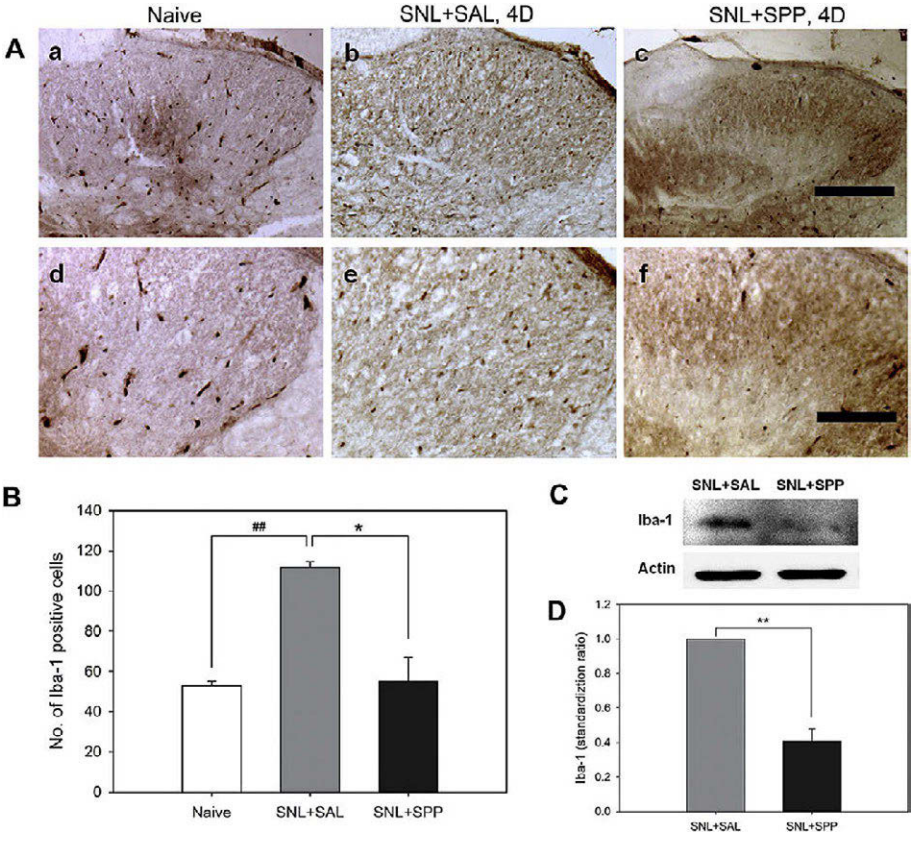


**Figure 2** Downregulated ionized calcium binding adaptor protein (Iba-1) on postoperative Day 4 in the spinal cords of rats receiving one shot of *Scolopendra* pharmacopuncture (SPP) immediately after spinal nerve ligation (SNL). (A) Immunohistochemistry photomicrographs of the L5 spinal dorsal horn in SNL rats administered one shot of saline (SAL) and SPP and incubated with antibody recognizing Iba-1. Images (a–c) and (d–f) were viewed by using a 10 × and a 20 × lens, respectively. Scale bars are 200 μm for (a–c) and 100 μm for (d–f). (B) Bar graph presenting the number of Iba-1 positive microglia cells ($^{\#\#}p < 0.001$, SNL+SAL *vs*. naïve group; $*p < 0.05$, SNL+SPP *vs*. SNL+SAL group), $n = 3$. (C) Protein levels of Iba-1 and actin in the L5 spinal cord of rats undergoing different treatments, as obtained using western blotting. (D) Standardized ratio of the relative integrated densities (Iba-1/actin) of the SNL+SPP group to those of the SNL+SAL group ($**p < 0.001$ *vs*. SNL+SAL group), $n = 3$. Iba-1 is the microglial marker.

