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Spinal cord stimulation attenuates below-level mechanical hypersensitivity in rats after thoracic spinal cord injury

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Abstract

Objectives: The burden of pain after spinal cord injury (SCI), which may occur above, at, or below injury level, is high worldwide. Spinal cord stimulation (SCS) is an important neuromodulation pain therapy, but its efficacy in SCI pain remains unclear. In SCI rats, we tested whether conventional SCS [50 Hz, 80% motor threshold (MoT)] and 1200 Hz, low-intensity SCS (40% MoT) inhibit hind paw mechanical hypersensitivity, and whether conventional SCS attenuates evoked responses of wide-dynamic range (WDR) neurons in lumbar spinal cord.

Materials and Methods: Male rats underwent a moderate contusive injury at the T9 vertebral level. Six to 8 weeks later, SCS or sham stimulation (120 min, n=10) was delivered through epidural miniature electrodes placed at upper-lumbar spinal cord, with using a cross-over design. Mechanical hypersensitivity was examined in awake rats by measuring paw withdrawal threshold (PWT) to stimulation with von Frey filaments. WDR neurons were recorded with *in vivo* electrophysiologic methods in a separate study of anesthetized rats.

Results: Both conventional SCS and 1200 Hz SCS increased PWTs from pre-stimulation level in SCI rats, but the effects were modest and short-lived. Sham SCS was not effective. Conventional SCS (10 min) at an intensity that evokes the peak Aα/β waveform of sciatic compound action potential did not inhibit WDR neuronal responses (n=19) to graded or repeated electrical stimulation that induces windup.

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Conclusions: Conventional SCS and 1200 Hz, low-intensity SCS modestly attenuated below-level mechanical hypersensitivity after SCI. Inhibition of WDR neurons was not associated with pain inhibition from conventional SCS.

Keywords

Spinal cord injury; pain; spinal cord stimulation; rat; dorsal horn neuron

INTRODUCTION

More than 30% of patients with spinal cord injury (SCI) develop debilitating pain, which is described as above-level, at-level, or below-level with respect to the location of the injury, according to the three-tiered system defined by the International Association for the Study of Pain (1–3). Below-level pain, which develops unilaterally or bilaterally in 30–40% of patients within 5 years of SCI, often affects the lower extremities (1, 4). Rodent SCI models have demonstrated similar sensory disturbances, including hypersensitivity to tactile stimulation at the hind paws that can persist for months after initial injury (5–7). The pathogenesis of SCI pain has been studied extensively, but the mechanism remains unclear (8–10). Moreover, current pharmacologic therapies are often ineffective for SCI pain or become intolerable to patients (11, 12).

Neuromodulation, which encompasses implantable and non-implantable technologies for delivering electrical or chemical stimuli, is a valuable option for treating pain. One form of neuromodulation—spinal cord stimulation (SCS)—is clinically effective for many refractory pain conditions (13, 14). Conventional SCS has been used for over 50 years to treat peripheral neurogenic pain and musculoskeletal pain conditions. When used at frequencies of 40–60 Hz, it activates Aβ-afferents in the dorsal columns and produces paresthesia. This stimulation is above the sensory threshold and therefore considered supra-sensory (14–16). In contrast, the new high-frequency (>1 kHz), low-intensity SCS produces pain relief without eliciting paresthesia (sub-sensory) (13, 17–19) and may not activate or change conduction properties of dorsal column fibers (20).

Mitigating SCI pain is important to improving quality of life in SCI patients. However, no consensus has been reached on the effectiveness of different SCS paradigms for SCI pain (21, 22), because clinical studies are limited and pre-clinical mechanistic investigations are lacking. Contusive SCI in rodents produces motor and sensory dysfunctions similar to those experienced by patients after traumatic SCI and has been widely used to study underlying etiologies (5–7, 23). However, paralysis below the injury level would prevent reflexive pain responses to stimulation at the hind limbs. Therefore, previous studies often used moderate SCI models to allow partial motor function recovery and measurement of SCI pain-related behavior (5, 8, 11, 12, 21). Accordingly, we produced a moderate contusive injury at the T9 vertebral level (~T10 spinal level) in male rats by using a computerized displacement method (23).

Previous studies showed that low-intensity SCS at 1000–1200 Hz inhibited mechanical hypersensitivity in nerve-injured rats (24, 25). Yet, clinical studies have reported variable efficacy of this new paradigm in pain patients (19, 26). Therefore, we compared the effect of

this new paradigm with that of conventional SCS for SCI pain inhibition in an identical experimental setting. The spinal cord dorsal horn is an important site for pain transmission and modulation. Because inhibition of spinal wide-dynamic range (WDR) neurons may contribute to pain inhibition by conventional SCS (15, 24, 27), we further performed *in vivo* electrophysiologic recording to determine whether conventional SCS also attenuates evoked responses of lumbar spinal WDR neurons in SCI rats.

MATERIALS AND METHODS

Animals

To avoid the potential impact of changes in estrogen level and estrous cycles in female rats on SCI pain and SCS, we used adult male Sprague-Dawley rats (220–280 g, Envigo, Indianapolis, IN) in this study. Rats were housed in groups of three before SCS lead implantation. After lead implantation, rats were housed in individual cages to prevent cagemates from biting or pulling out the leads. Rats were housed under optimal laboratory conditions with a 12-h light/dark cycle and free access to food and water. All behavioral experiments were performed during the light cycle between 9:00 a.m. and 5:00 p.m. All animal work was approved by the Animal Care and Use Committee of Johns Hopkins University (Baltimore, MD, USA) and complied with the National Institutes of Health's *Guide for the Use of Experimental Animals* to ensure minimal animal use and discomfort.

Spinal Cord Injury and Postsurgical Care

SCI was produced with a method we have described previously (23). Briefly, rats were deeply anesthetized with 2.0% isoflurane (Abbott Laboratories, North Chicago, IL), a partial laminectomy was made with fine tip rongeurs at the T9 vertebrae, and the dura was kept intact. To avoid severe SCI, which induces prolonged paralysis and prevents the study of evoked pain responses, we used a computer-controlled impactor to produce a moderate contusion injury (Impact One Stereotaxic Impactor, Leica, Buffalo Grove, IL, USA, tip diameter, 2.0 mm; speed: 4.0 m/sec; depth: 1.5 mm, dwell time: 0.1 sec). Muscle and subdural tissue were sutured with 4–0 PolySyn™ sutures (Angiotech, Reading, PA). The skin was closed with metal clips and the wound site was covered with antibiotic ointment. Because the rats often lose the micturition reflex, their bladders were manually expressed twice daily for 10 to 14 days until self-voiding resumed. Additionally, the animals were administered a prophylactic dose of enrofloxacin (Sigma-Aldrich, St. Louis, MO, 1 mg/kg) once daily for 7 days via subcutaneous injection to prevent urinary tract infection. Skin staples were removed approximately 2 week post-surgery.

