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The impact of electrical charge delivery on inhibition of mechanical hypersensitivity in nerve-injured rats by sub-sensory threshold spinal cord stimulation

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Abstract

Objectives: Spinal cord stimulation (SCS) represents an important neurostimulation therapy for pain. A new ultra-high frequency (10,000 Hz) SCS paradigm has shown improved pain relief without eliciting paresthesia. We aim to determine whether sub-sensory threshold SCS of lower frequencies can also inhibit mechanical hypersensitivity in nerve-injured rats and examine how electric charge delivery of stimulation may affect pain inhibition by different patterns of subthreshold SCS.

Materials and Methods: We used a custom-made quadripolar electrode (Medtronic Inc., Minneapolis, MN, USA) to provide bipolar SCS epidurally at the T10–12 vertebral level.

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According to previous findings, SCS was tested at 40% of the motor threshold (MoT), which is considered to be a sub-sensory threshold intensity in rats. Paw withdrawal thresholds (PWTs) to punctate mechanical stimulation were measured before and after SCS in rats that received an L5 spinal nerve ligation (SNL).

Results: Both 10,000 Hz (10 kHz, 0.024 ms) and lower frequencies (200 Hz, 1 ms; 500 Hz, 0.5 ms; 1200 Hz, 0.2 ms) of subthreshold SCS (120 min) attenuated mechanical hypersensitivity, as indicated by increased PWTs after stimulation in SNL rats. Pain inhibition from different patterns of subthreshold SCS was not governed by individual stimulation parameters. However, correlation analysis suggests that pain inhibition from 10 kHz subthreshold SCS in individual animals was positively correlated with the electric charges delivered per second (electrical dose).

Conclusions: Inhibition of neuropathic mechanical hypersensitivity can be achieved with low-frequency subthreshold SCS by optimizing the electric charge delivery, which may affect the effect of SCS in individual animals.

Keywords

spinal cord stimulation; pain; nerve injury; electrical charge; rat

INTRODUCTION

Spinal cord stimulation (SCS) is an important clinical modality for pain treatment (1–3). Traditional SCS delivers 40–70 Hz electrical pulses that activate low-threshold A β -fibers in the dorsal column; The antidromic and orthodromic action potentials (APs) carried in these fibers then activate spinal and supraspinal mechanisms to inhibit pain (3–5). An important principle for traditional SCS therapy is to evoke a paresthesia by activating A β -fibers (i.e., supra-sensory threshold), that overlaps the pain region (4, 6, 7). Limitations of traditional SCS include suboptimal pain relief and paresthesia that may be uncomfortable to some patients. A new pattern of SCS that uses ultra-high frequency (10 kHz) stimulation with short pulse width (0.024–0.030 ms) improves pain treatment without eliciting paresthesia (8–13). Because the amplitude of “paresthesia-free” SCS is likely below the sensory threshold (subthreshold), it may improve patients’ tolerance of the stimulation. However, its treatment time is significantly limited by high energy consumption, as ultra-high frequency stimulation requires frequent battery recharge. Questions remain as to whether subthreshold SCS may be effective at lower frequencies, which would be more energy efficient than 10 kHz stimulation.

The pattern or waveform of SCS may determine how electric charge is transmitted from the lead to the neural tissue (e.g., rate, amount of charge delivery), and can significantly influence its therapeutic effect. Waveform is characterized by frequency (Hz), pulse width (ms), and amplitude (mA), which have often been considered as discrete variables in programming the therapy. However, the way in which the integrated components of these parameters affect SCS-induced pain inhibition has been given little consideration. In particular, total amount of electric charge transmitted per time unit (charge-per-second) to neural substrate can be viewed as an “electrical dose,” and may be titrated to optimal pain relief by SCS(14). The electrical dose is calculated by multiplying pulse width by amplitude

and frequency. In this regard, SCS that delivers a large amount of charge-per-second is referred to as high-dose stimulation. The other two parameters are duty time and charge-per-pulse. So far, no study has examined how charge delivery affects pain inhibition by different frequencies of subthreshold SCS, and which parameter or component of stimulation governs the therapeutic actions. Answers to these questions will help in the development of new programming strategies to improve clinical efficacy of SCS. Mechanical hypersensitivity is one important manifestation of neuropathic pain (15–17), and has often been tested for determining the time course of pain inhibitory effect of SCS in previous animal studies (18–22). Accordingly, we compared the effects of different patterns of subthreshold SCS on mechanical hypersensitivity in rats after an L5 spinal nerve ligation (SNL) and determined whether pain inhibition by subthreshold SCS is related to the amount of electric charge delivery.