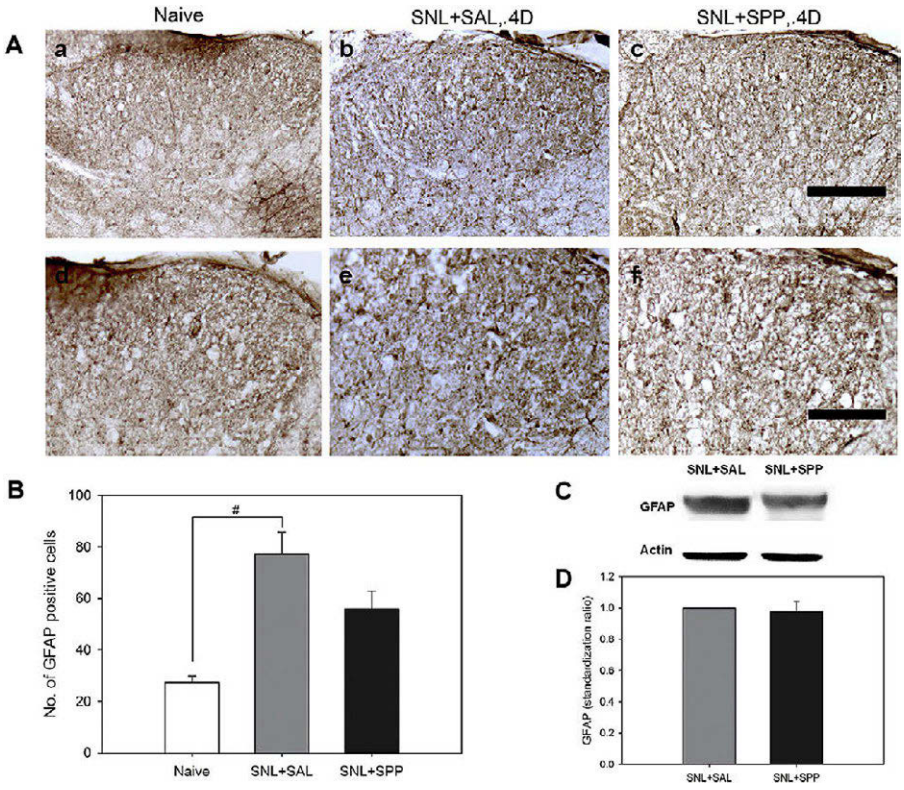


**Figure 3** Unchanged glial fibrillary acidic protein (GFAP) on postoperative Day 4 in the spinal cords of rats receiving one shot of *Scolopendra* pharmacopuncture (SPP) immediately after spinal nerve ligation (SNL). (A) Immunohistochemistry photomicrographs of the L5 spinal dorsal horn in SNL rates administered one shot of saline (SAL) and SPP and incubated with antibody recognizing GFAP. Images (a—c) and (d—f) were viewed by using a 10 × and a 20 × lens, respectively. Scale bars are 200 μm for (a—c) and 100 μm for (d—f). (B) Bar graph presenting the number of GFAP positive astrocytes [$^{\#}p < 0.05$, SNL+SAL *vs*. naïve group; naïve ($n = 3$), SNL+SAL ($n = 5$), SNL+SPP ($n = 5$)]. (C) Protein levels of GFAP and actin in the L5 spinal cords of rats undergoing different treatments, as obtained using Western blotting. (D) Standardized ratio of the relative integrated densities (GFAP/actin) of the SNL+SPP group to those of the SNL+SAL group, $n = 3$. GFAP is the astrocytic marker.

D). The emerging evidence leads us to speculate that the alleviated development of neuropathic pain after one SPP treatment seems to be attributed to the attenuation of microglial activation.

To assess the effect of SPP on late-stage neuropathic pain, we initiated five once-daily treatments with SPP (1 mg/200 μL) or SAL (200 μL) from Day 3 after SNL, when neuropathic pain had already developed. The mechanical allodynia and thermal hyperalgesia assessments of the SNL + SAL ($n = 6$) and the SNL + SPP ($n = 7$) groups that were done prior to surgery were compared with those done daily from POD 3 to POD 11 following SNL. As shown in Fig. 4A and B, in both the SAL- and the SPP-injected rats, SNL resulted in a significant decline in the threshold and the latency of hind-paw withdrawal on Day 3 after surgery, which represented both mechanical allodynia and thermal hyperalgesia [$p < 0.05$, $p < 0.001$ *vs*. presurgical baselines (POD 0), paired *t*-test]. Moreover, the paw-withdrawal threshold (PODs 8—11) and the paw-withdrawal latency on POD 5 and PODs 9—11 due to five treatments with SPP were distinguishable from those of the SAL-treated rats [$p < 0.05$, $p < 0.001$ *vs*. SNL + SPP (POD 3); Fig. 4A and B]. The one-way repeated measures ANOVA followed by Bonferroni's *post hoc* test showed a statistically significant difference between the SAL- and the SPP-treated SNL rats on some PODs ($p < 0.05$, $p < 0.001$ *vs*. SNL+SAL group, *t*-test; Fig. 4A and B). These data further support the notion that repeated SPP treatment inhibits the maintenance of neuropathic pain.