Behavior Tests

Mechanical hypersensitivity test—Prior to the behavioral testing, animals were acclimatized to their facilities for 1 week. In addition, animals were habituated to the test environment for 30–60 min before testing was begun on a given day. Hypersensitivity to punctate mechanical stimulation at the hind paws was determined by using the Up-Down method (24, 28, 29). von Frey filaments (0.38, 0.57, 1.23, 1.83, 3.66, 5.93, 9.13, 13.1 g) were applied in series for 4 to 6 sec each to the test area between the footpads on the plantar surface of the hind paw. The paw withdrawal threshold (PWT) was determined according to

the Dixon formula (30). Only SCI rats that could lift at least one hind paw by day 28 post-SCI were included in PWT testing. Because rats exhibit motor deficits after SCI, we defined a positive response as abrupt withdrawal, licking, or shaking of *either* hind paw when the filament was applied to one (23). The left hind paw was stimulated first, followed by the right hind paw, with no less than a 5-min interval. Data from both sides were included for analysis.

Basso, Beattle and Bresnahan (BBB) open-field locomotion score—The motor function of rats was assessed by using the BBB locomotor rating scale (7, 23, 31). Briefly, after acclimation in the testing room, rats were placed individually in an open field and assessed by two trained observers. Motor function in both hind limbs was scored during a 4-min period on a 21-point scale based on 10 categories of behavior (e.g., joint movement, stepping, coordination, paw rotation). Data from both sides were included for analysis. The two observers scored independently and then discussed and assigned consensus scores.

Implantation of SCS Electrodes

After rats were anesthetized with isoflurane (2%), a quadripolar electrode (Medtronic Inc., Minneapolis, MN, USA) was placed epidurally through a small laminectomy at the T13 vertebral level, as described previously (24, 29). The electrode was inserted in the rostral direction and adjusted so that the contacts were at the T13-L1/2 spinal cord level, which corresponds to the lower thoracic-upper lumbar region. The proximal end of the electrode exited the animal at the top of its head for later connection to an external neurostimulator (Model 2100, A-M Systems, Sequim, WA). Animals were allowed to recover from surgery for 10 days.

SCS Protocol for Animal Behavior Studies

SCS was applied to awake rats between 6 and 8 weeks post-SCI, which constitutes the maintenance phase of SCI pain (23, 32). Bipolar SCS (“twin-pairs” stimulation) was provided as described in our previous studies by using biphasic pulses at constant current mode (24, 29, 33). Motor threshold (MoT) was determined by slowly increasing the amplitude of 4 Hz (0.2 msec) stimulation from zero until muscle contraction was observed in the mid-lower trunk or hind limbs. Based on recent findings (20, 24, 25), we considered sensory threshold in awake rats to be near 50% MoT. Thus, conventional SCS (50 Hz, 0.2 msec) was conducted at 80% MoT (supra-sensory threshold), and 1200 Hz, low-intensity SCS (0.2 msec) was applied at 40% MoT (sub-sensory threshold). Sham stimulation (0 mA) was used as a control.

Of 16 rats subjected to SCI for behavioral studies, 12 (75%) recovered from complete paralysis and developed mechanical hypersensitivity (>50% reduction of PWT from pre-injury baseline) at day 28 post-SCI. These 12 rats received SCS lead implantation. At 10 days after electrode implantation, two rats showed deteriorating motor function, diminished mechanical hypersensitivity, or damage to the implanted lead and were eliminated from the subsequent studies. The remaining 10 rats (83.3%) recovered well from surgery and were randomized to receive three rounds of SCS treatment during days 40–54 after SCI in one of the following three sequences (3 cohorts): Cohort A (n=3): 50 Hz–1200 Hz–Sham; Cohort B

(n=3): 1200 Hz–Sham–50 Hz; Cohort C (n=4): Sham–50 Hz–1200 Hz. During each round, one cohort of animals received sham stimulation (0 mA) in order to blind the experimenter to animal treatment conditions. Each round consisted of two treatment days (120 min/day) followed by 4 treatment-free recovery days to limit potential carryover effect (Figure 1A). This cross-over design in which the order of treatment varied was intended to limit potential order effect. On each treatment day, rats were acclimated to the testing environment for 30 min before baseline PWT was measured. Then, MoT was determined and SCS or sham stimulation applied for 120 min. We tested PWT at 30, 60, and 90 min during SCS (intra-SCS) and at 0, 30, and 60 min after completion of SCS (post-SCS) to determine the carryover effect. The data from the different rounds were combined for analysis.

Extracellular Recordings of WDR Neurons

Electrophysiology study was conducted in a separate group of rats at 6–8 weeks post-SCI and in naïve rats. We conducted *in vivo* extracellular recordings of WDR neuronal activity as described previously (15). Briefly, after the rats were anesthetized with isoflurane (1.5%), they received a tracheotomy and were ventilated mechanically (Kent Scientific Corporation, Litchfield, CT). Then, a laminectomy was performed at vertebral levels T12–L1 corresponding to lumbar enlargements at spinal segments L3–L6. We examined neurons located at deep laminae (III–V, 400–1200 μm below the dorsal surface) and selected WDR neurons with defined receptive fields in the plantar region of the hind paw. WDR cells were identified by their characteristic responses to mechanical test stimulation at the skin receptive field. Analog data were collected with a real-time, computer-based data acquisition and processing system (CED Spike 2, United Kingdom).

The WDR neuronal response to a suprathreshold electrical test stimulus at the receptive field consists of an early Aβ-component (0–25 msec), Aδ- component (25–100 msec), and a later C-component (100–500 msec). Graded intracutaneous electrical stimuli (0.1–10 mA, 2 msec) were applied through a pair of fine, 31-guage needles inserted at the central plantar side of the hind paw (15, 34). WDR neurons also display an action potential (AP) windup phenomenon—a short-form neuronal sensitization to a train of 0.5 Hz electrical stimulation (16 pulses, 2 msec, 5–10 mA) at the hind paw. After 30 sec, a train of 0.1 Hz stimulation (12 pulses, same intensity), which does not induce windup, was applied as a control.

SCS Protocol for Electrophysiology Studies

To mimic the actions of SCS in the animal behavioral studies, we provided SCS in the electrophysiology experiments with the same type of miniature electrode (Medtronic Inc.) as that used during behavior test. After the rats underwent a laminectomy, the SCS electrode was placed caudally over the T10–12 vertebral level (~T12-L1/2 spinal level) with the dura mater preserved. The distal end of the lead was connected to an external stimulator (model 2100; A-M Systems, Sequim, WA).

