Conventional and Kilohertz-frequency Spinal Cord Stimulation Produces Intensity- and Frequency-dependent Inhibition of Mechanical Hypersensitivity in a Rat Model of Neuropathic Pain

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ABSTRACT

Background: Spinal cord stimulation (SCS) is a useful neuromodulatory technique for treatment of certain neuropathic pain conditions. However, the optimal stimulation parameters remain unclear.

Methods: In rats after L5 spinal nerve ligation, the authors compared the inhibitory effects on mechanical hypersensitivity from bipolar SCS of different intensities (20, 40, and 80% motor threshold) and frequencies (50, 1 kHz, and 10 kHz). The authors then compared the effects of 1 and 50 Hz dorsal column stimulation at high- and low-stimulus intensities on conduction properties of afferent Aα/β-fibers and spinal wide-dynamic–range neuronal excitability.

Results: Three consecutive daily SCS at different frequencies progressively inhibited mechanical hypersensitivity in an intensity-dependent manner. At 80% motor threshold, the ipsilateral paw withdrawal threshold (% preinjury) increased significantly from pre-SCS measures, beginning with the first day of SCS at the frequencies of 1 kHz (50.2 ± 5.7% from 23.9 ± 2.6%, n = 19, mean ± SEM) and 10 kHz (50.8 ± 4.4% from 27.9 ± 2.3%, n = 17), whereas it

What We Already Know about This Topic

- The optimal stimulus parameters and mechanisms by which spinal cord stimulation produces analgesia are unclear
- Whether high frequency (1–10 kHz) stimulation produces analgesia using different mechanisms than conventional frequency (50 Hz) is unknown

What This Article Tells Us That Is New

- In rats, both conventional and high-frequency stimulation reduced mechanical hypersensitivity after nerve injury
- High frequency compared with conventional stimulation had an earlier onset of effect and required a lower intensity to block peripheral Aβ fibers, but failed to significantly inhibit windup in spinal wide-dynamic–range neurons

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was significantly increased beginning on the second day in the 50 Hz group (38.9 ± 4.6% from 23.8 ± 2.1%, n = 17). At high intensity, both 1 and 50 Hz dorsal column stimulation reduced Aα/β-compound action potential size recorded at the sciatic nerve, but only 1 kHz stimulation was partially effective at the lower intensity. The number of actions potentials in C-fiber component of wide-dynamic–range neuronal response to windup-inducing stimulation was significantly decreased after 50 Hz (147.4 ± 23.6 from 228.1 ± 39.0, n = 13), but not 1 kHz (n = 15), dorsal column stimulation. **Conclusions:** Kilohertz SCS attenuated mechanical hypersensitivity in a time course and amplitude that differed from conventional 50 Hz SCS, and may involve different peripheral and spinal segmental mechanisms.

THE treatment of neuropathic pain remains challenging, and spinal cord stimulation (SCS) represents an adjustable and nondestructive procedure that attenuates pain by delivering small therapeutic doses of electrical current to spinal structures, primarily in the dorsal column.[1–3] The effectiveness of SCS depends on the stimulation frequency and intensity. The most common frequency range used clinically for SCS and tested in animal studies is 50–60 Hz,[2,4,5] referred to as conventional SCS. However, no consensus exists regarding whether this is the optimum frequency for SCS analgesia. Stimulation intensity is another important parameter, and SCS is often applied at the amplitude that elicits paresthesia over the painful area in patients and is titrated to the highest comfortable level. In animal studies, SCS is usually tested at an intensity slightly below the motor threshold (MoT), which is considered to be the tolerance threshold.[2,6] Although paresthesia may be largely from activation of afferent sensory fibers during SCS (*i.e.*, sensory threshold), muscle contraction at MoT can be caused by stimulation of dorsal column fibers that excite the motoneuronal pools or by spreading of the electric field to activate local nerve roots.[7] So far, the effects of changing stimulation frequency and intensity on SCS analgesia in neuropathic pain condition remain unclear.

It was shown that 500 Hz SCS induced greater peripheral vasodilation than did 50 Hz stimulation.[8] Transcutaneous electrical nerve stimulation at 100 Hz also produced greater pain inhibition than lower frequencies did.[9,10] Other than its use in reducing torticollis spasmodicus,[11] kilohertz-level SCS has not been tested systematically for pain inhibition, and hence its analgesic efficacy remains unknown. The gate-control theory represents the fundamental biological basis for conventional SCS-induced analgesia, and postulates that activity in large A-fibers attenuates spinal pain transmission by activating inhibitory dorsal horn interneurons.[12,13] Accordingly, we hypothesize that SCS at different frequencies may induce greater pain inhibition at the higher intensities due to activation of more A-fibers. In addition, because kilohertz-level SCS delivers many more electrical pulses than does 50 Hz SCS of the same stimulus intensity and treatment duration, it may also induce a stronger pain inhibition than 50 Hz SCS. Yet, it is also possible that different frequencies of SCS may have distinct mechanisms of action, as with transcutaneous electrical nerve stimulation and electroacupuncture.[14,15] Thus, we hypothesized that kilohertz-level SCS and conventional 50 Hz SCS may differently activate the gate-control mechanism and affect peripheral afferent conduction properties. Testing such a hypothesis in a human study would require a large-scale, well-controlled clinical trial because of the heterogeneity of genetics and pain etiology in the general population. Here, we examined the intensity-dependent pain inhibition of bipolar SCS of various frequencies (50, 1 kHz, and 10 kHz) in rats after an L5 spinal nerve ligation (SNL). We further compared the effects of conditioning stimulation of the dorsal column, the primary structure targeted by SCS, at 50 Hz and 1 kHz on the conduction property of afferent Aα/β-fibers, and on inhibition of dorsal horn wide-dynamic–range (WDR) neuronal responses in SNL rats.

Materials and Methods

All procedures were approved by the Johns Hopkins University Animal Care and Use Committee (Baltimore, Maryland) as consistent with the National Institutes of Health Guide for the Use of Experimental Animals. All animals were euthanized (sodium pentobarbital, 100–300 mg, intraperitoneal injection) at the end of the experiment. To minimize experimenter bias, the investigator who performed the behavioral tests was blinded to the treatment conditions.