MATERIAL AND METHODS

Animals.

Adult male Sprague-Dawley rats (300–350 g; Envigo, Indianapolis, IN) were housed under optimal laboratory conditions with a 12-hour light/dark cycle and free access to food and water. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee (Baltimore, MD, USA) as consistent with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council. Washington: National Academy Press, 1996) to ensure minimal animal use and discomfort.

Surgery of Spinal Nerve Ligation.

Animals were anesthetized with 2.0% isoflurane (Abbott Laboratories, North Chicago, IL). The left L5 spinal nerve was tightly ligated with a 6–0 silk suture and cut distally as described previously (6, 18). Care was taken not to pull the nerve or touch the L4 spinal nerve. The animals were monitored after surgery for signs of wound infection, inadequate food and water intake, or weight loss until the surgical site had healed.

Spinal Cord Stimulation.

At day 5–7 post-SNL, an SCS electrode was placed epidurally through a small laminectomy as described previously (18, 19, 23). The animals were anesthetized with isoflurane, a small laminectomy was performed at the level of T13, and the electrode was inserted epidurally in the rostral direction. We used a custom-made quadripolar electrode (contact diameter: 0.9–1.0 mm, center spacing: 2.0 mm, Medtronic Inc., Minneapolis, MN, USA) to provide bipolar SCS (“twin-pairs” stimulation) as shown in previous studies (18, 19, 22). The sterilized leads were placed at the T10–12 vertebra level, which corresponds to the upper lumbar spinal cord region (e.g., T13–L1, Fig. 1A). A subcutaneous tunnel was used to position the proximal end of the electrode in the upper thoracic region, where it exited the skin and was connected to an external stimulator (model 2100, A-M Systems, Sequim, WA). Animals were allowed to recover from surgery for > 1 week.

Based on stimulation frequency, pulse width, and amplitude, we calculated three components of SCS: charge per pulse = pulse width × amplitude, duty time = frequency ×

pulse width, and charge per second = charge per pulse $\times$ frequency (Table 1). The duty time describes the effective stimulation period (on-time) per time unit of treatment (14). To allow comparisons, we set the four patterns of high-dose subthreshold SCS frequencies to have similar duty times by adjusting the pulse width: 200 Hz, 1 ms; 500 Hz, 0.5 ms; 1200 Hz, 0.2 ms; and 10 kHz, 0.024 ms. Delivery was symmetrically biphasic with constant current. We also tested a low-dose subthreshold SCS (50 Hz, 0.2 ms) and sham stimulation (0 mA).

In animal behavioral studies, SCS is usually tested at an intensity slightly below the motor threshold (MoT), which is considered to be close to the tolerance threshold (18, 19, 21, 22, 24). MoT for each SCS pattern was determined in awake animals by slowly increasing the amplitude of 4 Hz stimulation with the corresponding pulse width from zero until muscle contraction is observed in the mid-lower trunk or hind limbs (e.g., MoT for 200 Hz stimulation was tested at 4 Hz with pulse width of 1 ms), as shown in previous studies (18, 19, 21, 24). Based on recent findings (18, 21, 25), we considered sensory threshold to be near 50% MoT. Thus, we used 40% MoT as sub-sensory threshold intensity for SCS.

Behavioral Test.

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, 13.1 g) (26). Briefly, the von Frey filaments were applied for 4 to 6 seconds to the test area between the footpads on the plantar surface of the hind paw. If a positive response occurred (e.g., abrupt paw withdrawal, licking, and shaking), the next smaller von Frey hair was used; if a negative response was observed, the next higher force was used. The test was continued until (1) the responses to five stimuli were assessed after the first crossing of the withdrawal threshold or (2) the upper/lower end of the von Frey hair set was reached before a positive/negative response had been obtained. The paw withdrawal threshold (PWT) was determined according to the Dixon formula (27). Rats that showed impaired motor function after surgery or did not develop mechanical hypersensitivity (i.e., mechanical allodynia, >50% reduction from pre-SNL PWT) on the hind paw ipsilateral (left) to the nerve injury by day 5 post-SNL were excluded from subsequent studies. Prior to the behavioral testing, animals were acclimatized to their facilities for 1 week. To minimize variability of the behavioral outcome measures, animals were trained for 3 to 5 days before baseline data were obtained. In addition, animals were habituated to the test environment for 30 minutes before testing was begun on a given day. We blinded the experimenter to the treatments (e.g., stimulation pattern) to reduce selection and observation bias.