Because anesthesia can affect MoT (29), we determined the intensities of SCS in the electrophysiology study by recording the antidromic sciatic compound APs evoked by graded electrical stimulation applied through the SCS electrode (0.01–3.0 mA, 0.2 msec) (15, 24, 27). Briefly, a monopolar silver hook electrode was placed on the ipsilateral sciatic

nerve for recording the antidromic compound APs. The reference electrode was placed in the nearby muscle. Different compound AP waveforms were distinguished on the basis of the activation threshold and the conduction velocity. The current thresholds that resulted in the first detectable Aα/β waveform (Ab0) and the peak Aα/β waveform (Ab1) without inducing an Aδ waveform were determined for each animal. The effects of conventional SCS (50 Hz, 0.2 msec, Ab1, 10 min) or sham SCS on stimulus-response (S-R) functions of Aβ-, Aδ-, and C-components to graded electrical stimuli (0.1–10 mA, 2.0 msec, 15 sec interval), and on windup response of WDR neurons were examined at 0–15 min (0 min) and 30–45 min (30 min) after treatment.

Statistical Analysis

We carried out statistical analyses with STATA version 14.1 and PRISM version 6. As PWTs are often at the cutoff value in naïve animals, the percentage of maximum possible effect [% MPE] for inhibiting mechanical hypersensitivity was calculated with the equation: %MPE = [1 – (Cutoff PWT – Post-SCS PWT)/(Cutoff PWT – Pre-SCS PWT)] × 100, where the calculated cutoff PWT = 21.72 g. Changes in %MPE after SCS were compared to baseline within each group and between different groups with a two-way mixed model ANOVA. Dunnett's multiple comparisons test or the Tukey honestly significant difference post-hoc test was used to compare specific data points. The methods for statistical comparisons in each study are given in the figure legends. We randomized animals to the different treatment groups and blinded the experimenter to treatment to reduce selection and observation bias. There were no data missing for any of the variables.

For WDR recording, the S-R functions of WDR neuronal response to graded electrical stimuli and the windup functions were compared between the pre- and post-treatment conditions in each group and between different groups with a two-way mixed model ANOVA. The Bonferroni and Fisher's LSD post-hoc tests were used to compare specific data points. The normally distributed data are expressed as mean ± SD, and the non-normally distributed data are expressed as median + interquartile range. All tests were two-tailed, and $p<0.05$ was considered statistically significant in all tests. The sample size was calculated based on the respective statistical power analysis [power = 0.080, $\alpha = 0.05$ (2-sided)] and previous similar studies (24, 29, 33).

RESULTS

Inhibitory Effects of SCS on Mechanical Hypersensitivity in SCI Rats

Motor function in both hind limbs of SCI rats was evaluated with the 21-point BBB scale. Rats exhibited paralysis (i.e., BBB score: 0) of both lower limbs immediately after SCI. Our previous studies have shown that motor dysfunction recovers and mechanical hypersensitivity in the hind paws reaches a plateau in rats at 3 weeks post-SCI and persists for at least 8 weeks (5, 23). Similarly, SCI rats in the current study showed motor function recovery by day 40, with BBB scores around 10 (Supplemental Figure 1). We then used a crossover design to administer three rounds of treatment over days 40–54 post-SCI (Figure 1A). BBB scores did not change significantly in any cohort after the three rounds, as

compared to pre-treatment scores on day. BBB scores were comparable between cohorts from day 40 to day 54 post-SCI (Supplemental Figure 1).

Both conventional SCS (50 Hz, 80% MoT, 120 min) and high-frequency SCS (1200 Hz, 40% MoT, 120 min) attenuated hind paw mechanical hypersensitivity in SCI rats on treatment days 1 and 2, as indicated by significant increases in %MPE at 60 and 90 min intra-SCS and at 0 min post-SCS when compared to pre-SCS baseline (Figure 1B,C). Furthermore, mechanical hypersensitivity was significantly lower in both SCS groups than in the sham stimulation group (n=10) across time points. Time course and peak pain inhibition from conventional 50 Hz SCS and 1200 Hz, low-intensity SCS were comparable on each treatment day. Average %MPE after SCS or sham stimulation on each treatment day is summarized in Table 1.

On treatment day 1, the average %MPE at 30, 60, and 90 min intra-SCS and at 0 min post-SCS was significantly higher in both 50 Hz and 1200 Hz SCS groups than in the sham stimulation group. On treatment day 2, %MPE was significantly higher in the 50 Hz SCS group than in the sham group (Figure 1D). Pre-SCS PWTs on days 2 and 3 did not differ from those on day 1 (Figure 1E). Changes in mechanical hypersensitivity after SCS are also presented as PWTs in Supplemental Figure 2. Overall, the inhibitory effects from both SCS paradigms in SCI rats were rather modest as compared to those we have reported for nerve-injured rats in our previous studies (24, 25, 29).

Changes in WDR Neuronal Response After SCI

In a separate group of rats, WDR neuronal activation showed an early A-fiber component and a later C-fiber component in response to an intracutaneous electrical stimulus (Figure 2A). The Aβ- and C-component S-R functions of WDR neuronal responses to graded intracutaneous electrical test stimuli (0.1–10 mA, 2 msec) were significantly depressed in SCI rats (n=12) compared to those in naïve rats (n=9, Figure 2B). WDR neurons showed a progressive increase in C-component in response to repeated electrical stimulation (0.5 Hz, 16 pulses) at the skin receptive field, representing the windup phenomenon (Figure 2C). The windup functions of the C-component were not significantly different between naïve and SCI rats (Figure 2D).

SCS Does Not Attenuate S-R Function or Windup Responses of WDR Neurons in SCI rats

We used another group of SCI rats to examine the effect of conventional SCS on lumbar spinal WDR neuronal response at 6–8 weeks post-SCI. The experimental setup for recording WDR neurons and applying SCS in the electrophysiology study is illustrated schematically in Figure 3A. Because isoflurane anesthesia increases MoT (29), we calibrated the stimulation intensity of SCS in electrophysiology experiments by recording the Aα/β- and Aδ-waveforms of sciatic compound APs evoked by the graded SCS and distinguished them based on the activation threshold and the conduction velocity (Figure 3B), as reported in our previous studies (15, 24, 29). Compared to the respective pre-SCS baseline values, the S-R functions (Figure 3C,D) in each component of WDR neuron response to graded electrical stimuli (0.1–10 mA, 2 msec) and total APs (i.e., area under the curve) were not significantly

changed at 0 min and 30 min after conventional SCS (50 Hz, 0.2 msec, Ab1, 10 min, N=19) or sham SCS (n=19, Figure 3E,F).

The windup functions of C-component and areas under the curve at 0 min and 30 min after 50 Hz SCS were not significantly different from those at the pre-SCS baseline (Figure 4A). However, windup functions at 0 min and 30 min after sham SCS were significantly different from those at pre-SCS baseline (Figure 4B).