L5 SNL

The animals were anesthetized with isoflurane (2%; Abbott Laboratories, North Chicago, IL), the left L5 spinal nerve of male Sprague–Dawley rats (350–450 g; Harlan Laboratories, Inc., Indianapolis, IN) was ligated with a 6-0 silk suture, and cut distally.[16]

Epidural SCS Lead Implantation

On day 5 post-SNL, a sterile rat SCS electrode (Medtronic Inc., Minneapolis, MN) was implanted as described in previous studies.[17,18] After a small laminectomy at the level of T13, the electrode was inserted epidurally in the rostral direction. The position of the electrode was adjusted so that the contacts were at the T10–12 spinal levels (see Supplemental Digital Content 1, http://links.lww.com/ALN/A948, which includes the figure for this experiment). A subcutaneous tunnel was used to position the proximal end of the electrode in the upper thoracic region, where it exited the skin and connected to an external stimulator (model 2100; A-M Systems, Sequim, WA).

Animal Behavioral Tests

Hypersensitivity to punctuate mechanical stimulation was determined with the up–down method by using a series of von Frey filaments (0.38, 0.57, 1.23, 1.83, 3.66, 5.93, 9.13,

and 13.1 g) as described previously.[19] The von Frey filaments were applied for 4–6 s 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 method and formula provided by Dixon.[20]

Recording of Sciatic Compound Action Potentials Evoked by Dorsal Column and Dorsal Root Stimulation

Titrating Dorsal Column Stimulation. Two tungsten needle electrodes (insulated except for the most distal 0.3–0.5 mm) were inserted into the (left) dorsal column at T13–L1 level.

Testing Changes of Peripheral Conduction Property. The test stimulation was applied through a pair of platinum hook electrodes to the (left) L4 dorsal root. A monopolar silver hook electrode was placed on the ipsilateral sciatic nerve for recording the antidromic compound action potentials (APs). The reference electrode was placed in the nearby muscle.

Spinal Dorsal Horn Recordings

Extracellular recordings of single dorsal horn neuron activity were obtained with microelectrodes as described previously.[21,22] Briefly, analog data were collected with a real-time computer-based data acquisition and processing system (DAPSYS 6; Brian Turnquist, the Johns Hopkins University, Baltimore, MD). WDR neurons located at deep laminae (III–V) in the ipsilateral L4 segment were identified by their characteristic responses.[21,23] Only WDR neurons with defined receptive fields in the plantar region of the hind paw were studied.

Experimental Design

Study 1. Comparing the effects of SCS at different intensities (20, 40, and 80% MoT) and frequencies (50, 1 kHz, and 10 kHz) on behavioral mechanical hypersensitivity in SNL rats. MoT was determined by slowly increasing the amplitude of 4 Hz electrical stimulation from zero until muscle contraction was observed in mid-lower trunk or hind limbs. In parallel, sensory threshold (SenT, the intensity at which an animal showed brief stoppage of ongoing activity, awaking, alerting, turning attention toward stimulation, or slightly changing body posture to accommodate the stimulation) was also monitored in a subgroup of animals (n = 52). Because SenT is nearly half of the MoT, and remained unchanged across 3 days of SCS (see Supplemental Digital Content 1, http://links.lww.com/ALN/A948, which includes the figure for this experiment), we chose 20% MoT (below SenT intensity), 40% MoT (half-maximum intensity and near SenT), and 80% MoT (maximum intensity without discomfort in awake animals) to examine the intensity–response relationship for SCS. To mimic the clinical actions of SCS,[24–26] we used a four-contact electrode (Medtronic Inc.) to provide bipolar SCS.[5] We set the first and third contacts (rostral to caudal) of the four-contact lead as an anode and the second and fourth as a cathode ("twin-pairs" stimulation). The pulse width of 0.024 ms (biphasic, constant current mode) was used in all studies to allow comparison of data among groups.

SCS may produce a cumulative pain-inhibitory effect after repetitive treatments.[5,18] Therefore, we used 3-day treatment protocol to examine the overall effect of SCS (see Supplemental Digital Content 1, http://links.lww.com/ALN/A948, which includes the illustration of this experiment). On each of consecutive days 12, 13, and 14 post-SNL (week-1 test), animals received one 30-min SCS after pre-SCS PWT measurement. A small group of animals received sham stimulation (0 mA). PWTs were measured again at 15 min (during SCS), 30 min (0 min post-SCS), and 60 min (30 min post-SCS) after the initiation of SCS. Because PWT returned to pre-SCS level at 5 days after the last SCS, on days 19, 20, and 21 post-SNL (week-2 test), animals received SCS at same intensity again but at a different frequency. To limit potential order effect, we used a cross-over design for switching SCS frequencies in different groups between the 2 weeks. The data from the 2 weeks were combined for analysis. Three SCS intensities were tested in different sets of animals. Our pilot experiment showed that if kilohertz-level SCS was applied directly at 80% MoT, rats often exhibited signs of discomfort (*e.g.*, frequent vocalizing, escaping, sudden and rigorous adjusting of body posture) during the first few minutes, which were different from signs of SenT. This "onset response" was largely prevented by gradually increasing the stimulus amplitude from a lower value (*e.g.*, 0–10% MoT) to the set value more than 2–3 min. To compare data among groups, we applied this procedure to all groups.

Study 2. Examining the effects of 50 and 1 kHz dorsal column conditioning stimulation on the conduction properties of large afferent fibers in SNL rats. Repetitive electrical stimulation of nerves may change their conduction properties. Because 1 kHz SCS induced the most different changes from 50 Hz SCS in behavior study, it was chosen for comparison with 50 Hz stimulation. The intensity for the conditioning stimulation was calibrated by recording sciatic compound APs to graded dorsal column stimulation[22]: The intensity that resulted in the first detectable Aα/β-waveform (Ab0), followed by the peak Aα/β-waveform without inducing an Aδ-waveform (Ab1) was determined. Different compound AP waveforms were distinguished on the basis of the activation threshold and the conduction velocity. The compound APs to the test stimulation at the ipsilateral L4 dorsal root (0.1–2.2 mA, 0.2 ms) were recorded before, at 0–5 min, and 10–15 min after dorsal column conditioning stimulation (5 min, 0.024 ms, Ab0, Ab1).