Experimental Protocol.

The behavioral testing paradigms were similar to those we have used in our previous studies (18, 19). Of all rats ($n = 45$) that received SNL, 5 rats did not develop significant mechanical hypersensitivity (>50% reduction of PWT from pre-SNL baseline) at day 7 post-SNL. The rest of rats ($n = 40$) received SCS lead implantation, and 8 rats showed impaired motor function or damage to the implanted lead after implantation surgery. These rats were eliminated from the subsequent behavioral studies. The remaining 32 rats were randomly selected to receive the first round of SCS on three consecutive days (120 min, 1 session/day) from days 14–17 post-SNL (Week-1 SCS). On each test day, rats were acclimated for 30

minutes before we measured the baseline PWT. MoT was determined first, SCS or sham stimulation was then applied for 120 minutes. We examined PWTs at 30, 60, and 90 minutes during the SCS (intra-SCS) and at 0, 30, and 60 minutes after the completion of SCS (post-SCS) to determine the carryover effect. Different frequencies were tested in different animals. After completing Week-1 SCS and allowing recovery from this treatment for 3 days, we tested the same group of animals with the second round of SCS (Week-2 SCS, Fig. 1B). To limit potential order effect, we used a cross-over design to switch SCS frequencies in different groups between the two weeks. A group of animals received sham stimulation (0 mA) in each test week in order to blind the experimenter to animal treatment conditions. The data from the two tests were combined for analysis.

Statistical Analysis.

To determine the PWT in behavioral studies, we converted the pattern of positive and negative von Frey filament responses to a 50% threshold value using the formula provided by Dixon (27). The PWT was compared between the pre- and post-SCS conditions and between groups by using a two-way mixed model analysis of variance (ANOVA). Correlation of pain inhibition with charge delivery was calculated with Spearman's correlation analysis. STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK) was used to conduct all statistical analyses. The Bonferroni and Fisher's LSD post-hoc tests were used to compare specific data points. All tests were two-tailed, and numerical data are expressed as mean $\pm$ SEM. $P < 0.05$ was considered 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 (18, 19, 21, 28, 29).

RESULTS

Pain Inhibition by Different Patterns of Subthreshold SCS

We applied four patterns of high-dose SCS (200 Hz, 500 Hz, 1200 Hz, 10 kHz) and one of low-dose SCS (50 Hz), all at sub-sensory threshold intensity (40% MoT) to examine the effects on mechanical hypersensitivity in SNL rats (Figs. 1–3). In animal groups that showed increased ipsilateral PWTs in response to subthreshold SCS, the peak effect usually appeared at 60–90 minutes after initiation of SCS and remained for a short period (0–30 minutes) after cessation (Fig. 2A–F). With 200 Hz and 1200 Hz SCS, the trend was for SCS-induced inhibition to increase gradually from the first day to the subsequent days (Fig. 2B,D).

The mean ipsilateral PWT during SCS (i.e., intra-SCS) was calculated by averaging PWT at 30, 60, and 90 minutes after the start of SCS (Fig. 3A–F). Intra-SCS PWT increased significantly from the pre-SCS level of day 1 on each of the three treatment days of 200 Hz (day 1: $P = 0.047$, day 2: $P < 0.001$, day 3: $P = 0.002$, $n=11$, Fig. 3B), 500 Hz (day 1: $P = 0.005$, day 2: $P = 0.002$, day 3: $P = 0.001$, $n=11$, Fig. 3C), and 10 kHz stimulation (days 1–3: $P < 0.001$, $n=11$, Fig. 3E). Intra-SCS PWT also increased significantly on the second ($P = 0.043$) and third days ($P = 0.009$) of 1200 Hz SCS ($n=11$, Fig. 3D), and on the second day of 50 Hz SCS ($P = 0.02$, $n=10$, Fig. 3A). Sham stimulation did not change PWT from pre-stimulation baseline ($n=10$, Fig. 3F). The mean post-SCS PWT was calculated by averaging

PWTs at 0, 30, and 60 minutes after SCS cessation. Post-SCS PWT was significantly higher than the pre-SCS baseline on the second ($P = 0.023$) and third ($P = 0.003$) treatment days of 200 Hz SCS (Fig. 3B), and on the third treatment day in the 10 kHz group ($P = 0.037$, Fig. 3E).