DISCUSSION

Although SCS has been well studied for the treatment of neuropathic pain with a peripheral origin, little is known about the usefulness of SCS for alleviating central neuropathic pain after SCI. Previous studies showed that conventional SCS at a moderate frequency (40–60 Hz) activates dorsal column fibers and produces satisfactory pain relief in some SCI patients (35, 36). However, others have reported a rather limited efficacy (22, 37). Thus, the clinical evidence for SCI pain inhibition by SCS remains mixed.

One study in a cohort of 127 patients suggested that pain in individuals with incomplete SCI at low thoracic to upper lumbar levels may benefit from conventional SCS (38). Those results are supported by our findings that rats with moderate SCI at T10 spinal cord had increased PWTs when conventional SCS was applied at a spinal segment below the injury (i.e., caudal SCS). Furthermore, 1200 Hz, low-intensity SCS also attenuated hind paw mechanical hypersensitivity in SCI rats. The maximum effect and time course of pain inhibition were comparable between the two SCS paradigms. To our knowledge, no preclinical or clinical study has tested the high-frequency, sub-sensory threshold SCS for SCI pain treatment. SCI pain is notoriously difficult to treat, as it is often refractory to commonly used medications such as opioids, antidepressants, and anticonvulsants. Our findings suggest that both conventional SCS and 1200 Hz, low-intensity SCS may have the potential to attenuate below-level mechanical hypersensitivity caused by incomplete thoracic SCI. Nevertheless, both paradigms produced only modest and short-lived inhibition of mechanical hypersensitivity in SCI rats. Therefore, studies for optimizing stimulation parameters will be needed. In particular, 10 kHz SCS and burst SCS are also effective paradigms for inhibiting pain without eliciting paresthesia (13, 17, 18, 39, 40). Their utility in pain treatment and functional recovery after SCI warrants future investigation. It is also important to test whether SCS applied above the injury level produces greater pain inhibition, and whether SCS efficacy persists after repetitive and long-term treatment.

Besides improving sensory function, SCS also promotes motor function recovery and attenuates SCI-associated autonomic dysregulation (21, 22, 41) by mechanisms such as enhancement of oligodendrocyte survival and differentiation (42). These beneficial effects may also facilitate the alleviation of SCI pain-related behavior. In addition to mechanical hypersensitivity, SCI pain symptoms can include sharp electrical, shooting, and burning spontaneous pain, which may occur in various body regions and affect musculoskeletal, visceral, and somatosensory systems (2, 3, 12). To date, research in this area is limited, and the mechanisms are still unknown.

A limitation of our study is that we examined only mechanical hypersensitivity. Because repetitive noxious heat stimulation may cause sensitization and tissue damage, we did not examine the time course by which SCS inhibits heat hypersensitivity in hind paws. Thus, future investigations are needed to determine the benefit of different SCS paradigms on other pain modalities (e.g., heat hypersensitivity, ongoing pain) and other pain categories (e.g., above- and at-level pain). Because no post-mortem histological quantification of the location and extent of injury was performed, the level and degree of SCI were not confirmed. Future studies need to determine how these factors may affect pain inhibition from SCS treatment. Such information may help clinicians to improve techniques and patient selection.

WDR neurons receive peripheral A-fiber and C-fiber inputs through mono- and polysynaptic transmissions, and play an important role in spinal nociceptive transmission (34, 43). Changes in WDR neuronal responses are often examined in mechanistic studies of SCS (15, 24, 27, 44, 45). In previous studies, neurons above and below the SCI epicenter have exhibited increased excitability that may partially underlie SCI pain (10, 43, 46, 47). Surprisingly, we found that the response of WDR neurons in L4–5 spinal cord to graded electrical stimulation was lower in SCI rats than in naïve rats. The reason for this apparent discrepancy is unclear but is unlikely due to injuries of the lumbar dorsal horn neurons or afferent fibers, which are several segments away from the epicenter. The mechanisms of SCI pain involve both spinal and supraspinal mechanisms (9) and may differ in the development and maintenance phases. It is possible that supraspinal mechanisms (9), hyperexcitability of WDR neurons near the epicenter and rostral to lesion site (47), and sensitization of nociceptive-specific and low-threshold dorsal horn neurons (43, 46) may also play important roles in the SCI pain that we observed.

In order to mimic the conventional SCS used in our animal behavioral studies, we also applied epidural SCS in our electrophysiology study at the lower thoracic to upper lumbar spinal level. This level is below the epicenter and above the lumbar segments where WDR neurons were recorded. Previous studies using similar parameters showed that conventional SCS delivered at this spinal level to rats with peripheral nerve injury decreased spontaneous activity, evoked responses, and windup of WDR neurons (15, 24, 27, 44). Because the electrophysiological recordings were conducted in different animals and with different parameters than the behavior experiments, whether these neuron inhibitory effects contribute to behavioral pain inhibition by conventional SCS warrants further examination. Here, the same treatment did not significantly attenuate S-R functions or inhibit windup in lumbar WDR neurons of SCI rats, as compared to pre-SCS level. The reasons for this difference are unclear but may be due in part to different etiologies of SCI and peripheral neuropathic pain (8, 9). For example, neurochemical and neurophysiologic changes in both central and peripheral nervous systems have been suggested to contribute to SCI pain, and some may differ from that of peripheral neuropathic pain (8–12, 46). Conventional SCS-induced therapeutic effects require the activation of dorsal columns and intact spinal pain transmission pathways (14–16). However, the pathologic changes after SCI may disrupt the functional integrity of dorsal column and spinal pain circuitry (8, 9, 21). In addition, essential spinal neuronal substrates and neurochemical mechanisms (e.g., GABAergic signaling) through which conventional SCS inhibits neuronal activity and pain may be compromised after SCI (7, 48, 49). Such loss of functionality may also contribute to the loss

of SCS-induced WDR neuronal inhibition that we observed. Intriguingly, windup was significantly increased from baseline in SCI rats at 0 and 30 min after sham stimulation. Yet, this facilitation of windup appeared to be prevented by conventional SCS. In addition to WDR neurons, nociceptive-specific and low-threshold dorsal horn neurons may also increase excitability and contribute to pathological pain after SCI (50). How their excitability is affected by SCS requires additional investigation.

Though spinal segmental mechanisms of pain inhibition may be compromised after SCI, it is possible that orthodromic action potentials may reach supraspinal structures after incomplete SCI via residual intact dorsal column fibers and ascending pathways. Thus, activation of supraspinal mechanisms may be important to SCI pain inhibition from conventional SCS. Such a mechanism would partially explain why conventional SCS induces better pain relief in patients with incomplete cord lesion than in those with complete cord transection (38, 51). Previous studies have shown that high-frequency, sub-sensory threshold SCS does not activate dorsal column fibers or change their conduction property, induce acute inhibition of WDR neurons, or inhibit spinal nociceptive transmission (13, 20, 24, 39, 52). Therefore, we did not test the effect of low-intensity, 1200 Hz SCS on WDR neurons in SCI rats. To date, the neuronal substrates and cellular and neurochemical mechanisms that underlie pain inhibition by high-frequency, sub-sensory threshold SCS remain unclear. It has been hypothesized that this paradigm may produce subtle, imperceptible conduction blockade of afferent fibers, desynchronize axonal activity, slowly change neurotransmitter content, and modulate neuronal intrinsic properties in the spinal cord (13, 39, 40, 45, 52).