Study 3. Comparing the inhibition of WDR neuronal activity by 50 and 1 kHz dorsal column conditioning stimulation in SNL rats. Inhibition of WDR neuronal activity may contribute to SCS analgesia.[22,27] The WDR neuronal response to a suprathreshold electrical stimulus consists of an early A-fiber component (0–75 ms) and a later C-fiber component (75–500 ms).[28] WDR neurons also display AP windup phenomenon, a short-form neuronal sensitization, to repetitive

C-fiber inputs.[21,22,28] The effects of 50 and 1 kHz dorsal column stimulation (5 min, 0.024 ms, Ab1) on stimulus–response (S–R) functions of C-fiber components to graded electrical stimuli (0.1–10 mA, 2.0 ms, 15-s interval, delivered to the receptive field), and on windup response to a train of 16 electrical pulses (0.5-Hz, supra–C-fiber threshold) were both examined at 0–15 and 30–45 min after the conditioning stimulation. In windup test, 12 pulses at 0.1 Hz (which does not induce windup) were delivered at 30 s after 0.5 Hz stimulation as a negative control.

Data Analysis

In Study 1, the primary analysis was to examine the overall impact of each SCS on mechanical hypersensitivity by comparing the "mean PWTs" among groups, which are calculated by averaging together the PWTs measured before SCS and at 15, 30, and 60 min after the initiation of SCS. PWTs were presented as percentage of pre-SNL baseline. Data from sham-stimulation group in different studies were combined for analysis. We also conducted exploratory analysis to separate responders and nonresponders to SCS. We first calculated the "mean post-SCS PWT of three SCS" by averaging the PWTs across the three SCS treatments. Then the percent change of post-SCS PWT was calculated as follows: percent change of post-SCS PWT = ([mean post-SCS PWT of three SCS) – (pre-SCS PWT of the first SCS)]/(pre-SCS PWT of the first SCS) × 100. We defined rats with more than 100% change in post-SCS PWT, which is more than 2 SD above the mean of sham-stimulation group (14.8 ± 35.5%; mean ± SD; n = 14), as responders. Those rats with a value less than 50% were defined as nonresponders. We plotted percent change of post-SCS PWT of each animal for different frequency groups, shown as the "Individual %Reversal." A one-way ANOVA was used to compare data among groups. The "mean PWT" were also compared with the pre-SCS value in each group by using one-way, repeated-measures ANOVA.

In Study 2, the areas under the Aα/β-waveforms of sciatic compound AP to the graded dorsal root stimulation were measured to establish the S–R functions. For each group, the S–R functions were compared between the pre- and postdorsal column stimulation conditions with a two-way, repeated-measures ANOVA.

In Study 3, the numbers of APs in the C-component evoked by graded intracutaneous stimulation and by each stimulus in the train for inducing windup were used to plot S–R function and windup functions, respectively. For each frequency group, the total C-components under S–R function and that under 0.5 Hz windup function were compared between the pre- and postdorsal column stimulation conditions with a one-way, repeated-measures ANOVA.

STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK) was used to conduct all statistical analyses. The Tukey honestly significant difference *post hoc* test was used to compare specific data points. Two-tailed tests were performed, and data are expressed as mean ± SEM; *P* value less than 0.05 was considered significant in all tests.

Results

In the behavioral study, 88 of the 110 rats that received SNL completed the experiment and were analyzed. Twenty-two animals were not used for the following reasons: nine rats (8.2%) did not develop mechanical hypersensitivity (>50% reduction of PWT from preinjury baseline) at day 5 post-SNL. Of all SNL rats that were implanted with an SCS lead (n = 101), eight rats (7.9%) showed impaired motor function, diminished mechanical hypersensitivity, or damage to the implanted lead before the week-1 test. These rats were eliminated from subsequent studies. Among the remaining animals (n = 93), five rats failed to complete week-1 test (data were excluded) and were not testable in week-2, due to later damages to the lead, undetectable MoT, or deteriorating health conditions; nine rats completed only the week-1 test (data were analyzed).

Time Course of Reversal of Neuropathic Mechanical Hypersensitivity by Different Frequencies of SCS

When SCS was applied at low amplitude 20% MoT, the overall inhibitory effect was marginal. Before the first SCS, the ipsilateral PWTs in SNL rats of each group were near 20% of pre-SNL baseline (19.5–23.8%), indicating the development of mechanical hypersensitivity (fig. 1A). The averaged PWTs on each treatment day associated with 50 Hz (n = 16; first: 25.3 ± 2.1%; second: 27.6 ± 2.4%; third: 30.4 ± 4.3%), 1 kHz (n = 16; first: 30.1 ± 3.5%; second: 36.4 ± 7.6%; third: 34.9 ± 6.6%), and 10 kHz (n = 16; first: 30.0 ± 2.2%; second: 35.1 ± 4.5%; third: 30.8 ± 4.2%) stimulation were not statistically significantly different from that of the sham-stimulation group (n = 14; first: 23.4 ± 3.0%; second: 24.9 ± 2.3%; third: 26.4 ± 2.0%), nor from the pre-SCS level (fig. 1B). Sham stimulation did not change PWT (n = 14; 23.8 ± 2.4%) from that in the pre-SCS level.

When SCS was applied at medium amplitude 40% MoT, the mean PWT was significantly increased from the pre-SCS level on the first (33.2 ± 4.7%; $P < 0.05$), second (37.9 ± 5.2%; $P < 0.01$), and third (45.3 ± 5.4%; $P < 0.001$) treatment days in the 1 kHz group (pre-SCS: 20.7 ± 1.7%; n = 19), but only on the second (36.3 ± 4.7%; $P < 0.05$) and third (43.9 ± 4.7%; $P < 0.001$) days in the 10 kHz group (pre-SCS: 23.8 ± 2.6%; n = 16), and only on the third (39.0 ± 4.5%; $P < 0.01$) day in the 50 Hz group (pre-SCS: 23.1 ± 2.9%; n = 17; fig. 1B). The averaged mean PWT across the 3 treatment days was increased from the pre-SCS level in all SCS groups, but was statistically significantly higher than that of sham stimulation (24.9 ± 2.0%) only in the 1 kHz (38.8 ± 4.6%; $P < 0.05$) and 10 kHz (36.5 ± 3.4%; $P < 0.05$) groups (fig. 1B). In each frequency group, the trend was for SCS-induced inhibition to increase gradually from the first to the third treatment; the mean PWTs did not become statistically significantly higher than that of the sham-stimulation group (26.4 ± 2.0%) until the third treatment day in 1 kHz (45.3 ± 5.4%) and 10 kHz (43.9 ± 4.7%) groups. In animals that showed increased ipsilateral PWT to

