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The Analgesic and Antispastic Effects of Epidural Spinal Stimulation Depending on the Frequency and Intensity After Spinal Cord Injury

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ABSTRACT

Objectives: Neuropathic pain and spasticity after spinal cord injury (SCI) are intractable conditions that represent distinct outcomes of sensory and motor dysfunction. Spinal cord stimulation (SCS) has been proposed as a potential therapy for these conditions. However, the therapeutic effects of different SCS parameters and their underlying mechanisms remain unclear. The present study aimed to investigate the therapeutic effects of a developed epidural SCS system on SCI-induced pain and spasticity, and to explore its underlying mechanisms in modulating microglia activation.

Materials and Methods: Male Sprague-Dawley rats underwent contusive SCI at T10 segment using an Infinite Horizons impactor, and an epidural SCS system was implanted two weeks after SCI. Mechanical sensitivity and spasticity were assessed using paw withdrawal threshold and modified Ashworth scale. Epidural SCS was applied at low frequency (LF; 50 Hz) or high frequency (HF; 1 kHz) with intensities of 40% or 80% of the motor threshold (MT). Sham control rats underwent SCS implantation without electrical stimulation. Immunohistochemistry was performed to evaluate microglia activation in the lumbar spinal segments.

Results: HF with 80% MT intensity showed a higher effect on neuropathic pain and spasticity, whereas LF with 40% MT intensity showed a lower effect. Although SCS did not alter the number of microglia in the lumbar spinal segments, both LF and HF with 80% MT intensity significantly decreased the proportion of activated microglia in both dorsal and ventral horns compared with the sham group.

Conclusion: These findings show that the epidural SCS with 80% MT effectively alleviates neuropathic pain and spasticity by reducing spinal microglial activation. These results highlight the importance of optimizing stimulation parameters to achieve therapeutic benefits in clinical settings.

Keywords: Epidural spinal cord stimulation, microglia, neuropathic pain, spasticity, spinal cord injury

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INTRODUCTION

Spinal cord injury (SCI) induces devastating and long-lasting impairments in both sensory and motor functions. A significant consequence of altered sensory processing after SCI is neuropathic pain, which affects approximately 53% of patients with SCI and severely compromises their quality of life.¹ Similarly, motor dysfunction manifests as paralysis and muscle hypertonia (spasticity) below the level of injury, with a prevalence >60% among patients with SCI.^{2,3} It is increasingly recognized that these major complications are not independent outcomes but interconnected consequences of altered spinal cord excitability after injury.⁴

SCI disrupts the communication of supraspinal signals within the spinal network, outsize the influence of sensory inputs.⁵ This maladaptive neuroplasticity contributes to spinal sensitization, a key mechanism underlying aberrant pain processing after SCI. Furthermore, increased activity of sensory afferents, coupled with decreased spinal inhibitory mechanisms, also causes the hyperexcitability of motoneurons.⁶ Consequently, this leads to increased muscle tone and involuntary muscle contractions, known as spasticity, particularly during rapid or passive movements.^{3,7,8} Despite extensive neurologic damage, the neuronal networks of the spinal cord below the injury site still retain a portion of intact spinal circuitry responsible for sensory-motor integration^{9,10}; Therefore, targeting these remaining circuits could offer a promising strategy for restoring neurologic function and alleviating secondary complications associated with SCI.

Spinal cord stimulation (SCS) is a potential treatment option for chronic pain and spasticity, given it modulates neuronal excitability, delivering electrical impulses directly to the spinal cord.¹¹ Clinical reports indicate that SCS significantly improves the quality of life for patients experiencing chronic pain or spasticity, especially when pharmacotherapies become intolerable, with additional benefits for motor function.^{12–14} The efficacy of SCS is critically dependent on stimulation parameters, such as frequency, which influence spinal cord excitability. Conventional low-frequency (40–60 Hz; LF) SCS has been widely used to manage neuropathic pain after SCI. It is known that this approach activates the γ -amino butyric acid-ergic neurons in spinal dorsal horns¹⁵ and promotes the release of endogenous opioids,¹⁶ despite inducing paresthesia.^{17–19} In comparison, high-frequency (kHz range; HF) SCS has emerged as an alternative approach that effectively inhibits mechanical hypersensitivity through distinct neural mechanisms from those of LF SCS (50 Hz).^{20,21} This technique can deliver stimulation below the sensory threshold, avoiding paresthesia. For example, 1-kHz SCS altered the conduction properties of sensory neurons, causing a more rapid onset of analgesic effects than that of 50-Hz stimulation.²¹ However, although HF SCS indicates robust efficacy in relieving neuropathic pain after SCI, the effects of SCS on spasticity remain less understood.

Microglia, the resident macrophages of the CNS, play a critical role in regulating the local microenvironment to maintain neural homeostasis.²² After SCI, microglia become activated in both the dorsal^{23,24} and ventral horns,^{25,26} releasing proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , not only at the level of injury but also below the level of injury (L5). This activation contributes to neuronal hyperexcitation and the development of neuropathic pain in the dorsal horn.^{23,27} In the ventral horn, activated microglia downregulate the potassium-chloride cotransporter (KCC2) through brain-derived neurotrophic factor-tropomyosin receptor kinase B receptor signaling,^{28–30} which contributes to increased excitability of motor neurons and the spasticity after SCI. Our previous study showed

that the transcutaneous electrical stimulation (TENS) applied to the hindlimb reduced spasticity and the activated microglia in the ventral horn of L5 and L6.²⁶ This suppressive effect of TENS suggests that the neuromodulation into aberrant spinal circuit can be effective in controlling the properties of neurons and microglia. Indeed, studies have shown that LF (50–60 Hz)^{31,32} and HF (kHz)³³ SCS modulate activated microglia. However, the region-specific effects of SCS on activated microglia, particularly in relation to different stimulation frequencies, remain poorly understood.

In this study, we aimed to evaluate the therapeutic effects of epidural SCS on neuropathic pain and spasticity after SCI. Using a fully implantable and custom-made SCS system,^{34,35} we applied stimulation with varying frequencies (LF and HF, low and high intensity) to examine their differential impacts. In particular, we explored region-specific activation of spinal microglia as a potential mechanism underlying both neuropathic pain and spasticity and investigated ways stimulation parameters influence microglial responses.

MATERIALS AND METHODS

Experimental Animals

The experiments were performed using adult male Sprague-Dawley rats ($N = 52$, Orient Bio Inc, Seoul, Korea) weighing 170 to 230 g at the time of operation. All the animals were treated under a 12 h/12 h light-dark cycle (8 AM–8 PM) with access to water and food ad libitum. The research procedures were approved by the Korea University Institutional Animal Care and Use Committee (KUIACUC-2021-0103), and all experiments were conducted following ethical principles.