In summary, our study showed that both conventional and 1200 Hz, low-intensity SCS induces modest inhibition of below-level mechanical hypersensitivity in rats after a moderate contusive SCI at thoracic level. Yet, pain inhibition from conventional SCS was not associated with a suppression of evoked responses in lumbar spinal WDR neurons after SCI. The neurophysiology and neurochemical mechanisms of SCI pain relief by both conventional SCS and high-frequency, sub-sensory threshold SCS warrant further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Wieseler J, Ellis AL, McFadden A, et al. Below level central pain induced by discrete dorsal spinal cord injury. J Neurotrauma. 9 2010;27(9):1697–1707. [PubMed: 20649467]
2. Widerstrom-Noga E, Felix ER, Adcock JP, Escalona M, Tibbett J. Multidimensional Neuropathic Pain Phenotypes after Spinal Cord Injury. J Neurotrauma. 3 1 2016;33(5):482–492. [PubMed: 26414803]
3. Widerstrom-Noga E, Biering-Sorensen F, Bryce TN, et al. The International Spinal Cord Injury Pain Basic Data Set (version 2.0). Spinal Cord. 4 2014;52(4):282–286. [PubMed: 24469147]
4. Siddall PJ, McClelland JM, Rutkowski SB, Cousins MJ. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain. 6 2003;103(3):249–257. [PubMed: 12791431]
5. Liu S, Huang Q, He S, et al. Dermorphin [D-Arg2, Lys4] (1–4) amide inhibits below-level heat hypersensitivity in mice after contusive thoracic spinal cord injury. Pain. 12 2019;160(12):2710–2723. [PubMed: 31365470]
6. Nakae A, Nakai K, Yano K, Hosokawa K, Shibata M, Mashimo T. The animal model of spinal cord injury as an experimental pain model. J Biomed Biotechnol. 2011;2011:939023. [PubMed: 21436995]
7. Hulsebosch CE, Xu GY, Perez-Polo JR, Westlund KN, Taylor CP, McAdoo DJ. Rodent model of chronic central pain after spinal cord contusion injury and effects of gabapentin. J Neurotrauma. 12 2000;17(12):1205–1217. [PubMed: 11186233]
8. Hulsebosch CE, Hains BC, Crown ED, Carlton SM. Mechanisms of chronic central neuropathic pain after spinal cord injury. Brain Res Rev. 4 2009;60(1):202–213. [PubMed: 19154757]
9. Yezierski RP. Spinal cord injury pain: spinal and supraspinal mechanisms. J Rehabil Res Dev. 2009;46(1):95–107. [PubMed: 19533523]
10. Gwak YS, Hulsebosch CE. Neuronal hyperexcitability: a substrate for central neuropathic pain after spinal cord injury. Curr Pain Headache Rep. 6 2011;15(3):215–222. [PubMed: 21387163]
11. Shiao R, Lee-Kubli CA. Neuropathic Pain After Spinal Cord Injury: Challenges and Research Perspectives. 7 2018;15(3):635–653.
12. Widerstrom-Noga E Neuropathic Pain and Spinal Cord Injury: Phenotypes and Pharmacological Management. Drugs. 6 2017;77(9):967–984. [PubMed: 28451808]
13. Chakravarthy K, Richter H, Christo PJ, Williams K, Guan Y. Spinal Cord Stimulation for Treating Chronic Pain: Reviewing Preclinical and Clinical Data on Paresthesia-Free High-Frequency Therapy. Neuromodulation. 1 2018;21(1):10–18. [PubMed: 29105244]
14. Guan Y Spinal cord stimulation: neurophysiological and neurochemical mechanisms of action. Curr.Pain Headache Rep. 6/2012 2012;16(3):217–225. [PubMed: 22399391]
15. Guan Y, Wacnik PW, Yang F, et al. Spinal cord stimulation-induced analgesia: electrical stimulation of dorsal column and dorsal roots attenuates dorsal horn neuronal excitability in neuropathic rats. Anesthesiology. 12/2010 2010;113(6):1392–1405. [PubMed: 21068658]
16. Foreman RD, Linderoth B. Neural mechanisms of spinal cord stimulation. Int Rev Neurobiol. 2012;107:87–119. [PubMed: 23206679]
17. Kapural L, Yu C, Doust MW, et al. Comparison of 10-kHz High-Frequency and Traditional Low-Frequency Spinal Cord Stimulation for the Treatment of Chronic Back and Leg Pain: 24-Month Results From a Multicenter, Randomized, Controlled Pivotal Trial. Neurosurgery. 11 2016;79(5):667–677. [PubMed: 27584814]
18. Kapural L, Yu C, Doust MW, et al. Novel 10-kHz High-frequency Therapy (HF10 Therapy) Is Superior to Traditional Low-frequency Spinal Cord Stimulation for the Treatment of Chronic Back and Leg Pain: The SENZA-RCT Randomized Controlled Trial. Anesthesiology. 10 2015;123(4):851–860. [PubMed: 26218762]
19. North JM, Hong KJ, Cho PY. Clinical Outcomes of 1 kHz Subperception Spinal Cord Stimulation in Implanted Patients With Failed Paresthesia-Based Stimulation: Results of a Prospective Randomized Controlled Trial. Neuromodulation. 10 2016;19(7):731–737. [PubMed: 27186822]