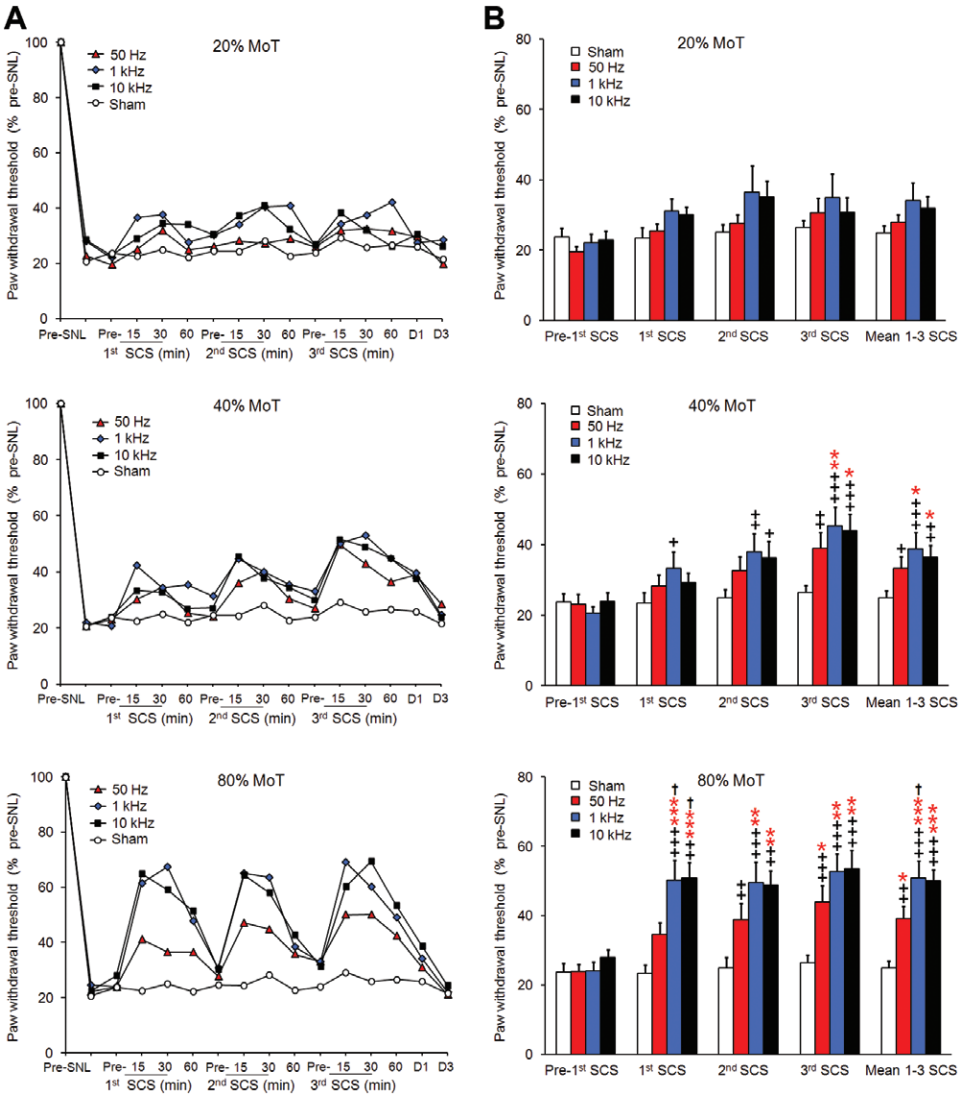

Fig. 1. Effects on paw withdrawal threshold of different frequencies of spinal cord stimulation (SCS) applied at 20–80% motor threshold in nerve-injured rats. (*A*) The paw withdrawal thresholds, shown as percent of prespinal nerve ligation (SNL) baseline, before and at different time points after SCS at one of three frequencies (50, 1 kHz, and 10 kHz) delivered at 20, 40, and 80% motor threshold (MoT, 0.024 ms, constant current, 30 min/session, 1 session/day). Data from animals that received sham stimulation in different studies were combined for analysis. Error bars are omitted to improve clarity. (*B*) On each treatment day, the overall effect of SCS on PWT was compared among groups by averaging together the PWTs at pre-SCS and 15, 30, and 60 min after the initiation of SCS applied at 20% MoT, 40% MoT, and 80% MoT. Data are expressed as mean + SEM in (*B*). +*P* < 0.05, ++*P* < 0.01, +++*P* < 0.001 *versus* pre-SCS PWT; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 *versus* sham stimulation; †*P* < 0.05 *versus* 50 Hz.

SCS, the peak effect usually appeared at 15 min after initiation of SCS and remained for a short period after cessation of SCS (*i.e.,* 30 min after initiation), but largely diminished by 30 min post-SCS (fig. 1A).

When SCS was applied at high amplitude 80% MoT, the ipsilateral PWT increased from the pre-SCS level at all three SCS frequencies (fig. 1A). Compared with pre-SCS level (50 Hz: 23.8 ± 2.1%; 1 kHz: 23.9 ± 2.6%; 10 kHz: 27.9 ± 2.3%), the mean PWT was statistically significantly increased beginning on the first day of SCS in both kilohertz-level groups (1 kHz: 50.2 ± 5.7%, n = 19; 10 kHz: 50.8 ± 4.4%, n = 17; fig. 1B), whereas it was significantly increased beginning on the second day in the 50 Hz group (38.9 ± 4.6%; n = 17). In all SCS groups, the averaged mean PWT across the 3 treatment days (50 Hz: 39.1 ± 3.6%; 1 kHz: 50.8 ± 4.9%; 10 kHz: 49.9 ± 3.3%) was statistically significantly increased from the pre-SCS level and was also significantly higher than that of sham-stimulation group (24.9 ± 2.0%; fig. 1B). It is important to note that the mean PWTs in the 1 kHz (50.2 ± 5.7%) and 10 kHz (50.8 0 ± 4.4%) groups were both higher than that of the 50 Hz group (34.5 ± 3.5%) on the first SCS day. The averaged mean PWT across the three treatment days was also statistically significantly higher in the 1 kHz group than in the 50 Hz group. The pain-inhibitory action increased

progressively in the 50 Hz group, as the mean PWT did not become statistically significantly higher than that of pre-SCS and sham-stimulation groups until the second and third days of treatment, respectively. However, the peak effect was reached and maintained from the first day of treatment in the 1 and 10 kHz groups (fig. 1B). SCS at all intensities did not affect PWT of the contralateral hind paw (data not shown).