Figs. 5A and 6A show marked increases in Iba-1 and GFAP positive cells of rats that underwent SNL as compared to naïve rats, as well as dramatic decreases in those, of SPP-treated SNL rats as compared with SAL-treated ones. These results are analyzed quantitatively in the accompanying graphs (Figs. 5B and 6B), where statistically significant increases in the numbers of Iba-1 and GFAP positive glial cells by 83.6% and 341.2% due to SNL, respectively ($p < 0.001$ SNL+SAL *vs*. naïve group, *t*-test). In addition, reductions in the numbers of those cells by 22.4% and 25.5% after repeated SPP treatment, respectively, were observed ($p < 0.05$ SNL+SPP *vs*. SNL+SAL group, *t*-test).

Moreover, exposure to repeated SPP treatment led to decreased GFAP protein levels, which were monitored using Western blot analysis (Fig. 6C). In Fig. 6D, where the levels of GFAP normalized to internal actin (GFAP/actin) were measured by using a densitometric analysis and expressed as a standardized ratio, shows a very significant reduction by 30.92% after repeated SPP treatment ($p < 0.05$, *t*-test).

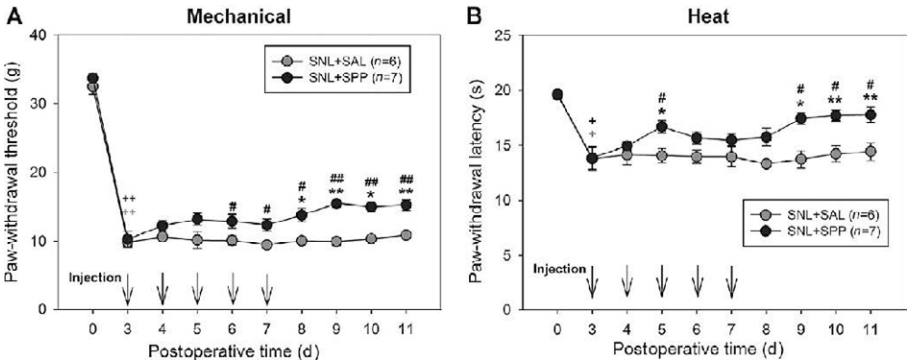


**Figure 4** Attenuated late-phase neuropathic pain behaviors induced by spinal nerve ligation (SNL) in rats treated repetitively by using postligation with *Scolopendra* pharmacopuncture (SPP). SNL resulted in significant reductions in the hind-paw-withdrawal threshold and the latency to mechanical and thermal stimuli, respectively, compared to those prior to saline (SAL) or SPP was administered [$^{+}p < 0.05$, $^{++}p < 0.001$ *vs*. the presurgical value (postoperative Day 0)]. Repeated daily injections of SPP suppressed SNL-induced (A) mechanical allodynia and (B) thermal hyperalgesia at late times in neuropathic rats ($*p < 0.05$, $**p < 0.001$ *vs*. SNL+SPP group (postoperative Day 3). The difference between the SNL+SPP and the SNL+SAL groups was statistically significant ($^{\#}p < 0.05$, $^{\#\#}p < 0.001$ *vs*. SNL+SAL group). Arrows indicate SAL or SPP injection time points.