20. Song Z, Viisanen H, Meyerson BA, Pertovaara A, Linderoth B. Efficacy of kilohertz-frequency and conventional spinal cord stimulation in rat models of different pain conditions. Neuromodulation. 4 2014;17(3):226–234; discussion 234–225. [PubMed: 24612269]
21. Huang Q, Duan W, Sivanesan E, et al. Spinal Cord Stimulation for Pain Treatment After Spinal Cord Injury. Neurosci Bull. 6 2019;35(3):527–539. [PubMed: 30560438]
22. Chari A, Hentall ID, Papadopoulos MC, Pereira EA. Surgical Neurostimulation for Spinal Cord Injury. Brain Sci. 2 10 2017;7(2).
23. Duan W, Huang Q, Chen Z, Raja SN, Yang F, Guan Y. Comparisons of motor and sensory abnormalities after lumbar and thoracic contusion spinal cord injury in male rats. Neurosci Lett. 8 24 2019;708:134358. [PubMed: 31269465]
24. Shechter R, Yang F, Xu Q, et al. Conventional and kilohertz-frequency spinal cord stimulation produces intensity- and frequency-dependent inhibition of mechanical hypersensitivity in a rat model of neuropathic pain. Anesthesiology. 8 2013;119(2):422–432. [PubMed: 23880991]
25. Chen Z, Huang Q, Yang F, et al. The Impact of Electrical Charge Delivery on Inhibition of Mechanical Hypersensitivity in Nerve-Injured Rats by Sub-Sensory Threshold Spinal Cord Stimulation. Neuromodulation. 2 2019;22(2):163–171. [PubMed: 30556616]
26. Kapural L, Harandi S. Long-term efficacy of 1–1.2 kHz subthreshold spinal cord stimulation following failed traditional spinal cord stimulation: a retrospective case series. Reg Anesth Pain Med. 1 2019;44(1):107–110. [PubMed: 30640661]
27. Yang F, Xu Q, Cheong YK, et al. Comparison of intensity-dependent inhibition of spinal wide-dynamic range neurons by dorsal column and peripheral nerve stimulation in a rat model of neuropathic pain. Eur J Pain. 8 2014;18(7):978–988. [PubMed: 24390782]
28. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J.Neurosci.Methods 7/1994 1994;53(1):55–63. [PubMed: 7990513]
29. Yang F, Carteret AF, Wacnik PW, et al. Bipolar Spinal Cord Stimulation Attenuates Mechanical Hypersensitivity at an Intensity That Activates a Small Portion Of a-Fiber Afferents In Spinal Nerve-Injured Rats. Neuroscience. 12 29 2011;199:470–480. [PubMed: 22001681]
30. Dixon WJ. Efficient analysis of experimental observations. Annu.Rev.Pharmacol.Toxicol 1980 1980;20:441–462. [PubMed: 7387124]
31. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma. 2 1995;12(1):1–21. [PubMed: 7783230]
32. van Gorp S, Deumens R, Leerink M, Nguyen S, Joosten EA, Marsala M. Translation of the rat thoracic contusion model; part 1-supraspinally versus spinally mediated pain-like responses and spasticity. Spinal Cord. 7 2014;52(7):524–528. [PubMed: 24819511]
33. Yang F, Xu Q, Shu B, et al. Activation of cannabinoid CB1 receptor contributes to suppression of spinal nociceptive transmission and inhibition of mechanical hypersensitivity by Abeta-fiber stimulation. Pain. 11 2016;157(11):2582–2593. [PubMed: 27589093]
34. Guan Y, Borzan J, Meyer RA, Raja SN. Windup in dorsal horn neurons is modulated by endogenous spinal mu-opioid mechanisms. J.Neurosci 4/19/2006 2006;26(16):4298–4307. [PubMed: 16624950]
35. Buchhaas U, Koulousakis A, Nittner K. Experience with spinal cord stimulation (SCS) in the management of chronic pain in a traumatic transverse lesion syndrome. Neurosurg Rev. 1989;12 Suppl 1:582–587. [PubMed: 2812434]
36. Meglio M, Cioni B, Prezioso A, Talamonti G. Spinal cord stimulation (SCS) in deafferentation pain. Pacing Clin Electrophysiol. 4 1989;12(4 Pt 2):709–712. [PubMed: 2470055]
37. Richardson RR, Meyer PR, Cerullo LJ. Neurostimulation in the modulation of intractable paraplegic and traumatic neuroma pains. Pain. 2 1980;8(1):75–84. [PubMed: 6966047]
38. Tasker RR, DeCarvalho GT, Dolan EJ. Intractable pain of spinal cord origin: clinical features and implications for surgery. J Neurosurg. 9 1992;77(3):373–378. [PubMed: 1506884]
39. Sdrulla AD, Guan Y, Raja SN. Spinal Cord Stimulation: Clinical Efficacy and Potential Mechanisms. Pain Pract. 11 2018;18(8):1048–1067. [PubMed: 29526043]
40. Ahmed S, Yearwood T, De Ridder D, Vanneste S. Burst and high frequency stimulation: underlying mechanism of action. Expert Rev Med Devices. 1 2018;15(1):61–70. [PubMed: 29249191]

41. Tator CH, Minassian K, Mushahwar VK. Spinal cord stimulation: therapeutic benefits and movement generation after spinal cord injury. Handb Clin Neurol. 2012;109:283–296. [PubMed: 23098720]

42. Li G, Fan ZK, Gu GF, et al. Epidural Spinal Cord Stimulation Promotes Motor Functional Recovery by Enhancing Oligodendrocyte Survival and Differentiation and by Protecting Myelin after Spinal Cord Injury in Rats. Neurosci Bull. 4 2020;36(4):372–384. [PubMed: 31732865]

43. Hao JX, Kupers RC, Xu XJ. Response characteristics of spinal cord dorsal horn neurons in chronic allodynic rats after spinal cord injury. J Neurophysiol. 9 2004;92(3):1391–1399. [PubMed: 15331646]

44. Yakhnitsa V, Linderoth B, Meyerson BA. Spinal cord stimulation attenuates dorsal horn neuronal hyperexcitability in a rat model of mononeuropathy. Pain. 2 1999;79(2–3):223–233. [PubMed: 10068168]

45. Li S, Farber JP, Linderoth B, Chen J, Foreman RD. Spinal Cord Stimulation With “Conventional Clinical” and Higher Frequencies on Activity and Responses of Spinal Neurons to Noxious Stimuli: An Animal Study. Neuromodulation. 7 2018;21(5):440–447. [PubMed: 29164752]

46. Wang J, Kawamata M, Namiki A. Changes in properties of spinal dorsal horn neurons and their sensitivity to morphine after spinal cord injury in the rat. Anesthesiology. 1 2005;102(1):152–164. [PubMed: 15618799]

47. Zhang H, Xie W, Xie Y. Spinal cord injury triggers sensitization of wide dynamic range dorsal horn neurons in segments rostral to the injury. Brain Res. 9 7 2005;1055(1–2):103–110. [PubMed: 16083864]

48. Lee-Kubli CA, Ingves M, Henry KW, et al. Analysis of the behavioral, cellular and molecular characteristics of pain in severe rodent spinal cord injury. Exp Neurol. 4 2016;278:91–104. [PubMed: 26808661]

49. Meisner JG, Marsh AD, Marsh DR. Loss of GABAergic interneurons in laminae I-III of the spinal cord dorsal horn contributes to reduced GABAergic tone and neuropathic pain after spinal cord injury. J Neurotrauma. 4 2010;27(4):729–737. [PubMed: 20059302]

50. Lu Y, Perl ER. Modular organization of excitatory circuits between neurons of the spinal superficial dorsal horn (laminae I and II). J.Neurosci 4/13/2005 2005;25(15):3900–3907. [PubMed: 15829642]

51. Cioni B, Meglio M, Pentimalli L, Visocchi M. Spinal cord stimulation in the treatment of paraplegic pain. J Neurosurg. 1 1995;82(1):35–39. [PubMed: 7815131]