Preparation of the Spinal Cord Contusion Model

SCI was performed two weeks before implanting the electrode and stimulator (Fig. 1a). Under deep anesthesia with isoflurane (a mixture of 2% isoflurane and 98% oxygen), a total laminectomy was performed at the T10 segment without dura mater rupture. Contusive SCI was accomplished by Infinite Horizons impactor (Precision Systems & Instrumentation, Lexington, KY). To induce the SCI, the impact force was desired at 150 kDyn. After the SCI, rats were kept under a warm floor to maintain body temperature. Passive bladder control was manually performed twice a day after SCI, and Unasyn (ampicillin/sulbactam, 100 mg/kg, Pfizer, Seoul, Korea) was intraperitoneally injected once a day until self-voiding resumed.

Implantation of Stimulator and Electrode

For SCS, a custom epidural electrode and stimulator were used (Fig. 1b). The electrode primarily comprised gold conductors and polyimide insulation. The shape of the electrode was newly designed from the previous version.³⁴ The stimulator can deliver current stimulation, wirelessly charged through magnetic induction.³⁵ To assess biocompatibility, the electrode was implanted for one or three weeks, and tissue responses were evaluated (Fig. 2a). Spinal cord segments at the implantation site (L4 and L5) were collected from rats at each timepoint, and from normal rats with no surgical procedures. The tissues were stained with hematoxylin and eosin. The tissue response of the stimulator was validated in a previous study.³⁵

The electrode and stimulator were implanted two weeks after SCI, when SCI-injured rats had recovered sufficiently to bear their weight and walk (Fig. 2c). A partial laminectomy was performed at

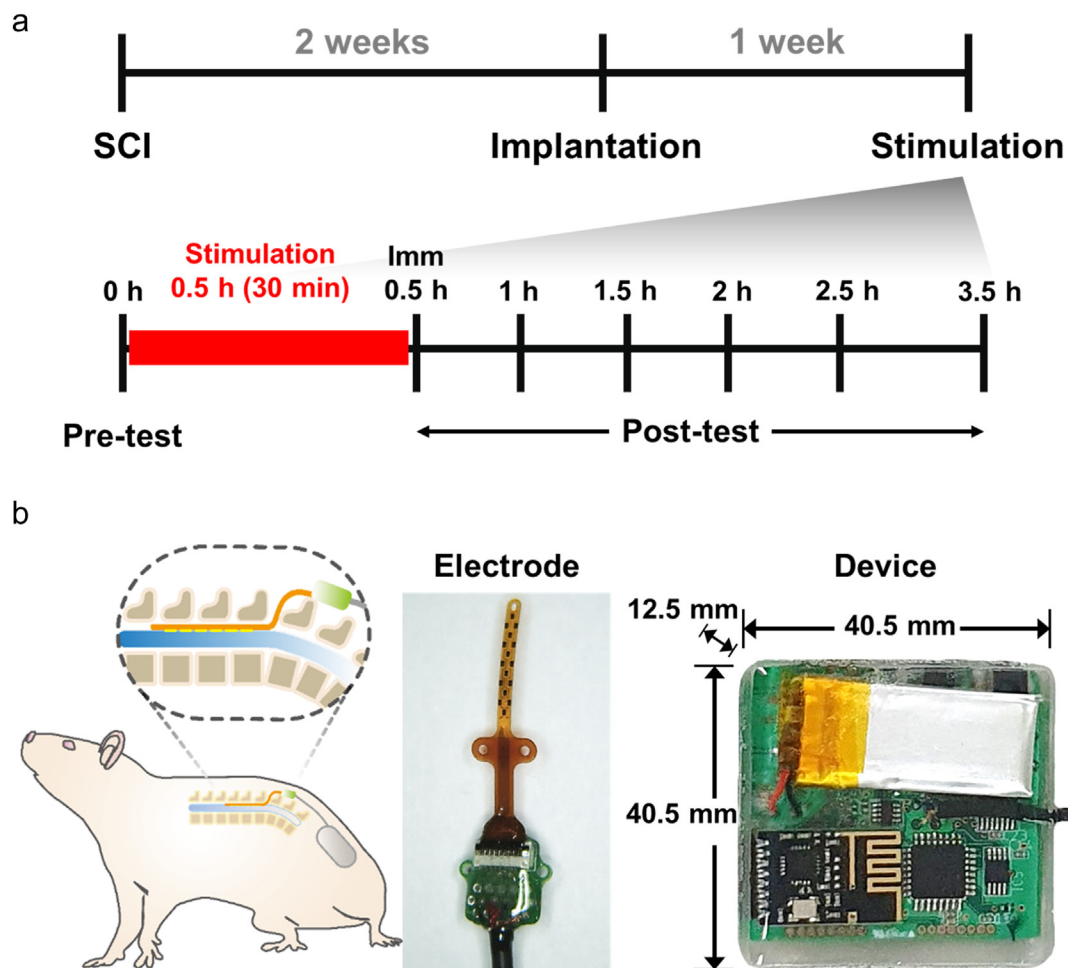


Figure 1. Experimental timeline and images of SCS system. Experimental design to assess the effects of SCS on neuropathic pain and spasticity after SCI in rats. a. The implantation of the epidural electrode was performed two weeks after SCI, and rats were allowed to rest for one week after implantation. To assess the SCS effects, pretests were performed before the stimulation, and posttests were performed 0.5, 1, 1.5, 2, 2.5, and 3.5 hours after stimulation (0.5 hours means immediately after SCI). SCS was applied for 0.5 hours (30 minutes). b. Images of the customized electrode and stimulator. [Color figure can be viewed at www.neuromodulationjournal.org]

the L1 vertebra. The electrode was inserted into the epidural space. After electrode implantation, bilateral muscle contractions with gradual electrical stimulation (4 Hz, 0.2 milliseconds) were measured to ensure the accuracy of the electrode position. Next, the electrode was fastened with sutures using holes on the body at both sides for immobilization and covered with the adjacent muscles. For the stimulator implantation, the skin on the left back muscle was separated from the muscle, and the stimulator was inserted. The overlying skin was sutured, covering the stimulator.

Experimental Protocol of SCS

Stimulation was initiated one week after implantation of the SCS system to allow postoperative recovery. The stimulating parameters are listed in Table 1. Before stimulation, the motor threshold (MT) was measured in awake rats by gradually increasing the intensity of electrical stimulation until muscle contraction was observed, with the fixed parameter (4 Hz, 0.2 milliseconds) to establish a reference point for quantitative analysis.^{19,21,36} The commonly used 50 Hz with a pulse width of 0.2 milliseconds was assigned to the LF group. Among the kHz-frequency SCS protocols (1, 5, and 10 kHz), 1 kHz with the same pulse width was selected for

the HF group owing to limitations in waveform delivery at higher frequencies. Two different intensities were used for stimulation: 40% MT, which is considered a subsensory threshold, and 80% MT, which is considered an above-sensory threshold (inducing paresthesia) in awake animals.^{37,38} Five different parameters were applied: sham control ($n = 6$), LF-low intensity (LF40, $n = 9$), LF-high intensity (LF80, $n = 10$), HF-low intensity (HF40, $n = 10$), and HF-high intensity (HF80, $n = 11$) for 0.5 hours (30 minutes duration), respectively. Sham control also underwent implantation of the SCS system, but the system was turned off (sham stimulation). During the SCS, the rats were carefully monitored for any signs of painful responses, such as vocalization or excessive grooming.