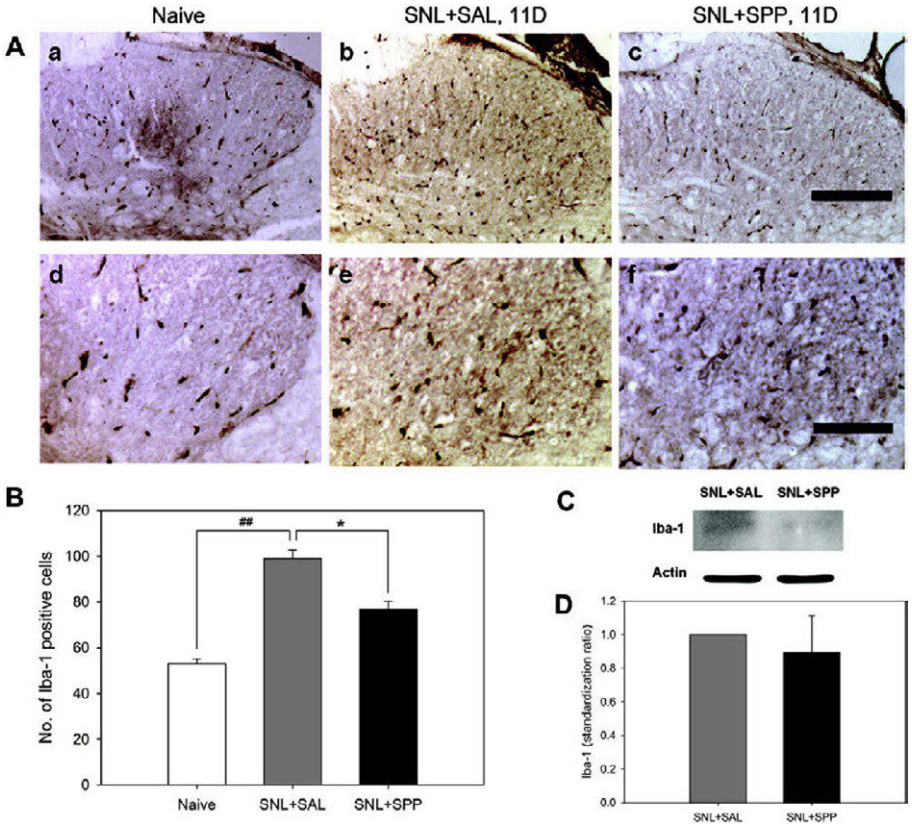


**Figure 5** Downregulated ionized calcium binding adaptor protein (Iba-1) on postoperative Day 11 in the spinal cords of rats receiving repeated *Scolopendra* pharmacopuncture (SPP) after spinal nerve ligation (SNL). (A) Immunohistochemistry photomicrographs of the L5 spinal dorsal horn in SNL rats repeatedly administered saline (SAL) and SPP and incubated with antibody recognizing Iba-1. Images (a–c) and (d–f) were viewed by using a 10 × and a 20 × lens, respectively. Scale bars are 200 μm for (a–c) and 100 μm for (d–f). (B) Bar graph presenting the number of Iba-1 positive microglia cells [$^{\#\#}p < 0.001$, SNL+SAL *vs*. naïve group; $*p < 0.05$, SNL+SPP *vs*. SNL+SAL group; naïve ($n = 3$), SNL+SAL ($n = 5$), SNL+SPP ($n = 5$)]. (C) Protein levels of GFAP and actin in the L5 spinal cord of rats undergoing different treatments, as obtained using western blotting. (D) Standardized ratio of the relative integrated densities (Iba-1/actin) of the SNL+SPP group to those of the SNL+SAL group, $n = 3$. Iba-1 is the microglial marker.