52. Yang F, Duan W, Huang Q, et al. Modulation of Spinal Nociceptive Transmission by Sub-Sensory Threshold Spinal Cord Stimulation in Rats After Nerve Injury. Neuromodulation, 1 2020;23(1):36–45. [PubMed: 31162783]

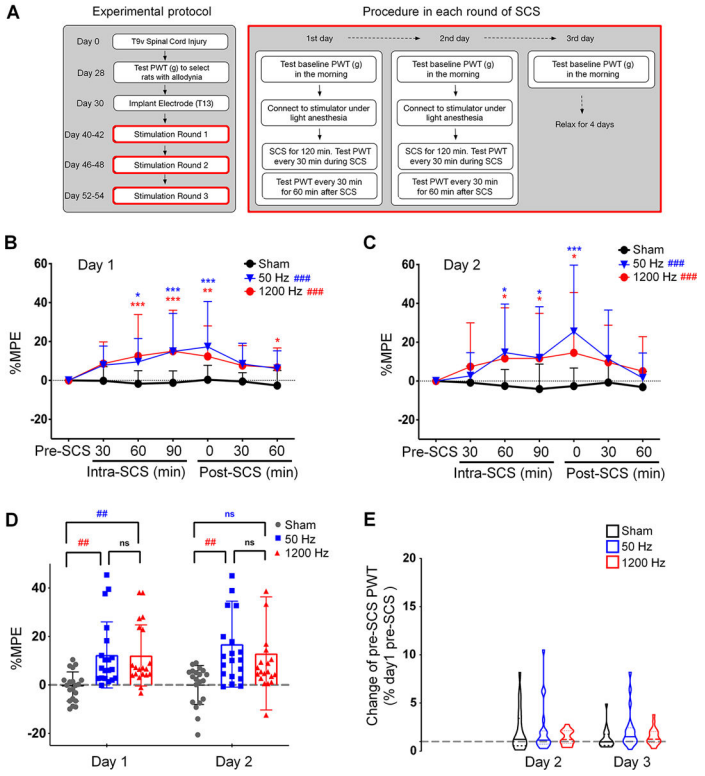

Figure 1. SCS attenuates mechanical hypersensitivity in the hind paws of rats with SCI.

(A) Experimental protocol of SCS in SCI rats. In each round, conventional SCS (50 Hz, 80% MoT), high-frequency SCS (1200 Hz, 40% MoT), or sham stimulation was applied on two consecutive days (120 min/day). Paw withdraw threshold (PWT) was measured before, during, and after stimulation on each day. (**B**, **C**) Changes in the percentage of maximum possible effect (%MPE) of mechanical hypersensitivity inhibition during (intra-SCS) and after SCS in each group (n=10 rats, 2 sides/rat) on day 1 **(B)** and day 2 **(C)**. %MPE = [1 – (Cutoff PWT – Post-SCS PWT)/(Cutoff PWT – Pre-SCS PWT)] × 100. Two-way mixed model ANOVA. Data are expressed as median + interquartile range. *p<0.05, **p<0.01, ***p<0.001 versus pre-SCS. #p<0.05, ##p<0.01, ##p<0.001 versus sham stimulation. **(D)** The average %MPE at 30, 60, and 90 min intra-SCS and 0 min post-SCS in each group on days 1 and 2. One-way ANOVA. Data are expressed as mean ± SD. ##p<0.01 versus indicated group; ns, not significant. **(E)** PWT before SCS on days 2 and 3 did not differ significantly from that on day 1 in any group.

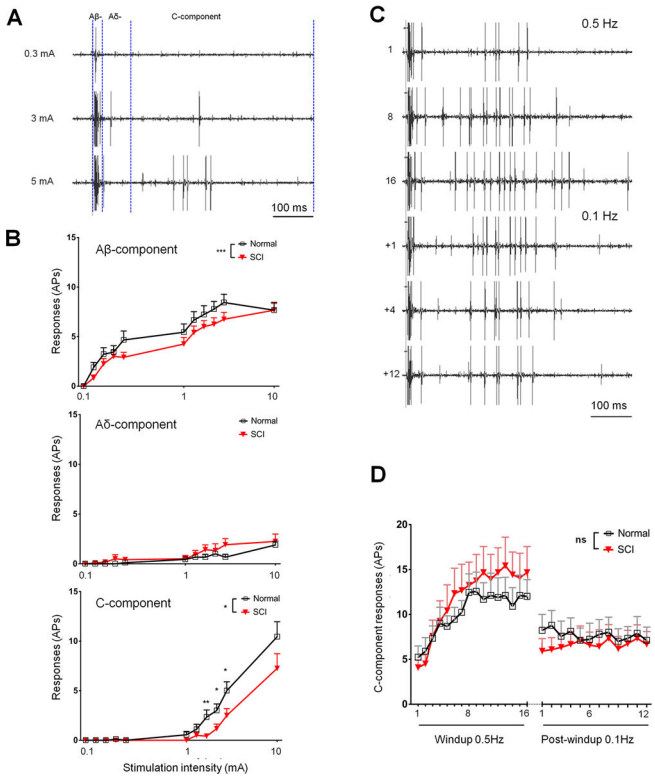

Figure 2. Changes in WDR neuronal response to electrical test stimulation in rats after SCI.

(A) Analog recordings of WDR neuronal responses to intracutaneous electrical test stimuli (0.3, 3, and 5 mA, 2.0 msec) in a naïve rat. WDR neuronal responses can be divided into Aβ- (0–25 msec), Aδ- (25–100 msec), and C-components (100–500 msec) based on the activation threshold and response latency. **(B)** The stimulus-response (S-R) curves of action potentials (APs) in each component to graded intracutaneous electrical test stimuli (0.1–10 mA, 2 msec) in SCI (n=12) and naïve rats (n=9). **(C)** Analog recordings in a naïve rat of WDR neuronal responses to the first, eighth, and sixteenth stimulus of a train of intracutaneous electrical test stimuli (0.5 Hz, 16 pulses, 2.0 msec) that induces windup, as well as to the first, fourth, and twelfth stimulus of a train of 0.1 Hz electrical stimuli (12 pulses) at the post-windup phase. **(D)** The APs of C-component to windup and post-windup stimulation were plotted against the stimulation number. B, D: Two-way mixed model ANOVA. Data are expressed as mean + SEM. *p<0.05, **p<0.01, ***p<0.001 versus indicated group; ns, not significant.