SCS Attenuated Mechanical Hypersensitivity in Both Frequency- and Amplitude-dependent Manner

The response to SCS analgesia varied among individual animals. In SNL rats, we defined rats with a more than 100% change in post-SCS PWT as responders and those with a value less than 50% as nonresponders. When SCS was applied at low amplitude (20% MoT), more rats responded to 1 kHz (4 of 16, 25%) and to 10 kHz (4 of 16, 25%) than to 50 Hz SCS (1 of 16, 6%; see Supplemental Digital Content 2, http://links.lww.com/ALN/A949, which includes all figures of this experiment). Regardless, the "Group %Reversal" values (*i.e.*, the averaged Individual %Reversal of each group) were not statistically significantly different among different frequency groups (50 Hz: 51.8 ± 13.2; 1 kHz: 75.8 ± 27.6; 10 kHz: 61.3 ± 19.2). However, the Group %Reversal value was significantly higher in the 1 kHz SCS group than in the sham-stimulation group (14.7 ± 9.5), indicating a pain inhibitory effect ($P = 0.032$). When SCS was applied at medium amplitude (40% MoT), 3 of 17 rats (18%) in the 50 Hz group, 6 of 19 (32%) in the 1 kHz group, and 5 of 16 (31%) in the 10 kHz group were responders. The Group %Reversal value was significantly higher in the 1 kHz (89.3 ± 19.5; $P = 0.005$) and 10 kHz (70.1 ± 19.8; $P = 0.043$) groups, but not in the 50 Hz group (61.2 ± 17.9; $P = 0.082$), compared with that in the sham-stimulation group. At high amplitude (80% MoT), the number of responders in the 50, 1 kHz, and 10 kHz groups was 6 of 17 (35%), 9 of 19 (47%), and 6 of 17 (35%), respectively. The Group %Reversal value was statistically significantly higher in each SCS group (50 Hz: 73.5 ± 14.5; 1 kHz: 132.9 ± 24.8; 10 kHz: 105.8 ± 37.7) than in the sham-stimulation group, and it was also significantly higher in the 1 kHz group than in the 50 Hz group. The MoTs were not significantly different among different frequency groups. The averaged MoTs across 3 SCS days were also not statistically significantly different between responders and nonresponders to different frequencies of SCS at 80% MoT.

Dorsal Column Conditioning Stimulation Reduced Aα/β-compound APs Measured in the Peripheral Nerve

The Aα/β- and Aδ-waveforms of sciatic compound APs generated by the graded dorsal column stimulation and L4 dorsal root stimulation were distinguished on the basis of the activation threshold and the conduction velocity (fig. 2A). Both 50 Hz (n = 6) and 1 kHz (n = 6) dorsal column conditioning stimulation at Ab1 intensity decreased the size of Aα/β-waveforms evoked by the L4 dorsal root stimulation (fig. 2B), and significantly depressed the S–R functions at 0–5 min poststimulation (fig. 2, C and D). However, no inhibition occurred to 50 Hz stimulation at the lower stimulus intensity (Ab0, n = 6; fig. 2C). Although the S–R function did not significantly change at 0–5 min after 1 kHz stimulation at Ab0 ($P = 0.12$; n = 6), the size of Aα/β-waveforms to the lower intensity (0.2–0.6 mA) dorsal root test stimulation was significantly reduced (fig. 2D; $P < 0.05$).

Dorsal Column Conditioning Stimulation of 50 Hz, but Not 1 kHz, Inhibited Windup in WDR Neurons

The experimental setup for recording WDR neurons in the dorsal horn of SNL rats is illustrated in the schematic diagram (fig. 3A). Windup of the C-fiber component of the WDR neuronal response was induced by a train of 0.5 Hz electrical stimulation (16 pulses; fig. 3, B and C). The windup function was depressed and total C-fiber component to 0.5 Hz stimulation was statistically significantly decreased at 0–15 min (147.4 ± 2 3.6 APs) and 30–45 min (157.1 ± 18.2 APs) after 50 Hz dorsal column stimulation (fig. 3, C and D; n = 13), as compared with the prestimulation level (228.1 ± 39.0 APs). However, 1 kHz stimulation did not significantly inhibit windup from prestimulation level (233.4 ± 25.8 APs) at 0–15 min (199.3 ± 27.4 APs) and 30–45 min (215.8 ± 26.1 APs, n = 15; fig. 3, C and D). In the same experimental setting, there was a trend that 50 Hz stimulation also inhibited the total C-fiber components under the S–R function at 0–15 min (22.7 ± 9.2 APs, n = 13; fig. 3, E and F). Yet, the decrease was not statistically significantly different from the prestimulation level (34.6 ± 12.1 APs; $P = 0.06$). Due to the shorter pulse width (0.024 ms), the Ab1 intensity (1.85 ± 0.18 mA) was much higher than that to the stimulation of 0.2 ms pulse width (*e.g.*, near 0.2 mA).[22]

Discussion

Impact of Stimulation Frequency and Intensity

The pain-inhibitory action of kilohertz-level SCS has not been systematically examined in chronic pain conditions. We demonstrated for the first time that kilohertz-level SCS alleviated mechanical hypersensitivity in a preclinical model of neuropathic pain. In addition, the time course and magnitude of pain inhibition from 1 kHz SCS differed from that of conventional (50 Hz) SCS. First, at high amplitude (80% MoT), 1 kHz SCS induced greater inhibition than did 50 Hz SCS. Second, the peak pain inhibition was achieved by kilohertz-level SCS (80% MoT) with the first treatment, suggesting an early onset of its inhibitory action. Finally, only kilohertz-level SCS induced pain inhibition earlier at the medium amplitude (*e.g.*, 40% MoT), suggesting a different time course than conventional SCS. Yet, it remains to be determined whether SenT in rats is comparable with the paresthesia threshold in humans, and whether kilohertz-level SCS induces pain inhibition at an intensity that does not induce paresthesia in patients. As reported previously,