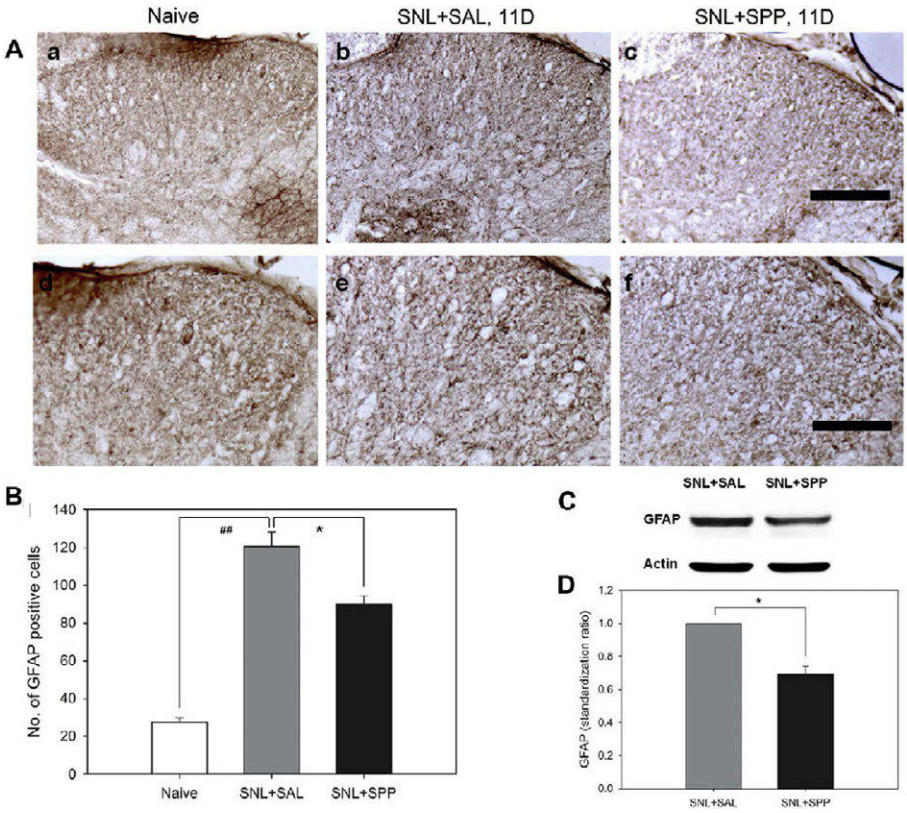


**Figure 6** Downregulated glial fibrillary acidic protein (GFAP) on postoperative Day 11 in the spinal cord of rats receiving repeated *Scolopendra* pharmacopuncture (SPP) after spinal nerve ligation (SNL). (A) Immunohistochemistry photomicrographs of the L5 spinal dorsal horn in SNL rats repeatedly administered saline (SAL)and SPP and incubated with antibody recognizing GFAP. Images (a—c) and (d—f) were viewed by using a 10 × and a 20 × lens, respectively. Scale bars are 200 μm for (a—c) and 100 μm for (d—f). (B) Bar graph presenting the number of GFAP positive astrocytes [$^{\#\#}p < 0.001$, SNL+SAL *vs*. the naïve group; $*p < 0.05$, SNL+SPP *vs*. SNL+SAL group; naïve ($n = 3$), SNL+SAL ($n = 5$), SNL+SPP ($n = 5$)]. (C) Protein levels of GFAP and actin in the L5 spinal cords of rats undergoing different treatments, as obtained using western blotting. (D) Standardized ratio of the relative integrated densities (GFAP/actin) of the SNL+SPP group to those of the SNL+SAL group ($*p < 0.05$ *vs*. SNL + SNL group), $n = 3$. GFAP is the astrocytic marker.

However, the phenotype was not reduced in the Iba-1 protein levels presented in Fig. 5C and D. Current data increase the plausibility that suppression of microglial and astrocytic activations participates in the suppressive potential of repeated SPP treatment for managing neuropathic pain during its maintenance phase.

## 4. Discussion

As observed, a single post-SNL treatment with SPP induced suppression of mechanical allodynia and thermal hyperalgesia paralleling microglial deactivation on POD 4. However, although microglial activation had fallen to near the basal level for naïve rats as the result of SPP treatment, intriguingly, the symptoms of neuropathic pain did not decrease to the degrees seen in naïve rats or to the pre-baseline levels. Two concerns have been raised. Activated satellite glial cells have been well documented to play a role in the early maintenance (PODs 3—7) of allodynia after SNL [27]. In addition, the role of Schwann cells in neuropathic pain have gradually received close attention in the latest study [21], although few researchers have investigated the contributions of SPP treatment to the separate phases of neuropathic pain. The assertion that other cell phenotypes, just like satellite or Schwann glial cells, will play additional roles in the early phase neuropathic pain, on which SPP treatment has no beneficial effect, seems plausible. Another possible interpretation is that spinal Iba-1 upregulation after SNL, which is shown in our data, may be associated with nerve injury, but may not be a cause of neuropathic pain. This is supported by the results that, although SNL-induced phosphorylation of c-Jun in spinal astroglia, the peptide JNK inhibitor D-JNKI-1, can block neuropathic pain, it is not accompanied by a reduction of GFAP expression [5]. Therefore, the method using changes in the expressions of spinal glial cells markers as indicators of the magnitude of pain remains controversial. It is the changes in the signaling molecules, not the structural proteins, in spinal glial cells, that are important for pain sensitization. Therefore, two highly interesting perspectives are opened: the upstream one in which spinal glial cells are activated and the downstream one in which products are released by activated glial cells.