Behavioral Tests for Motor Function, Pain, and Spasticity

The behavioral tests were conducted blindly to avoid experimental bias. Initially, tests were performed one day before surgery (pre-SCI) and at 1, 3, 5, 7, 14 (post-SCI), and 21 days (postimplantation) to evaluate baseline changes. For the effect of SCS, behavioral assessments were conducted before and after SCS (pre-SCS, and at 0.5, 1, 1.5, 2, 2.5, and 3.5 hours after SCS; 0.5 hours indicates immediately after a 30-minute application of SCS). Basso, Beattie, and Bresnahan locomotor

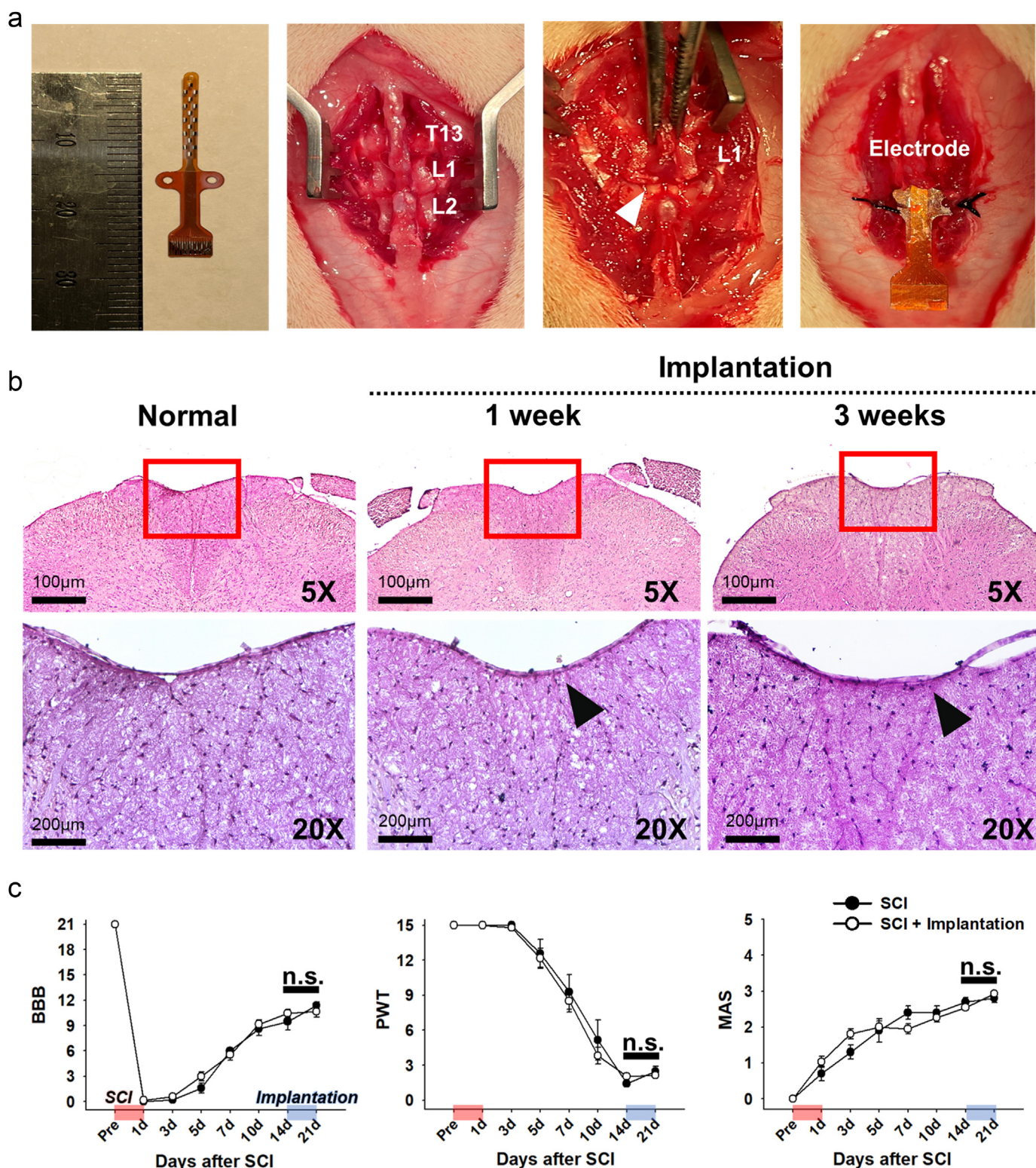


Figure 2. Biocompatibility of SCS system. **a.** Photograph of the implantation in rats with the customized epidural electrodes. White arrows indicate the space where epidural electrodes were inserted. **b.** Morphology of spinal cord stained with hematoxylin and eosin staining before (normal), one, and three weeks after implantation. Red boxes highlight the locations of 20x images. Black arrows indicate the dorsal columns where epidural electrodes were attached after implantation. **c.** Changes in motor, sensory functions, and spasticity after SCI until implantation of the stimulation electrode. "Pre" refers to the time before SCI surgery. Implantation was conducted 14 days (two weeks) after SCI. The red and blue boxes on the x-axis in panel c indicate the timing of SCI surgery and SCS system implantation. Values are presented as mean $\pm$ SEM. [Color figure can be viewed at www.neuromodulationjournal.org]

Table 1. Stimulation Parameters for Different Experimental Groups.