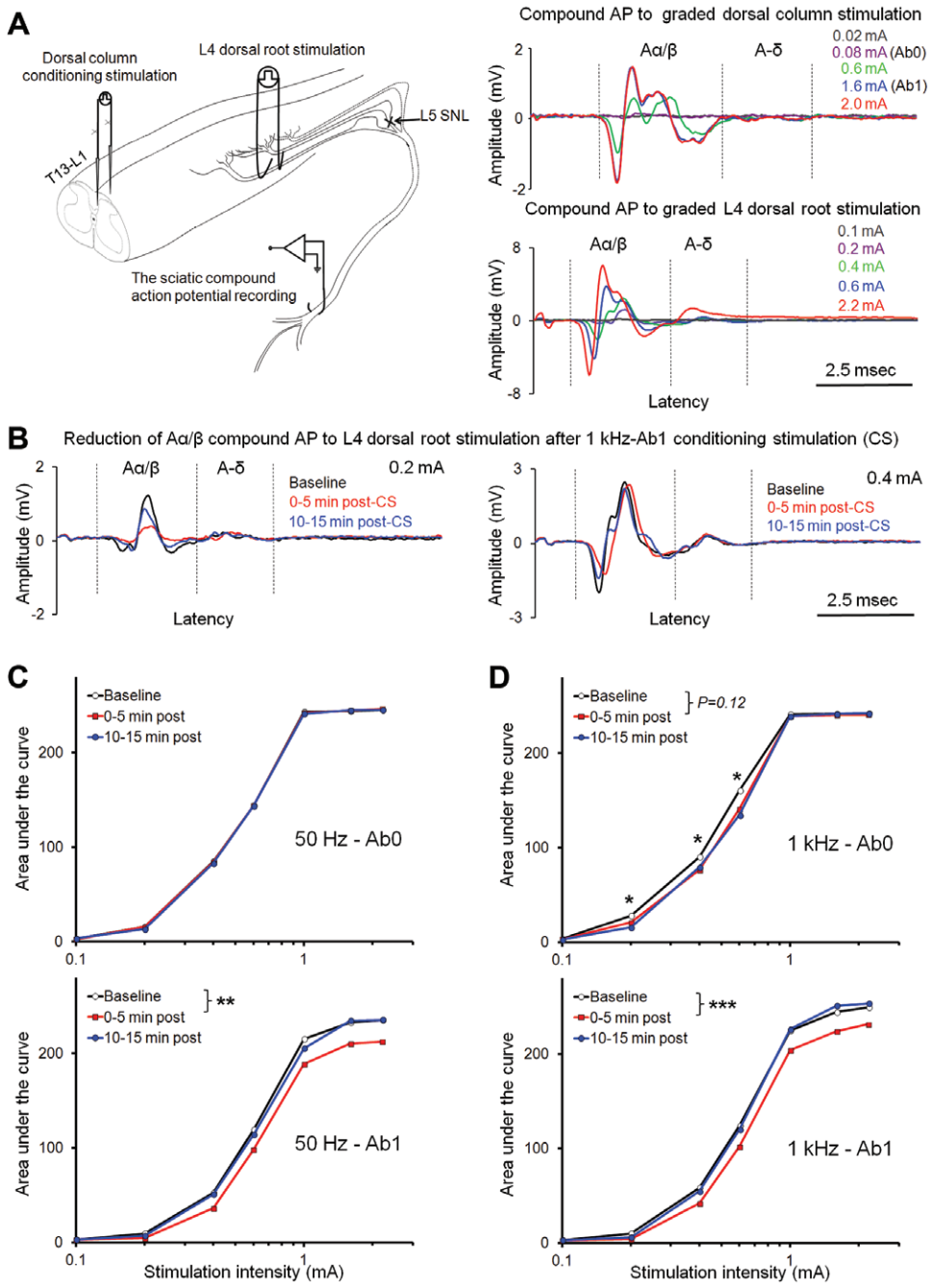

Fig 2. Dorsal column conditioning stimulation (CS) of 1 Hz and 50 Hz frequencies changed Aα/β-fiber conduction property in nerve-injured rats. (*A*) *Left*: schematic diagram illustrating the experimental setup for recording antidromic sciatic compound action potentials (APs) evoked by graded test electrical stimulation (0.1–2.2 mA, 0.2 ms) applied at the L4 dorsal root in rats that received an L5 spinal nerve ligation (SNL). The CS (5 min, 50 and 1 kHz, 0.024 ms, biphasic pulse) was delivered to the ipsilateral dorsal column at T13–L1 level. *Right*: examples of different compound AP waveforms corresponding to Aα/β- and Aδ-fiber activation to increasing intensities of dorsal column CS and dorsal root test stimulation. The intensity for CS was calibrated by recording sciatic compound AP to graded dorsal column stimulation (0.01–2.0 mA, 0.024 ms, biphasic pulse): The intensity that resulted in the first detectable Aα/β waveform (Ab0), followed by the peak Aα/β waveform (Ab1, the highest Aα/β waveform intensity without inducing an Aδ waveform), to dorsal column stimulation was determined. (*B*) Examples of sciatic compound APs evoked by 0.2 and 0.4 mA dorsal root test stimulation before and after dorsal column CS (1 kHz, Ab1, 5 min) were shown. (*C*) In the off-line analysis, the areas under the Aα/β waveforms generated by graded dorsal root stimulation were measured to establish the stimulus–response (S–R) functions. The S–R functions were not changed after 50 Hz CS of Ab0 intensity, but were significantly depressed at 0–5 min after 50 Hz CS at Ab1 intensity. (*D*) The size of Aα/β waveform to the lower intensities of dorsal root stimulation (0.2–0.6 mA) was significantly decreased from the prestimulation baseline at 0–5 min after 1 kHz dorsal column CS of Ab0 intensity. The S–R function was significantly depressed by 1 kHz CS of Ab1 intensity. Data are expressed as mean, and error bars are not shown to improve clarity. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 *versus* pre-CS baseline.