In addition, we found that the SNL rats that were treated only once with SPP did not return to the post-baseline of the saline-treated rats in POD 6, which indicates the long-term analgesic potential of SPP. However, a long longitudinal study will be needed. The results present an experimental paradigm for investigating the advantages of early clinical intervention, through which chronic neuropathic pain processes will be better prevented.

As predicted, SNL produced stable mechanical allodynia and thermal hyperalgesia, which was in agreement with previous studies [5,7,25,27]. After the neuropathic pain had been established, we performed SPP treatment on Days 3—7 when microglial and astrocytic cells should be at high levels in the L5/6 dorsal horn. This treatment attenuated mechanical allodynia from Day 6 to Day 11. By contrast, the treatment only partly suppressed thermal hyperalgesia on POD 5 and PODs 9—11, but the suppression was greater after the final treatment, suggesting a cumulative effect of SPP treatment.

In parallel with the behavioral results, repeated SPP treatments reduced both microglial and astrocytic activations, as indicated by the immunohistochemistry analysis. However, a discrepancy existed between the results of the immunohistochemistry and the Western blot analyses. Even though similar trends were observable, only the reductions in the GFAP protein levels showed statistical differences in Western blot results, which suggested that the deactivation of astrocytes mainly contributed to the maintenance of neuropathic pain. To some extent, microglial deactivation may be involved in the attenuated mechanical allodynia and thermal hyperalgesia in the early maintenance phase (PODs 5—7) of neuropathic pain.

Among the various pharmacopuncture formulae, this study used *Scolopendra* pharmacopuncture because the extracts or ingredients of *Scolopendra* have been purified or identified, such as a serine protease [28], peptide SQL (Ser-Gln-Leu) [29], and centipede acidic protein [30] and have been reported to improve the hemorrheological disturbances, polysaccharide level (which has specific effects on tumor cells [31]), and the levels of two quinoline alkaloids (3,8-dihydroxyquinoline and 2,8-dihydroxy-3,4-dimethoxyguinoline) and 2,4-di-tertbutylphenol (which have antioxidant effects) [32]. In addition, several lines of evidence have indicated that extracts of *Scolopendra* might effectively relieve the pain associated with joint injury by increasing expressions of IL-2, IL-4, and IL-10 [33], might suppress the growth of cervical tumors by regulating Bax- and Caspase-3-medicated mitochondrial signal transit pathways [34], and might have antisenility effects by decreasing the contents of lipid peroxide in the serum and of lipofuscin in the liver and brain tissues of rats [35]. The extracts also play a protective role during the development of cerulean-induced acute pancreatitis and acute pancreatitis-associated lung injury by deactivating c-Jun $NH_2$-terminal kinase, p38 and nuclear factor $\kappa$B, thereby inhibiting the release of high-mobility group box protein-1 and proinflammatory cytokine [36]. Furthermore, the ingredient centipede acidic protein, isolated from *Scolopendra*, has anti-atherogenic effects through the inhibition of lipid peroxidation and the regulation of the nitric oxide and the endothelin-1 systems [30].

Recent *in vitro* and *in vivo* assays have reported *Scolopendra* to have a positive effect for treating many disorders, but no evidence of its effect on neuropathic pain has been reported. Furthermore, as reported previously [37], scolopendrid water—alcohol extract applied to the ipsilateral BL23 acupoint produced a more significant improvement of neuropathic pain sensitivity than its application to the contralateral BL23, ST25, or LR3 acupoint did, which demonstrates the specific effect of the acupoint used for the treatment. Based on these considerations, we thought that extracts from *Scolopendra* might have constituents contributing to the suppression of neuropathic pain. Thus, in this study, *Scolopendra* extract was used to manufacture pharmacopuncture for injections on KI1 and was tested for its efficacy in neuropathic rats. As predicted, the main effect of SPP is thought to be the interaction between the action of the KI1 acupoint and the injected medicine, *Scolopendra* extract.

In summary, the inhibitory effects of SPP on the development and maintenance of neuropathic pain may be associated with the deactivations of microglia and astroglia, respectively. Additionally, microglia inactivation seems to be, at least in part, responsible for the effects of SPP in preventing neuropathic pain during the maintenance phase. The potential application of SPP in separate phases of neuropathic pain is implied by the results of this study; hence, future works are expected to shed light on identifying which constituents in *Scolopendra* extracts are effective for the management of neuropathic pain and to lead to the development of direct and exact therapeutic strategies for the management of such pain.

## Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

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