Group	Frequency (Hz)	Pulse width (ms)	Duration (h)	Intensity (% of MT)	Stimulating intensity (mA) ± SEM
LF40	50	0.2	0.5	40	0.0435 ± 0.001
LF80	50	0.2	0.5	80	0.0684 ± 0.011
HF40	1,000	0.2	0.5	40	0.0562 ± 0.006
HF80	1,000	0.2	0.5	80	0.0916 ± 0.001

Stimulation protocols with varying frequencies, pulse widths, durations, and intensities. The intensity was determined on the basis of the MT measured before stimulation, ensuring consistent application across groups. The resulting stimulating intensity was calculated for each protocol.

rating scale (BBB) test was used to investigate the locomotor function.³⁹ For assessing mechanical sensitivity, the paw withdrawal threshold (PWT) test was used. Von Frey filaments were bilaterally applied according to the Dixon up-and-down method on the plantar surface of the foot.⁴⁰ A brisk withdrawal of the hindlimb accompanying the supraspinal responses was regarded as a positive sign. For the evaluation of PWT at 0.5 hours (immediately after SCS), rats were transferred to individual cages and allowed to adapt for 5 minutes before the end of SCS. The spasticity, characterized by increased resistance against the rapid and forced movement of the hindlimbs, was assessed by the modified Ashworth scale (MAS).⁴¹

Tissue Processing for Histology and Immunohistochemistry

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection) and perfused with 0.9% saline including heparin (0.2 mL/L, JW Pharmaceutical Corporation, Gwacheon-si, Gyeonggi-do, Korea) followed by 4% buffered paraformaldehyde (PFA, Merck KGaA, Darmstadt, Germany). The spinal cord tissues (L4 and L5) were extracted, postfixed in 4% PFA, and dehydrated with 30% sucrose at 4 °C. After being embedded with the optimal cutting temperature compound (Sakura Finetechnical, Tokyo, Japan), the spinal tissues were cross-sectioned. For morphologic analysis, sectioned tissues were stained with hematoxylin (Abcam, Cambridge, UK) and eosin (Sigma-Aldrich, St Louis, MO).

For the immunohistochemistry, rats had SCS applied (LF80 and HF80) for 0.5 hours, then were deeply anesthetized, and their spinal cords were immediately extracted after the SCS as previously described. After blocking with 3% normal goat serum and Avidin-Biotin block (Vector Laboratories, Burlingame, CA), the sections were incubated overnight at 4 °C with the following primary antibodies: mouse anti-OX42 (1:2000, Abcam) or rabbit antiphosphorylated p38 (p-p38; 1:500, Cell Signaling Technology, Danvers, MA). Next, sections were incubated with the following secondary antibodies: biotinylated anti-mouse immunoglobulin (Ig) G (1:1000, Vector Laboratories) or biotinylated antirabbit IgG (1:1000, Vector Laboratories) for one hour at room temperature. Finally, Alex Fluor® 488 or Alex Fluor® 568 conjugate streptavidin (1:2000, Invitrogen Life Technologies, Carlsbad, CA) was applied for one hour at room temperature. The microglia were analyzed in laminae I to II of the dorsal horn and lamina X of the ventral horn and separated and counted into two different types (resting or activated). The resting type of the microglia had a small cell body with long, thin processes (soma diameter was greater than processes length), and the activated type of the microglia showed hypertrophy in the cell body (soma diameter greater than processes length), colocalized with p-p38 expression, which is deemed a microglia activation marker.^{26,42} The proportion of activated microglia was calculated with the number of activated microglia/number of total microglia.

Data Analysis and Statistics

All results were expressed as the mean ± SEM. The percentage maximal possible effect (% MPE) was calculated from the PWT and MAS using the following formula: $100 \times (\text{post-SCS response} - \text{baseline}) / (\text{maximal response} - \text{baseline})$. The maximal responses were 15 g in PWT. The maximal response in MAS was defined as grade 0 because the maximal effect of SCS was scored as grade 0, which meant the normal state. The overall effects, which were analgesia and antispastic efficacy, were determined using the area under the curve (AUC) for the time course of % MPE. Data normality was assessed using the Shapiro–Wilk test. The effects of SCS were analyzed using two-way repeated measures (RM) analysis of variance (ANOVA), followed by Bonferroni post hoc test, which was applied to BBB scores, PWT, MAS, and their respective MPE values. Depending on the normality of the data, group differences in the AUC and the number of microglia were assessed using either one-way ANOVA with Bonferroni post hoc test or Kruskal–Wallis test with Dunn’s post hoc test. Bonferroni correction was applied for multiple comparisons. Moreover, the relationship between AUC values (PWT or MAS) and electrical parameters (amplitude and charge per second) was analyzed using a univariate general linear model (UGLM). All statistical analyses were performed using IBM SPSS Statistics (version 27.0; IBM Corp, Armonk, NY).

RESULTS

Motor and Sensory Functions After SCI

Contusive SCI was induced in rats by the impact force of 150 kDyn. Before the injury, rats showed normal ranges of BBB (score of 21; Fig. 2c), PWT (15 g), and MAS (score of 0). One day after SCI, the rats showed paralysis in their hindlimbs, and their BBB scores decreased to 0. Motor functions such as slight joint movements in the hindlimbs recovered at five days post operation (PO). Locomotion recovery gradually progressed by PO14 (10.45 ± 0.46). The PWT in response to mechanical stimuli gradually decreased until PO14 ($2.02 \text{ g} \pm 0.16$). MAS began to increase on PO1 and gradually increased until PO14 (2.54 ± 0.09).

Biocompatibility of the Implanted Epidural Electrode and Stimulation Device

We investigated the biocompatibility of custom-designed electrodes and devices for epidural stimulation. Before spinal cord extraction, the implantation site was reopened under deep anesthesia. No visible tissue responses, such as swelling or tissue encapsulation, were observed around the implanted electrode. There was no visible compression or dense fibrine deposition on the dorsal column at the electrode attachment site one and three weeks after implantation (black arrow in Fig. 2b). Moreover, no remarkable increases of inflammatory infiltrates were detected in