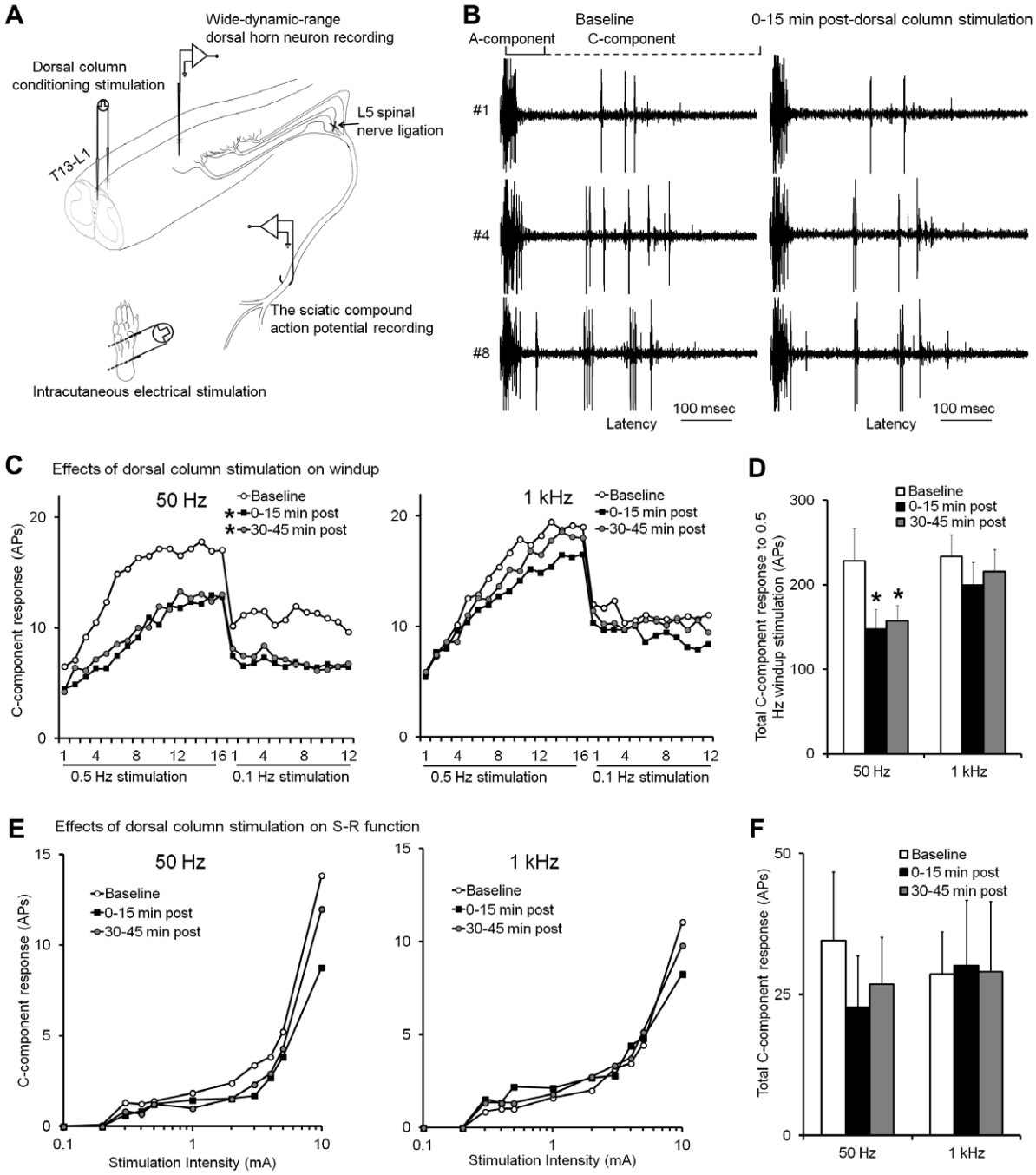

Fig 3. Dorsal column conditioning stimulation of 50 Hz, but not of 1 kHz, inhibited windup in wide-dynamic–range (WDR) neurons of nerve-injured rats. (*A*) Schematic diagram illustrating the experimental setup for recording WDR neuron in the L4 spinal segment of rats that received an L5 spinal nerve ligation. The conditioning stimulation (50 and 1 kHz, 0.024 ms, Ab1, 5 min) was delivered through two tungsten needle electrodes inserted in the ipsilateral dorsal column at T13–L1 level. (*B*) An analog recording of the WDR neuronal response to the first, fourth, and eighth stimulus of a train of intracutaneous electrical stimuli (0.5 Hz, 16 pulses, 2.0 ms, supra-C threshold) before and 0–15 min after 50 Hz dorsal column stimulation (Ab1, 0.024 ms, 5 min). (*C*) The number of action potentials (APs) in C-component of WDR neuronal response to 0.5 Hz stimulation and 0.1 Hz stimulation were plotted against the stimulation sequence number of each trial. For clarity, error bars are not shown. (*D*) At 0–15 min and 30–45 min after 50 Hz (n = 13), but not 1 kHz (n = 15), dorsal column stimulation, the total C-component of WDR neuronal response evoked by 0.5 Hz (16 pulses) windup-inducing stimulation delivered to the skin receptive field (*i.e.*, area under the windup function) was –significantly decreased from the prestimulation baseline. (*E*) The stimulus–response (S–R) functions of the C-fiber component of WDR neurons to graded intracutaneous electrical stimulation (0.1–10 mA, 2.0 ms) are shown before and after 50 Hz (n = 13) and 1 kHz (n = 15) dorsal column stimulation. (*F*) The area under the S-R function (*i.e.,* total numbers of APs in C-fiber component) was not significantly decreased from baseline after dorsal column stimulation. Data are presented as mean +SEM. **P* < 0.05 *versus* prestimulation baseline.

the pain inhibition from 50 Hz SCS (80% MoT) increased progressively from the first to the third day of treatment, indicating a cumulative effect.[18] Kilohertz-level SCS also did not lose efficacy on the second and third days of treatment, though it remains to be determined whether tolerance develops in response to kilohertz-level SCS with prolonged continuous stimulation. Our findings are in line with previous observations that electrical acupuncture induced stronger analgesia at 2–5 kHz than at lower frequencies,[29] and TENS at 100 Hz also reduced hyperalgesia better than did lower frequencies.[14,30] However, the kilohertz-level SCS did not appreciably increase the percentage of responders to SCS across 3 treatment days. Therefore, whether kilohertz SCS is likely to improve clinical outcomes merits further preclinical and clinical investigations, including testing different stimulation paradigms (*e.g.*, low-intensity, long-term SCS for hours/days) and other neuropathic pain manifestations (ongoing pain, heat hypersensitivity).

A correlation may exist between the intensity of SCS and the duration of pain relief.[2,31] SCS of both conventional frequency and kilohertz-level frequency induced intensity-dependent pain inhibition. Compared with sham stimulation, 50 Hz SCS significantly increased PWT only at the medium 40% MoT and high intensity of 80% MoT, supporting one clinical observation that conventional SCS at subthreshold intensity provides measurable but not clinically sufficient pain inhibition.[32] Subthreshold SCS also elicits less pain relief than does supraperception threshold SCS in patients with neuropathic pain.[32] It should be noted that even at the highest intensity, neither kilohertz-level or 50 Hz SCS achieved complete reversal of neuropathic mechanical hypersensitivity in most animals. That is, PWT did not return to pre-SNL levels, and there were nonresponders at all frequencies and intensities. Similarly, in clinical situations, SCS usually produces partial but satisfactory pain relief.[2,33] Thus, the efficacy of SCS analgesia can still be improved.