During SCS at an intensity of 40% MoT, there was no muscle twitching and animals showed normal behavior at the beginning and throughout SCS, without brief stoppage of ongoing activity, awaking, alerting, turning attention toward stimulation, or changing body posture to accommodate the stimulation.

Pain Inhibition by Different Patterns of Subthreshold SCS is not Governed by Individual Parameters or Components of Stimulation

To compare the effects from different patterns of subthreshold SCS, we calculated maximum possible effect (MPE) of inhibition of mechanical hypersensitivity by SCS in each animal based on intra-SCS PWTs and the respective pre-SCS baseline (Fig. 4A). $MPE (\%) = [1 - (\text{Pre-injury PWT} - \text{Intra-SCS PWT})] / [\text{Pre-injury PWT} - \text{Pre-SCS PWT}] \times 100$. Compared to that of the sham stimulation group, intra-SCS %MPEs significantly increased on each of the three treatment days with 200 Hz (day 1: $P = 0.046$, day 2: $P < 0.001$, day 3: $P = 0.006$, $n=11$) and 10 kHz SCS (day 1: $P < 0.001$, day 2: $P < 0.001$, day 3: $P = 0.009$, $n=11$), and on the second ($P = 0.049$) and third ($P = 0.018$) days with 1200 Hz SCS ($n=11$, Fig. 4A). Intra-SCS %MPE of the 10 kHz group was significantly higher than that of the 50 Hz group on the first ($P = 0.009$) and second day ($P = 0.008$). Intra-SCS %MPE of the 200 Hz group was also significantly higher than that of the 50 Hz group on the second day ($P = 0.007$).

Based on stimulation frequency, pulse width, and amplitude, we calculated charge-per-pulse, duty time, and charge per second for the four patterns of subthreshold SCS (Table 1). We set the four patterns of high-dose subthreshold SCS frequencies (200 Hz, 500 Hz, 1200 Hz, and 10 kHz) to have similar duty times by adjusting the pulse width. Among them, 200 Hz SCS had the longest pulse width (1 ms) and highest charge per pulse (Fig. 4B), but the lowest charge-per-second (Fig. 4C, Table 1). In contrast, 10 kHz SCS had the shortest pulse width (0.024 ms) and lowest charge-per-pulse, but the highest amplitude and charge-per-second. Regardless of these differences, 10 kHz and 200 Hz SCS induced comparable inhibitory effects on mechanical hypersensitivity.

We then conducted correlation analysis of individual data (Fig. 5A–E) and found a positive correlation between intra-SCS %MPE and charge per second (i.e., electrical dose) in animals that received 10 kHz SCS ($P = 0.017$, Fig. 5D). We also found a positive correlation between intra-SCS %MPE and electrical dose of individual animals when data from the four high-dose subthreshold SCS groups were combined ($P = 0.002$, Fig. 5E).

DISCUSSION

Paresthesia-free SCS applied at ultra-high frequency (10 kHz) provides improved pain relief as compared to traditional SCS, and is a significant technological advance in the neuromodulation field (1, 3, 9). By performing behavioral tests in neuropathic rats, we provide proof of principle that using low-frequency subthreshold SCS and longer pulse

widths could be a more energy efficient stimulation paradigm for inhibition of mechanical hypersensitivity, as compared to high-frequency stimulation. Our group data comparisons suggest that the efficacy of inhibition of mechanical hypersensitivity from different patterns of subthreshold SCS cannot be explained by the difference in individual stimulation parameters or components. However, correlation analysis indicates that pain inhibition may be positively correlated with the electrical dose that individual animals received during 10 kHz SCS. We also found a positive correlation between inhibition of mechanical hypersensitivity and electrical dose in individual animals when data from the four high-dose subthreshold SCS groups were combined for analysis.

We determined the MoT by increasing the intensity of the electrical stimulation until muscle contraction in mid-lower trunk or hind limbs was observed. The muscle contraction at MoT was considered to be a reflex response to stimulation of dorsal column fibers (i.e., primary afferents), which potentially excite the motoneuronal pools that innervate muscles (30). Individual muscle twitch could not be readily observed at high-frequency (e.g., > 100 Hz). Rather than muscle twitching threshold, the MoT in response to high-frequency stimulation may represent a “titanic muscle threshold” which can be affected by stimulation frequency. Accordingly, we measured MoT by applying stimulation at 4 Hz so that muscle twitching in response to each individual pulse could be clearly identified as shown in previous studies (18, 19, 21, 28). Using this method, two separate studies have suggested that 40% MoT may represent the sub-sensory threshold intensity of SCS in awake rats (18, 21). At this intensity, we found that in addition to 10 kHz, the lower frequencies of 200, 500, and 1200 Hz SCS also can significantly attenuate neuropathic mechanical hypersensitivity in SNL rats.