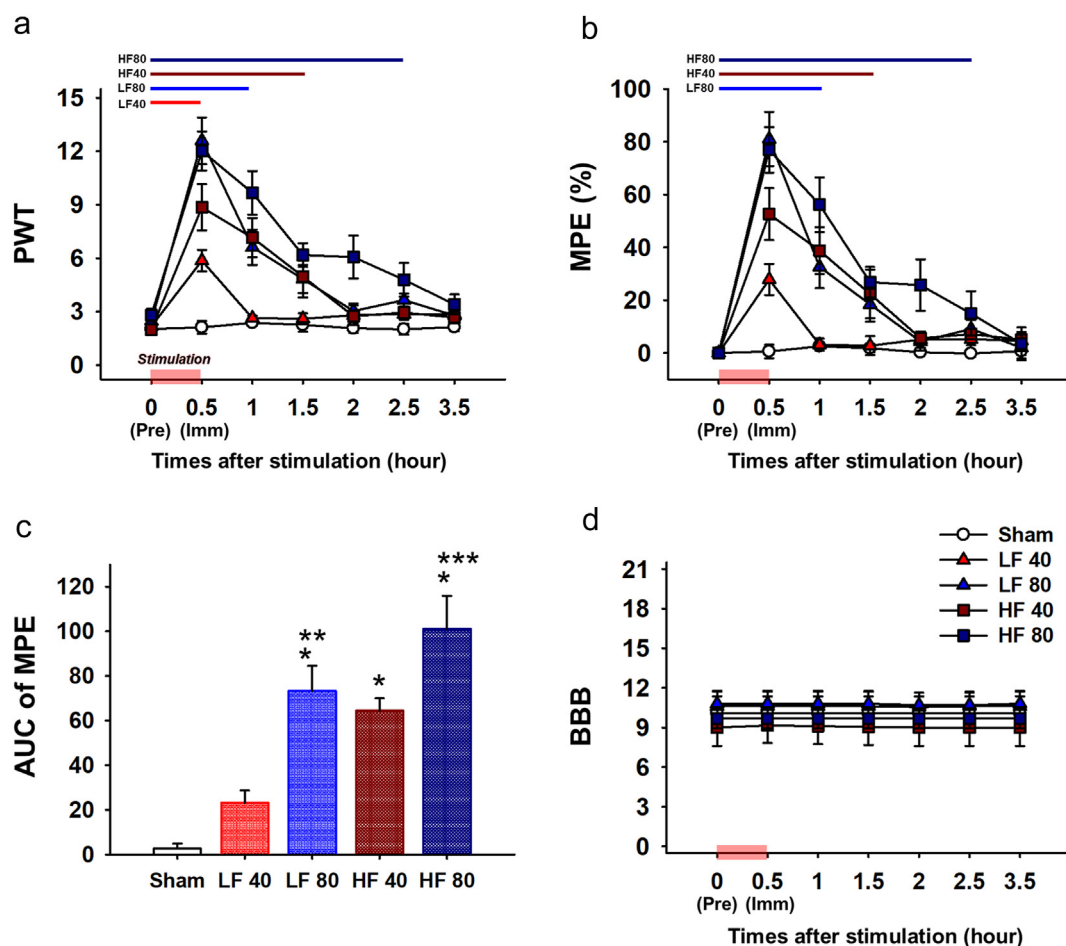


Figure 3. Changes in sensory thresholds and motor functions after SCS. a. Changes in PWT depending on the different parameters. b. MPE. c. A comparison of the AUC for the % MPE on the analgesic effects of SCS application. d. Changes in BBB scores depending on the different parameters. The red boxes on the x-axis in panels a and b indicate the timepoints at which stimulation was applied. Color bars in panels a and b indicate the duration for which each group showed statistically significant differences ($p < 0.05$) from the prestimulation baseline. Values are presented as mean $\pm$ SEM. * $p < 0.01$ compared with the sham group, and ** $p < 0.05$ and *** $p < 0.01$ compared with the LF 40 group. [Color figure can be viewed at www.neuromodulationjournal.org]

implanted rats compared with the normal rats (without surgical intervention). Under SCS-off conditions, no significant differences in BBB scores, PWT, or MAS were observed between PO14 (pre-implantation) and PO21 (postimplantation) after SCI ($p = 1$; Fig. 2c).

Effects of Epidural SCS on Neuropathic Pain and Spasticity

We tested whether epidural SCS affects PWT in the hindlimbs. During SCS, rats receiving high-intensity stimulation (LF80: $n = 2$; HF80: $n = 2$) briefly exhibited behaviors such as directed attention, paw rubbing, or body repositioning. However, these responses subsided within 30 seconds and were not accompanied by any abnormal muscle contractions. All five groups exhibited PWT values < 3 g, with no significant differences across the groups before SCS. According to two-way RM ANOVA, all groups exhibited significant increases in PWT at 0.5 hours (immediately after SCS) compared with pre values (LF40; 0.5 hours vs pre: $p < 0.05$; LF80, HF40, HF80: $p < 0.01$; Fig. 3a). The changes in PWT values were LF40 (from average 2.18 g–5.86 g), LF80 (2.46 g–12.59 g), HF40 (2.00 g–8.85 g), and HF80 (2.81 g–12.01 g). The analgesic effect of LF40 was observed only at 0.5 hours, indicating the shortest duration among all groups. In contrast, HF80 exhibited the longest-lasting effect,

with a significant increase in PWT maintained up to 2.5 hours (each timepoint vs pre: $p < 0.05$). The analgesic effects in each group remained significant up to the following timepoints: 0.5 hours (LF40), 1 hour (LF80), 1.5 hours (HF40), and 2.5 hours (HF80; each time vs pre: $p < 0.05$). The effects of SCS on the MPE also showed a similar pattern (Fig. 3b). The AUC of MPE in HF80 was the highest among the groups, and all SCS groups, except for LF40, showed significant differences from sham control (one-way ANOVA, LF80, HF40, HF80 vs sham: $p < 0.01$; Fig. 3c). Both LF80 ($p = 0.13$) and HF80 ($p < 0.01$) also showed significant differences from the value of LF40. However, the motor score of BBB was not altered in all groups after SCS from the value before SCS (Fig. 3d).

All five groups exhibited MAS values at approximately three grades before SCS, and no significant differences were observed among the groups. All SCS groups exhibited the greatest reductions in MAS values at 0.5 hours (immediately after SCS), with significant differences from prestimulation values (two-way RM ANOVA; $p < 0.01$; Fig. 4a). The changes in MAS were LF40 (2.94–2.05), LF80 (2.95–1.10), HF40 (2.95–1.05), and HF80 (3–0.77). Significant effects remained significant up to the following timepoints: 0.5 hours (LF40), 2 hours (LF80), 2 hours (HF40), and 2.5 hours (HF80; and each time vs pre: $p < 0.05$). A similar trend was

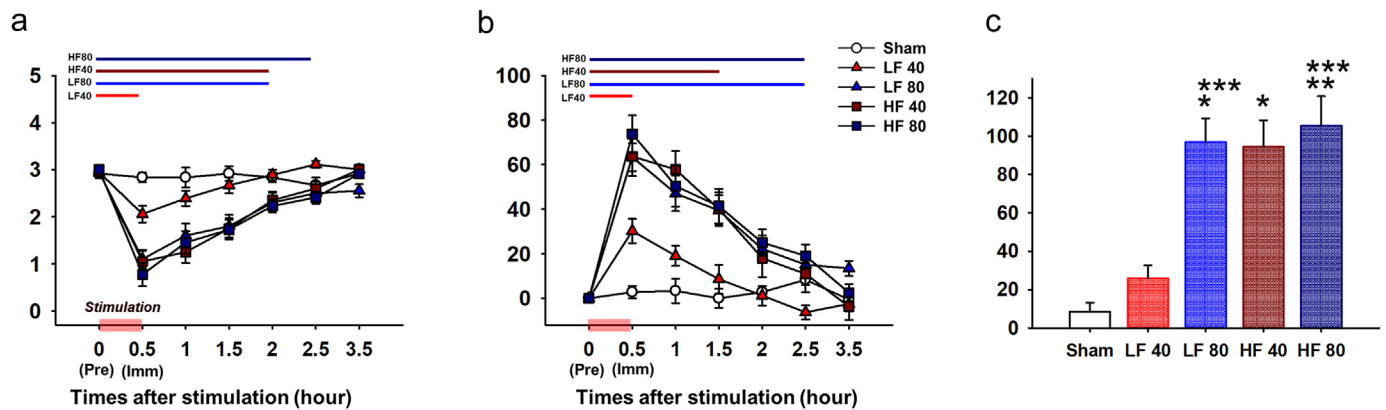


Figure 4. Changes in the spasticity after SCS. a. Changes in MAS depending on the different parameters. b. MPE. c. A comparison of the AUCs for the % MPE on the antispastic effects of SCS application. The red boxes on the x-axis in panels a and b indicate the timepoints at which stimulation was applied. Color bars in panels a and b indicate the duration for which each group showed statistically significant differences ($p < 0.05$) from the prestimulation baseline. Values are presented as mean $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$ compared with the sham group, and *** $p < 0.05$ compared with the LF 40 group. [Color figure can be viewed at www.neuromodulationjournal.org]