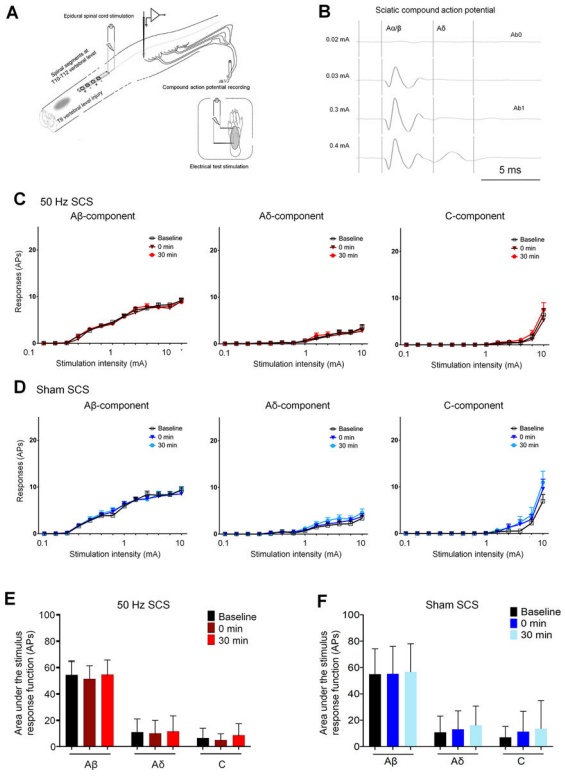

Figure 3. SCS does not inhibit WDR neuronal responses to graded electrical test stimulation in rats with SCI.

(A) Schematic diagram illustrating experimental setup for recording WDR neurons in SCI rats. **(B)** The antidromic compound action potentials (APs) evoked by bipolar electrical stimulation (0.01–3.0 mA, 0.2 msec) at the dorsal spinal cord were recorded at the sciatic nerve with a monopolar recording electrode. (**C**, **D**) The stimulus-response (S-R) curves of APs in the Aβ- (0–25 msec), Aδ- (25–100 msec), and C-components (100–500 msec) of the WDR neuronal response to graded intracutaneous electrical stimuli (0.1–10 mA, 2 msec) were not significantly changed from baseline at 0 min and 30 min after conventional SCS (50 Hz, Ab1, 0.2 msec, 10 min, N=19) **(C)** or after sham SCS (n=19) **(D)**. (**E**, **F**) The total number of APs in the Aβ-, Aδ-, and C-components of the WDR neuronal response to graded intracutaneous electrical stimuli (0.1–10 mA, 2 msec) was not significantly changed from baseline at 0 min and 30 min after conventional SCS (50 Hz, Ab1, 0.2 msec, 10 min) **(E)** or after sham SCS **(F)**. Two-way repeated measures ANOVA. Data are expressed as mean + SEM.

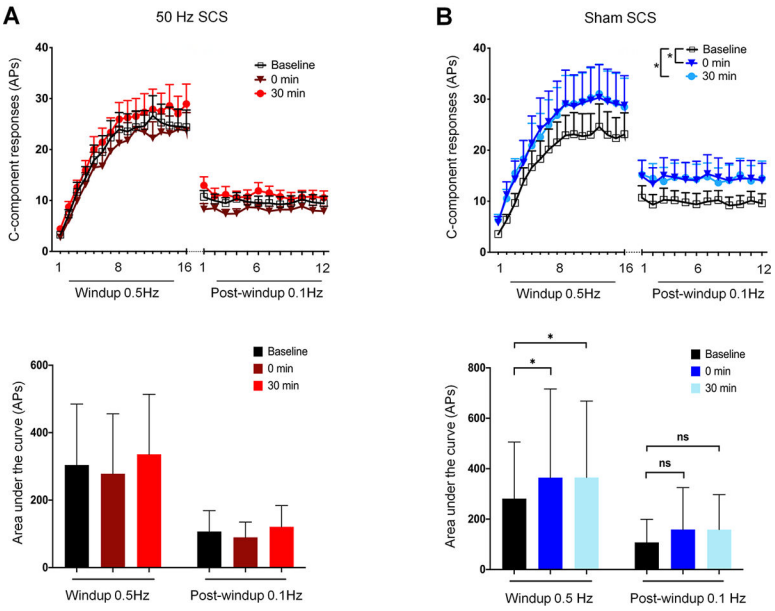

Figure 4. SCS does not inhibit windup of WDR neuronal responses to repeated electrical test stimulation in SCI rats.

(A) Upper: The C-component response of WDR neurons (N=19) to 0.5 Hz windup stimulation (16 pulses, 0.2 msec) and the following 0.1 Hz post-windup control stimulation (12 pulses, 0.2 msec) were plotted against the stimulation number before (baseline) and after 50 Hz SCS (Ab1, 0.2 msec,10 min) in SCI rats. Lower: The total number of APs (area under the windup curve) of C-components to 0.5 Hz and 0.1 Hz stimulation before (baseline) and after SCS. (**B**) Upper: The windup curves in WDR neurons (n=19) before and after sham SCS in SCI rats. Lower: The total number of APs of C-components to 0.5 Hz and 0.1 Hz stimulation before and after sham SCS. Windup curve: Two-way repeated measures ANOVA. Area under the curve: One-way repeated measures ANOVA. Data are expressed as mean + SEM. *p<0.05 versus indicated group; ns, not significant.

Table 1.

Percent of Maximal Possible Effect of Spinal Cord Stimulation on Mechanical Hypersensitivity in Rats With Spinal Cord Injury

	50 Hz			1200 Hz			Sham		
	mean	**95% CI**	**5%CI**	**mean**	**95% CI**	**5%CI**	**mean**	**95% CI**	**5%CI**
Day 1									
Intra-30 min	7.83	12.44	3.22	8.65	13.87	3.44	−0.16	3.22	−3.55
Intra-60 min	9.55	15.18	3.91	12.59	22.53	2.64	−1.68	1.46	−4.81
Intra-90 min	14.9	24.06	5.73	15.01	24.88	5.14	−1.17	1.69	−4.03
Post-0 min	17.28	28.18	6.39	12.38	19.7	5.06	0.36	3.84	−3.13
Post-30 min	8.48	13.44	3.52	7.66	12.47	2.85	−0.52	1.6	−2.65
Post-60 min	6.11	10.39	1.83	6.66	11.35	1.96	−2.58	1.04	−6.21
Day 2									
Intra-30 min	2.67	8.26	−2.91	7.4	17.98	−3.17	−0.84	3.14	−4.81
Intra-60 min	14.66	26.35	2.97	11.66	23.85	−0.54	−2.51	1.44	−6.46
Intra-90 min	11.92	24.25	−0.41	11.71	22.55	0.88	−4.13	1.89	−10.14
Post-0 min	25.48	41.51	9.46	14.54	29.08	0.01	−2.61	1.74	−6.96
Post-30 min	11.41	23.2	−0.39	9.68	18.6	0.77	−0.72	4.42	−5.87
Post-60 min	1.58	7.61	−4.44	5.04	13.38	−3.29	−3.15	0.62	−6.91

The duration of each treatment was 120 min. Intra- indicates a time point during spinal cord stimulation, and post- indicates a time point after cessation of stimulation.