Due to possible temporal and spatial summations from high-frequency stimulation, the “titanic muscle threshold” may be lower than the MoT to 4 Hz stimulation. If so, high-frequency SCS applied at 40% MoT may not be sub-sensory threshold. However, to our knowledge, there has been no evidence that the “titanic muscle threshold” is lower than the MoT in animal studies of SCS. Importantly, a recent electrophysiology study showed that high-frequency SCS at 40% MoT produced no activation in the gracile nucleus, and did not block conduction of peripherally evoked afferent activity in dorsal column (21). In line with this notion, animal did not show muscle twitching, nor changes in normal behavior (e.g., brief stoppage of ongoing activity, awaking, alerting, turning attention toward stimulation, or changing body posture to accommodate the stimulation) to high-frequency SCS at 40% MoT in current study, suggesting that the treatment is likely sub-sensory threshold.

Because clinical and animal studies have suggested that pain inhibition from subthreshold SCS may have a slower onset than traditional suprathreshold SCS (1, 3, 21), we maintained SCS for a longer duration in our current animal behavioral (120 minutes) and electrophysiologic (30 minutes) experiments than we did for studies of traditional SCS (6, 18, 19, 28). Inhibition of neuropathic mechanical hypersensitivity by traditional SCS (e.g., 50 Hz, 0.2 ms) is dependent on the stimulation intensity (18, 31). It was shown that the traditional SCS brings some benefits even at sub-sensory amplitudes; If it is permitted to increase the intensity just above the sensory threshold, the outcome in clinical studies gets better (3, 32, 33). One of our major findings was that mechanical hypersensitivity was inhibited not only by subthreshold SCS of ultra-high frequency (10 kHz), but also by that of

much lower frequencies (e.g., 200 Hz). These findings support recent observations in animal studies and in a randomized, 2×2 crossover clinical study demonstrating the effectiveness of low-frequency subthreshold SCS (18, 21, 34).

Group data comparisons suggested that inhibition of mechanical hypersensitivity by different patterns of subthreshold SCS cannot be attributed to individual stimulation parameters (e.g., frequency, pulse width). For example, both 200 Hz and 10 kHz SCS induced significant and comparable inhibition of mechanical hypersensitivity. Yet, the pulse width of 200 Hz SCS is the longest (1 ms) and that of 10 kHz SCS the shortest (0.024 ms). Thus, low-frequency SCS might also be effective at subthreshold intensity if the pulse width is increased. It remains to be determined whether an optimal pulse width is needed for some frequencies of subthreshold SCS to be the most effective.

The amount of electric charge delivered per second reflects the electrical dose that affects pain inhibition by SCS (14, 35). SCS that delivers a large amount of charge-per-second may be viewed as high-dose stimulation. In behavioral testing, we used a subthreshold 50 Hz SCS to deliver a lower charge-per-second (low-dose) than the other four patterns of high-dose SCS (200 Hz, 500 Hz, 1200 Hz, and 10 kHz). Among high-dose SCS, the 10 kHz SCS delivered the highest amount of charge-per-second, whereas 200 Hz SCS delivered the lowest. Nevertheless, the 10 kHz and 200 Hz SCS induced similar pain inhibition. Thus, from the group analysis, electrical dose cannot fully explain the efficacies of pain inhibition from different patterns of subthreshold SCS. However, correlation analysis suggested that pain inhibition may be positively correlated with the electrical dose that individual animals received during 10 kHz SCS. We obtained similar results when we combined data from the four high-dose subthreshold SCS groups for analysis. It should be noted that the correlation coefficient measures only the degree of linear association between two variables; neither regression nor correlation analysis can be interpreted as establishing cause-and-effect relationships.