expressed in MPE except for LF80 (2.5 hours, Fig. 4b). The AUC of MPE in LF40 was not significantly different from the sham control; in contrast, LF80, HF40, and HF80 showed significant increases (Kruskal–Wallis test; LF80, HF40: $p < 0.05$; HF80: $p < 0.01$ Fig. 4c). Both LF80 and HF80 also significantly differed from LF40 ($p < 0.05$).

Moreover, we investigated whether other factors of SCS, such as the applied amplitude or charge per second, affected PWT or MAS output. UGLM analysis showed that there was no relationship between AUC of PWT and the applied amplitude ($r^2 = 0.06$) or charge per second ($r^2 = 0.17$), or between AUC of MAS and the applied amplitude ($r^2 = 0.56$) or charge per second ($r^2 = 0.338$; data not shown).

SCS Effects on Spinal Microglia Expression

We examined whether the SCS-induced attenuation in neuropathic pain can be associated with a change in microglia phenotype in the dorsal horn. We found that the sham control, LF80, and HF80 groups—all comprising SCI rats—showed a significant increase in the total number of microglia in the dorsal horn compared with normal rats (one-way ANOVA; $p < 0.01$; Fig. 5b). However, there were no significant differences among the three SCI groups. After SCS, both LF80 and HF80 significantly reduced the number of activated microglia in the dorsal horn compared with the sham group (sham: 61.90 ± 3.15 ; LF80: 36.5 ± 1.99 ; HF80 42.3 ± 2.76 ; Kruskal–Wallis test; $p < 0.01$; Fig. 5b). Consistent with this, the proportions of activated microglia in the dorsal horn also were significantly lower in LF80 and HF80 than in the sham control (Kruskal–Wallis test; $p < 0.01$; Fig. 5c). Specifically, the proportion of activated microglia was 13% in the normal group, 41% in the sham control, 24% in LF80, and 27% in HF80.

We also investigated whether the SCS-induced reduction in spasticity is associated with a change in microglial phenotype in the ventral horn. Sham control, LF80, and HF80, which all underwent SCI, significantly expressed an increase in the total number of microglia in the ventral horn compared with normal rats, with no significant difference among them (one-way ANOVA; sham, HF80: $p < 0.01$; LF80: $p = 0.46$; Fig. 6b). However, both LF80 and HF80 significantly reduced the number of activated microglia in the ventral horn compared with the sham group (sham: 51.60 ± 1.55 ; LF80: 28.60 ± 1.47 ; HF80 23.63 ± 1.45 ; one-way ANOVA; $p < 0.01$; Fig. 6b). Consistent with this, the

proportions of activated microglia in the ventral horn also were significantly lower in LF80 and HF80 than in the sham control (one-way ANOVA; $p < 0.01$; Fig. 6c). In addition, there was no significant difference between normal and HF80. Specifically, the proportion of activated microglia was 13% in the normal group, 42% in the sham control, 24% in LF80, and 18% in HF80.

DISCUSSION

The present study showed that SCS, delivered through a fully implanted system, effectively alleviated neuropathic pain and spasticity in the contusive SCI model. Despite the clinical significance of these findings, the precise mechanism underlying the simultaneous modulation of neuropathic pain and spasticity remains elusive.⁴³ It has been suggested that neuronal hyperexcitability in spinal neurons after SCI or spinal nerve injury results from a loss of spinal inhibition,⁴⁴ increased descending facilitation,⁴⁵ and increased excitatory transmission.⁴⁶ However, it is important that microglia activation initiates neuronal hyperexcitability in the spinal cord, contributing to the development and maintenance of both neuropathic pain and spasticity.^{23,24,26,27,47} Microglia also are responsive to SCS and may play a key role in mediating its effects.^{31,32} In the present study, both LF80 and HF80 SCS produced inhibitory effects on neuropathic pain and spasticity, with HF80 showing superior efficacy to that of LF80. Notably, both effective stimulation paradigms were associated with changes in microglial activity. The results suggest that the underlying mechanism of the therapeutic effects of both LF and HF SCS involves microglial modulation.

Impact of Frequency and Intensity of SCS on Neuropathic Pain and Spasticity

SCS at LF80, HF40, and HF80 significantly reduced neuropathic pain and spasticity, with HF80 exhibiting the most pronounced effects (Figs. 3c and 4c). In contrast, LF stimulation with subsensory threshold intensity (LF40) was ineffective in alleviating both neuropathic pain and spasticity. Consistent with these results, a previous study reported that the efficacy of 10-kHz SCS for patients with leg and back pain was superior to conventional SCS (40–60 Hz) for a long-term period of 12 months.²⁰ Similarly, in a spinal nerve