Mechanisms Underlying Pain Inhibition from SCS of Different Frequencies

Application of high-frequency alternating current waveforms to nerves may block conduction of APs.[34,35] The size of compound AP is proportional to the number of fibers activated by electrical stimulation. This is the first study to demonstrate that both 50 and 1 kHz dorsal column stimulation reduced Aα/β compound AP recorded at peripheral nerve. Conventionally, conduction blockade occurs primarily under the stimulating electrodes and diminishes quickly after stimulation stops. Yet, the stimulating site and the recording site for compound AP are both peripheral to the conditioning stimulation at dorsal column in the current study, and the decrease of Aα/β compound AP remained at 0–5 min poststimulation. Thus, the mechanisms underlying the decreased Aα/β compound AP after dorsal column stimulation remain to be examined. Nevertheless, our findings suggest that SCS may change certain afferent conduction properties, potentially lead to conduction failure both at axon and the branch point where myelinated afferents bifurcate to enter the spinal cord or project up the ascending columns. Because branch points are particularly susceptible to high-frequency conduction failure, the afferent signals may not gain access to the nociceptive pathway in the spinal cord.[36] Because mechanical hypersensitivity may be signaled by abnormal activity in myelinated afferent fibers (*e.g.*, Aβ-fibers),[37,38] and the dorsal column contain axons that originate in these large-diameter afferent sensory neurons, inhibition of A-fiber inputs may partially contribute to SCS analgesia, especially for inhibition of mechanical hypersensitivity. Before conduction block, high-frequency alternating current typically induces a brief but intense burst of axonal firing, the so-called onset response,[39] which results from initially stimulating the nerve at as fast a rate as the refractory period of the nerve allows.[40,41] Behaviorally, we observed similar "onset response" in rats receiving kilohertz-level SCS at 80% MoT. Previously, we showed that MoT in behavioral testing is near the Ab0 of compound AP.[5] Here, 1 kHz, but not 50 Hz, conditioning stimulation at Ab0 also inhibited Aα/β compound AP. Thus, reversal of mechanical hypersensitivity from kilohertz-level SCS at 80% MoT may involve a peripheral mechanism. Yet, the frequency-related blocking mechanism remains to be confirmed in rats receiving epidural SCS. We did not observe significant reduction of Aα/β compound AP to 50 Hz stimulation at Ab0, nor the onset response to 50 Hz SCS, but 50 Hz SCS may affect other conduction properties that were not detected by the current method. Because traditional SCS primarily activates A-fibers traveling in the dorsal column, an intervention of all large afferent inputs by SCS is unlikely. In fact, rats in all groups still sensed and responded to mechanical stimuli applied to their hind paws. However, a greater blockade of peripheral afferent inputs, including those mediated by C-fibers, may be achievable by applying high-intensity, high-frequency stimulation near the dorsal root or dorsal root entry zone, as that demonstrated by a recent study.[42]

Although paresthesia elicited by SCS may not necessarily relate to the pain-relieving effect, a basic principle for conventional SCS is to create paresthesia, presumably by activating myelinated afferent fibers in the dorsal column, which overlap the affected pain region. As the fundamental biological basis for conventional SCS-induced analgesia, the gate-control theory postulates that some of these afferent sensory neurons send collateral branches to the affected spinal segments, and activities of these large fibers drive onto inhibitory dorsal horn interneurons to inhibit spinal pain transmission.[12,13] Previously, we showed that 50 Hz dorsal column stimulation (Ab1, 0.2 ms) inhibited WDR neuronal activity in SNL rats.[3,22] Here, 50 Hz stimulation of a much shorter pulse width (0.024 ms) also inhibited windup in WDR neurons of SNL rats. Thus, the gate-control mechanism may underlie pain inhibition from 50 Hz SCS of both pulse widths. High-frequency stimulation was more effective

than conventional stimulation at terminating seizures in an animal model,[43] suggesting that it induces greater neuronal inhibition. Because the pulse width and SCS duration are fixed, kilohertz-level SCS delivers many more electrical pulses than does 50 Hz SCS. Thus, one may expect that kilohertz-level SCS would induce a greater activation of the same segmental pain-inhibitory mechanisms that also underlie 50 Hz SCS–induced analgesia. However, 1 kHz stimulation did not significantly attenuate windup in WDR neurons in the same experimental setting. The reason for this is unclear, but may be partially due to conduction property changes on large afferent fibers induced by high-frequency stimulation may compromise A-fiber activation of the spinal "gate-control" mechanism.

Compared with pre-SCS measures, 1 kHz, 10 kHz, and 50 Hz SCS significantly reduced mechanical hypersensitivity at 40% MoT (mean 1–3 SCS), an intensity that is approximately at SenT. When compared with sham stimulation, only 1 kHz, 10 kHz differed significantly. Whether there is a mechanism of low-intensity, possibly just "subsensory," SCS over a dorsal column target is unclear. Putative mechanisms could include both imperceptible conduction blockade and modulation of neural mechanisms unique to kilohertz-level stimulation. However, traditional mechanisms would consist of some form of dorsal column activation. For example, because many neural tissues may not faithfully track high-frequency stimulation over a long period, kilohertz-level stimulation may lead to asynchronous neuronal activation. It is possible that activation of afferent fibers, dorsal horn and dorsal column neurons, and neurons in supraspinal pain modulatory structures in a stochastic, asynchronous manner could exert different pain-inhibitory effects from those produced when nerves fire synchronously at lower rates of stimulation.[6,44,45] Future studies are needed to examine whether pain inhibitions from kilohertz-level SCS and conventional SCS also involve different neurochemical mechanisms, and induce different changes in gene expression that underlie neuronal plasticity.[46] It is important to examine with psychophysical testing in patients whether kilohertz-level SCS may be more suitable for treating certain pain manifestations or modalities, such as tactile allodynia or pain due to A-fiber neuropathy, than conventional 50 Hz SCS, which may be better in alleviating heat hyperalgesia or pain related to C-fiber neuropathy.

Conclusions

The current study shows that SCS analgesia in SNL rats depends on both intensity and frequency of stimulation, and high-intensity, kilohertz-level SCS was shown to provide earlier inhibition of mechanical hypersensitivity than conventional 50 Hz SCS. Importantly, pain inhibition resulting from kilohertz-level and 50 Hz SCS may involve different peripheral (afferent conduction property change) and spinal segmental mechanisms (dorsal horn neuronal inhibition), though the exact mechanisms will require additional research.

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