Other features of neuropathic pain, such as spontaneous and ongoing pain, in patients are difficult to measure and not represented well by animal models. Since repetitive noxious heat stimulation may induce sensitization of nociceptors which would confound the finding, whereas mechanical hypersensitivity can be measured frequently to examine the time course of SCS actions (18–22), we focused on testing mechanical hypersensitivity in current study. Heat, cold and mechanical hypersensitivity involve different peripheral and central mechanisms (36–38), and may respond differently to SCS. Accordingly, the effects of different patterns of subthreshold SCS on neuropathic pain manifestations other than mechanical hypersensitivity need to be determined in future studies.

So far, the precise mechanisms of pain relief by subthreshold high-frequency SCS remain unclear. Putative mechanisms are multiple including imperceptible conduction blockade of afferent fibers, desynchronization of axonal activity, and other neuronal modulatory mechanisms unique to subthreshold high-frequency stimulation(1, 39). Subthreshold SCS may also exert its actions through slowly modulation of neurotransmitter content and neuronal intrinsic properties in the spinal cord. Yet, there has been no enough biological evidence that would support any of these notions. Future studies are also needed to examine

whether pain inhibition from subthreshold SCS involve any neurochemical mechanisms and glial-neuronal interactions. The mechanisms of inhibition of mechanical hypersensitivity may also differ between low- and high-frequency subthreshold SCS and warrant further study. Nociceptive afferent inputs are mostly mediated by C-fibers that terminate in the superficial dorsal horn (40–42). Future electrophysiology studies are needed to determine how different patterns of subthreshold SCS modulate *in vivo* spinal nociceptive transmission under neuropathic pain condition, such as by measuring spinal local field potentials that correspond to C-fiber inputs (28), and by examining responses of wide-dynamic range neurons and nociceptive-specific neurons in the dorsal horn (6, 43, 44).

Traditional SCS is an important non-opioid therapy for certain chronic pain conditions which became refractory to pharmacotherapies, but it is limited by inadvertent paresthesia and suboptimal efficacy (4, 45). Waveform pattern innovation is important for developing new stimulation paradigms that will improve SCS-induced pain relief (14, 46). New electrical waveforms such as 10 kHz paresthesia-free SCS may offer therapeutic advantages over traditional SCS by increasing clinical utility for indications that do not respond well to traditional SCS, rescuing a subset of patients who fail to benefit from traditional SCS, and improving patient tolerance to the treatment. Our findings suggest that paresthesia-free pain inhibition may not be limited to ultra-high frequency SCS. Lower frequencies of SCS may achieve similar effects if the electrical dose is optimized. Furthermore, we found a positive correlation between inhibition of mechanical hypersensitivity and charge-per-second in individual animals in the 10 kHz group and in the combined four patterns of high-dose subthreshold SCS groups, indicating that electrical dose may be important to subthreshold SCS to alleviate this neuropathic pain manifestation. Our study may inspire the future exploration of optimal waveform, therapeutic potentials, and mechanisms by which subthreshold SCS modulates pain transmission. A better understanding of how different waveforms affect pain inhibition by SCS is important for developing new programming strategies that will improve clinical efficacy and increase clinical indications.

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B Protocol for behavioral study of SCS

Figure 1. Experimental setup and protocol.

(A) Schematic diagram illustrating the experimental setup. The miniature SCS lead with four contacts (Medtronic, Minneapolis, MN) was implanted epidurally over the dorsal spinal cord at the T10–T12 vertebral level. Paw withdrawal threshold (PWT) was measured in response to mechanical stimuli (von Frey filaments, 0.38–13.1 g) applied to the mid-plantar area of the hind paw. (B) Illustration of the experimental procedure. In Week-1 SCS (days 14–16 post-injury), rats received one pattern of SCS or sham stimulation on three

consecutive days (120 minutes, 1 session/day) at 40% motor threshold (MoT) intensity. In Week-2 SCS (days 21–23 post-injury), the pattern of SCS was changed.

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Figure 2. Time course of changes in paw withdrawal threshold (PWT) after different patterns of subthreshold SCS.

(A-F) Changes in PWT on the ipsilateral hind paw in nerve-injured rats (days 14–28 post-injury) before and after 50 Hz SCS (0.2 ms, 40% MoT, $n=10$, A), 200 Hz SCS (1 ms, 40% MoT, $n=11$, B), 500 Hz SCS (0.5 ms, 40% MoT, $n=11$, C), 1200 Hz SCS (0.2 ms, 40% MoT, $n=11$, D), 10 kHz SCS (0.024 ms, 40% MoT, $n=11$, E), or sham stimulation ($n=10$, F). SCS or sham stimulation was applied for 3 consecutive days (120 minutes/session). Data from animals that received sham stimulation in different studies were combined for analysis.