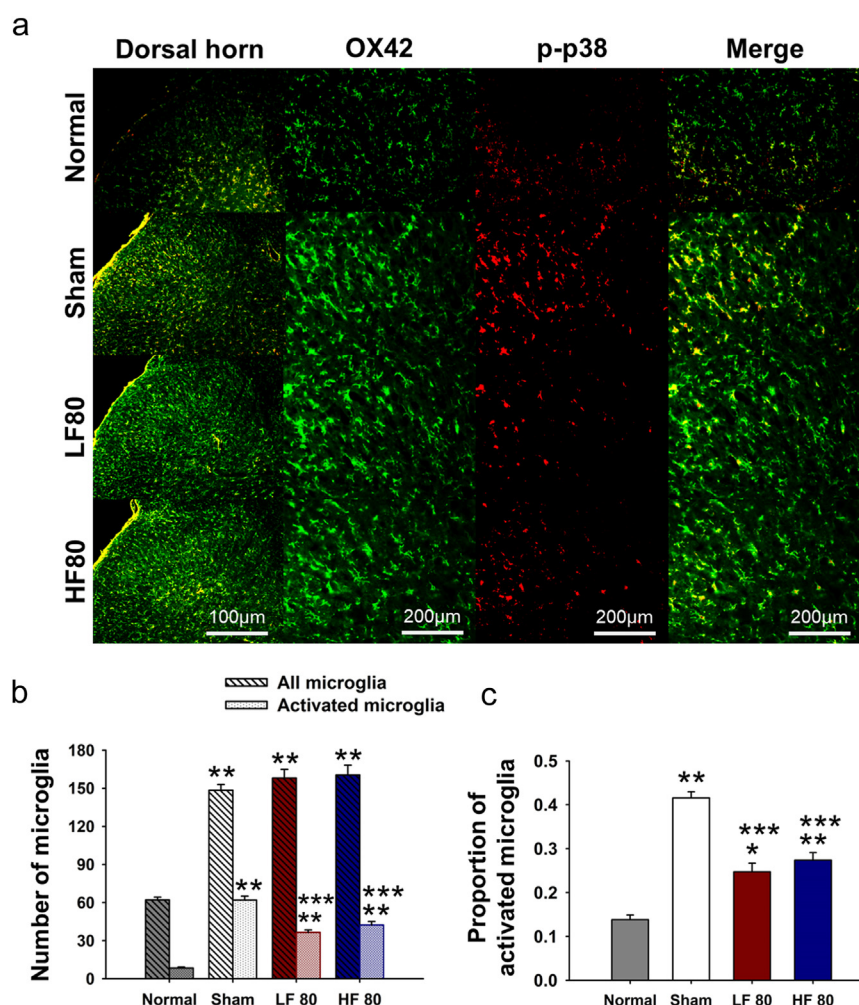


Figure 5. Changes in activation of microglia after SCS in dorsal horns. a. The microglia and p-p38 expression in the spinal dorsal horn at L4–L5 segments. b. The total number of all microglia and activated microglia in the normal, sham, LF80, and HF80 groups. c. The proportion of activated microglia. * $p < 0.05$ and ** $p < 0.01$ compared with the normal group, and *** $p < 0.01$ compared with the sham group. [Color figure can be viewed at www.neuromodulationjournal.org]

ligation (SNL) model, epidural 1-kHz and 10-kHz SCS significantly reduced neuropathic pain compared with pre-SCS levels and yielded greater efficacy than did LF (50 Hz) SCS.²¹

On the basis of the gate control theory, LF stimulation (<100 Hz) has been clinically used.⁴⁸ It is postulated that LF stimulation activates large-diameter A-fibers, which attenuate nociceptive signals through inhibitory interneurons that suppress wide-dynamic range (WDR) neurons in the spinal dorsal horn.⁴⁹ WDR neurons are sensitive to stimulation frequency and are effectively suppressed at 30 to 100 Hz. These neurons are crucial in engaging reciprocal circuits between the spinal and supraspinal regions involved in pain modulation, which mediate the effects of LF stimulation through descending cholinergic, serotonergic, and opioidergic pathways.^{50,51} However, a study reported inconsistent effects of LF SCS on the pain threshold. In an SNL model, epidural 50-Hz SCS delivered at 80% MT effectively alleviated ipsilateral pain, whereas lower intensities (20%–40% MT) had no meaningful effect.²¹ Similarly, our findings indicated that both the analgesic and antispastic effects of LF SCS differed depending on whether the stimulation intensity was 40% or 80% MT (Figs. 3c and 4c). This suggests that subthreshold conventional SCS (50 Hz) may produce

detectable changes in pain perception but often fails to achieve meaningful therapeutic outcomes in clinical settings.⁵² These heterogeneous effects of LF SCS can be influenced by multiple factors, such as stimulation intensities (associated with MT) and duration of application (acute vs long-term), the origin of pain (spinal cord vs spinal nerve damage), and stimulation site (epidural vs percutaneous).

Unlike LF stimulation, HF stimulation does not rely on A-fiber activation and operates at subsensory threshold intensities. HF stimulation may involve the desynchronization of nociceptive C-fiber signaling by delivering electrical impulses at a rate exceeding the refractory period of C-fibers.^{53,54} For example, a report showed that the transmission of nociceptive signals in the superficial spinothalamic tract was specifically inhibited with 10-kHz SCS.⁵⁵ Another proposed mechanism involves the selective activation of inhibitory interneurons in the dorsal horn without engaging dorsal column fibers.⁵⁶ The rapid and repetitive stimulation of HF induces the temporal summation of action potentials in inhibitory neurons, facilitating paresthesia-free relief.

Explaining the antispastic effect of SCS in a frequency-dependent manner remains challenging. A previous study showed that SCS