*P < 0.05, **P < 0.01, ***P < 0.001 versus pre-SCS on day 1. One-way repeated measures analysis of variance with Bonferroni post hoc test. Data are expressed as mean ± SEM.

Figure 3. Effects of different patterns of subthreshold SCS on mechanical hypersensitivity in rats after nerve injury.

(A-F) PWTs at pre-SCS, intra-SCS (averaged PWTs at 30, 60, and 90 minutes after initiating SCS), and post-SCS (averaged PWTs at 0, 30, and 60 minutes after cession of SCS) in rats treated with 50 Hz SCS (0.2 ms, $n=10$, **A**), 200 Hz SCS (1 ms, $n=11$, **B**), 500 Hz SCS (0.5 ms, $n=11$, **C**), 1200 Hz SCS (0.2 ms, $n=11$, **D**), 10 kHz SCS (0.024 ms, $n=11$, **E**), or sham stimulation ($n=10$, **F**). Data from animals that received sham stimulation in different studies were combined for analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus pre-SCS on day 1. One-way repeated measures analysis of variance with Bonferroni post hoc test. Data are expressed as mean $\pm$ SEM.

Figure 4. Comparisons of pain inhibition and charge delivery by different patterns of subthreshold SCS.

(A) The maximum possible effect (MPE) of SCS was calculated at 30, 60, and 90 minutes after initiation on each treatment day (120 minutes, 1 session/day, 40% MoT). Then %MPE at each of these times was averaged for each group: 50 Hz (0.2 ms, n=10), 200 Hz (1 ms, n=11), 500 Hz (0.5 ms, n=11), 1200 Hz (0.2 ms, n=11), 10 kHz (0.024 ms, n=11), and sham stimulation (n=10). *P < 0.05, **P < 0.01, ***P < 0.001 versus sham stimulation. ##P < 0.01 versus 50 Hz. (B) Charge-per-pulse (pulse intensity) in each group was calculated as:

pulse width $\times$ 40% MoT. (C) Charge-per-second (pulse density) was calculated as: charge-per-pulse $\times$ frequency. 1 mA is equal to the flow of 1 millicoulomb (mC) of electric charge in 1 second. * $P < 0.05$, *** $P < 0.001$ versus 10 kHz. One-way analysis of variance with Fisher's LSD post hoc test. Data are expressed as mean $\pm$ SEM. MoT, motor threshold.

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Figure 5. Correlation between pain inhibition and charge delivery per second by subthreshold SCS.

The maximum possible effect (MPE) of SCS was calculated at 30, 60, and 90 minutes after initiation (intra-SCS %MPE, 120 minutes, 1 session/day, 40% MoT) on each treatment day. Scatterplots show the averaged %MPE across three treatment days plotted against the charge per second for each animal in four high-dose subthreshold SCS groups: **(A)** 200 Hz (1 ms, $n=11$), **(B)** 500 Hz (0.5 ms, $n=11$), **(C)** 1200 Hz (0.2 ms, $n=11$), and **(D)** 10 kHz (0.024 ms, $n=11$). **(E)** When data from these groups were combined, scatterplots showed a significant linear correlation between intra-SCS %MPE and charge per second. MoT, motor threshold; HD-SCS, high-dose spinal cord stimulation.

Table 1.

Parameters and key components of subthreshold spinal cord stimulation.

Charge-per-pulse, duty time, and charge per second for different subthreshold SCS were calculated based on stimulation frequency, pulse width (PW), and amplitude. Data are expressed as mean. * $P < 0.05$, *** $P < 0.001$ versus 10 kHz. One-way analysis of variance with Bonferroni *post hoc* test.

Frequency (Hz)	Pulse width (ms)	Amplitude (mA)	Charge/pulse (μC) (PW × Amplitude)	Duty time (PW × Frequency)	Charge/second (μC/s) (Charge/pulse × Frequency)
50 (n=10)	0.2	0.104***	0.021*	10	1.04***
200 (n=11)	1.0	0.074***	0.074***	200	14.85***
500 (n=11)	0.5	0.091***	0.051***	250	25.73***
1200 (n=11)	0.2	0.095***	0.019*	240	22.73***
10,000 (n=11)	0.024	0.482	0.012	240	115.64
Sham (n=10)	0	0	0	0	0