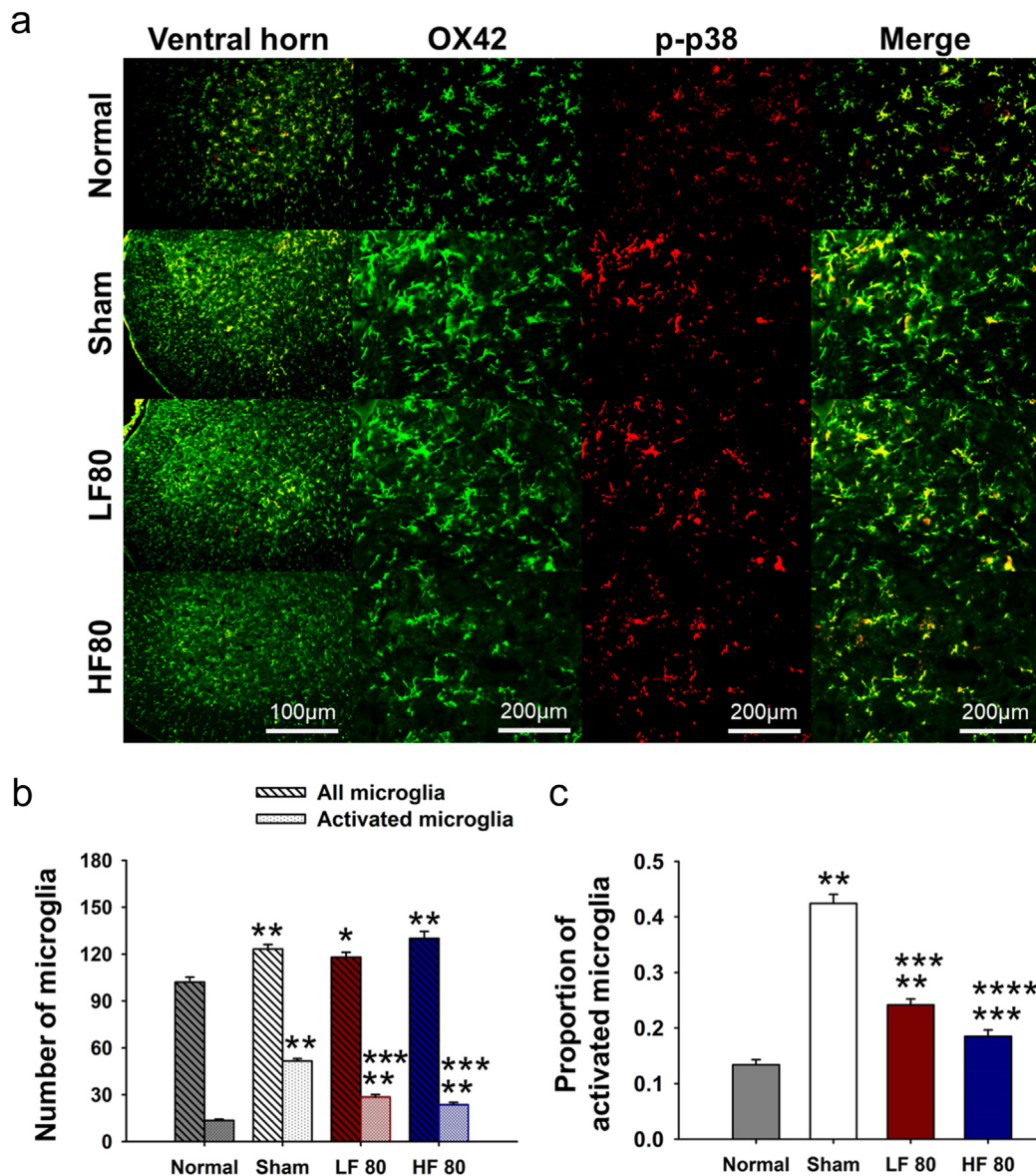


Figure 6. Changes in activation of microglia after SCS in ventral horns. a. The microglia and p-p38 expression in the spinal ventral horn at L4–L5 segments. b. The total number of microglia and activated microglia in the normal, sham, LF80, and HF80 groups. c. The proportion of activated microglia. * $p < 0.05$ and ** $p < 0.01$ compared with the normal group, *** $p < 0.01$ compared with the sham group, and **** $p < 0.01$ compared with the LF 80 group. [Color figure can be viewed at www.neuromodulationjournal.org]

significantly relieved spasticity in individuals with cerebral palsy.⁵⁷ One proposed mechanism involves restoring inhibitory control or disrupting the pathologic reflex loop by targeting the inhibitory interneurons in the spinal cord and modulating the conductive properties of afferent neurons. More recently, clinical findings have supported the therapeutic potential of frequency-specific modulation.⁵⁸ In particular, 1.2-kHz stimulation attenuated spasticity by blocking afferent activity, and combining both HF (1.2 kHz) and LF (40 Hz) components yielded superior outcomes in patients with SCI.

Changes in Microglia Activation After SCS

To explore a potential common mechanism underlying the effects of SCS on neuropathic pain and spasticity, we analyzed microglial activation in the spinal cord. Our findings revealed that

both LF80 and HF80 SCS effectively reduced microglial activation in laminae I to II of the dorsal horn. Specifically, the percentage of activated microglia in the LF80 and HF80 groups significantly decreased to 24% and 27% compared with 41% in the sham control group, as shown in the Results section (Fig. 5c). In the ventral horn, microglial activation also was significantly reduced compared with the sham group (42%), with LF80 and HF80 decreasing the proportion to 24% and 18%, respectively (Fig. 6c). Notably, HF80 decreased microglia activation to a level comparable to that of the normal group (13%), with no statistically significant difference between them.

Several studies explained that SCI-induced pain and spasticity are associated with an increase in activated microglia.^{23,24,27} This activation contains p38 mitogen-activated protein kinase (MAPK) activation-dependent signaling pathway,⁵⁹ and a remarkable upregulation of

cytokines and chemokines.^{60,61} Proinflammatory cytokines such as IL-1 β and -6 and TNF- α , released by activated microglia,⁶² contribute to the central sensitization of spinal neurons.^{59,63} Our study revealed an increase in resting and activated microglia in both dorsal and ventral horns of the SCI group. Notably, this phenomenon was observed in L4-to-L5 spinal segments located at remote sites from the epicenter. Consistent with these findings, previous studies have shown that microglial and p-p38 MAPK activation of L5 superficial dorsal horns was highly correlated with mechanical allodynia after SCI.²³ Because inhibition of the p38 MAPK signaling pathway has been shown to decrease the activation of M1 microglia (proinflammatory) and promote the polarization of M2 microglia (antiinflammatory),^{64–67} the analgesic or antispastic effect of epidural SCS in our present study may be attributed to the attenuation of M1 microglia activation.

We cannot fully explain whether HF80 SCS has a distinct mechanism to effectively suppress the activated microglia in the dorsal and ventral horns. However, it was reported that HF SCS (1.2 kHz) generated a shift toward an antiinflammatory state and reduced RNA levels of microglial proliferation markers (Irf-8 and Adgre-1) in a rat study with painful diabetic peripheral neuropathy, whereas LF SCS generated a shift toward a proinflammatory state.³⁶ In addition, a recent rat study revealed that HF SCS (10 kHz, 40% MT) significantly reduces immune responses in the spinal dorsal horn by inactivating the Kairo/P2X purinoceptor 7 (P2X7R)-mediated pathology in microglia.³³ Interestingly, knockdowning Kairo/P2X7R in microglia remarkably improved the efficacy of LF SCS (40 Hz, 80% MT), leading to a shift toward reduced neuroinflammation and long-lasting pain relief.³³

Study Limitations

Although LF and HF SCS exhibit distinct effects on neuropathic pain and spasticity, several issues, particularly regarding long-term efficacy and physiologic tolerance, remain to be fully elucidated. To ensure consistent therapeutic outcomes, the effects of repetitive stimulation and key parameters—such as frequency, pulse width, and amplitude—should be systematically investigated. Therefore, future research is essential to assess the long-term impact of diverse stimulation parameters, ultimately optimizing SCS outcomes.

CONCLUSION

In summary, we have indicated the therapeutic effects of epidurally applied SCS with different parameters on both neuropathic pain and spasticity. Our results suggest that HF and LF SCS with high intensity contribute to effectively relieving pain and spasticity by modulating the microglial activation. Given that the effects of repetitive SCS can differ between short- and long-term applications, further studies are required to investigate the effects of repetitive SCS and establish optimal parameters for long-term use.

Authorship Statements

Jinseung Lee made substantial contributions to the conception and design of the study; acquisition, interpretation, and analysis of data; and drafting the article. Wonok Kang made substantial contributions to the conception and design of the study and to acquisition and interpretation of data. Wonsuk Choi made substantial contributions to the conception and design of the study and to acquisition and interpretation of data. Eui Ho Park

contributed to the analysis of data and revising it critically for important intellectual content. Jinju Kwon contributed to the acquisition, interpretation, and analysis of data, and revising it critically for important intellectual content. Minji Kwon contributed to the analysis of data and revising it critically for important intellectual content. Hogwang Ok analyzed the data. Jinseok Kim made substantial contributions to the conception and design of the study. Sung-Min Park made substantial contributions to the conception and design of the study. Junesun Kim made substantial contributions to the conception and design of the study, interpretation, and analysis of data, and to revising it critically for important intellectual content. All authors approved the final version of the manuscript.

Conflict of Interest

The authors reported no conflict of interest.

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