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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

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Abstract

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Conflicts of interest

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Author contributions

S.R. and Y.G. designed the experiments and oversaw the overall execution of the project. F.Y. and Y.G. prepared the manuscript. F.Y., Q.X., Y-K.C., R.S., A.S., V.T. and S-Q.H. performed electrophysiological recording, animal surgery, drug injection and data analysis. P.W., R.M. and X.D. contributed to the experimental design and the interpretation of the results. All authors discussed the results and commented on the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Effects of different intensities of dorsal column conditioning stimulation on wide-dynamic range (WDR) neuronal response in nerve-injured rats. (A) Windup function was created by plotting the C-fibre component of WDR neurons to successive electrical stimuli applied to the skin receptive field at a frequency of 0.5 Hz (16 pulses, supra-C-fibre activation threshold, 2.0 ms) and then at 0.1 Hz (12 pulses, 30 s after the cessation of 0.5 Hz stimulation) against the stimulation trial number. Windup functions at baseline and at 0–15 and 30–45 min after dorsal column stimulation are shown for different intensity groups [0.5Ab0: $n = 18$, Ab0: $n = 19$, 0.5(Ab0 + Ab1): $n = 18$, Ab1: $n = 24$, Ab2: $n = 19$]. (B) The stimulus-response functions of the C-fibre component of WDR neurons evoked by graded electrical stimulation (0.1–10 mA, 2.0 ms) are shown at baseline and at 0–15 min and 30–45 min after dorsal column stimulation at different intensities. Error bars are omitted to improve the clarity.

Figure S2. Effects of different intensities of tibial nerve conditioning stimulation on wide-dynamic range neuronal response in nerve-injured rats. (A) Windup functions at baseline and at 0–15 and 30–45 min after tibial nerve stimulation at different intensities (Ab0: $n = 28$, Ab1: $n = 34$, Ab2: $n = 30$). (B) The stimulus-response functions of the C-fibre component of wide-dynamic range neurons evoked by graded electrical stimulation (0.1–10 mA, 2.0 ms) after tibial nerve stimulation at different intensities. Error bars are omitted to improve the clarity.

Background—Spinal cord stimulation (SCS) and peripheral nerve stimulation (PNS) are thought to reduce pain by activating a sufficient number of large myelinated (A β) fibres, which in turn initiate spinal segmental mechanisms of analgesia. However, the volume of neuronal activity and how this activity is associated with different treatment targets is unclear under neuropathic pain conditions.

Methods—We sought to delineate the intensity-dependent mechanisms of SCS and PNS analgesia by *in vivo* extracellular recordings from spinal wide-dynamic range neurons in nerve-injured rats. To mimic therapeutic SCS and PNS, we used bipolar needle electrodes and platinum hook electrodes to stimulate the dorsal column and the tibial nerve, respectively. Compound action potentials were recorded to calibrate the amplitude of conditioning stimulation required to activate A-fibres and thus titrate the volume of activation.

Results—Dorsal column stimulation (50 Hz, five intensities) inhibited the windup (a short form of neuronal sensitization) and the C-component response of wide-dynamic range neurons to graded intracutaneous electrical stimuli in an intensity-dependent manner. Tibial nerve stimulation (50 Hz, three intensities) also suppressed the windup in an intensity-dependent fashion but did not affect the acute C-component response.

Conclusions—SCS and PNS may offer similar inhibition of short-term neuronal sensitization. However, only SCS attenuates spinal transmission of acute noxious inputs under neuropathic pain conditions. Our findings begin to differentiate peripheral from spinal-targeted neuromodulation therapies and may help to select the best stimulation target and optimum therapeutic intensity for pain treatment.

1. Introduction

Chronic neuropathic pain often results in significant suffering. When pharmacological therapies fail to relieve chronic pain, or side effects associated with these therapies substantially impair quality of life, spinal cord stimulation (SCS) or peripheral nerve stimulation (PNS) are considered as alternative strategies for pain management (Long et al., 1981; Weiner, 2003; Kumar et al., 2008). SCS and PNS with parameters similar to those used in the clinic (e.g., 50–60 Hz, 0.2 ms) attenuate behavioural hypersensitivity to mechanical and thermal stimuli in nerve-injured rats (Maeda et al., 2008; Yang et al., 2011; Rosellini et al., 2012). In addition to initiating a feed forward inhibition of spinal nociceptive transmission (Melzack and Wall, 1965), synchronized electrical stimulation may also induce inhibitory postsynaptic potentials in dorsal horn neurons (Foreman et al., 1976; Narikawa et al., 2000), facilitate primary afferent depolarization to elicit presynaptic inhibition of incoming afferent inputs, activate descending pain modulation (Barchini et al., 2012; Song et al., 2013) and change afferent conduction properties (Campbell, 1981; Shechter et al., 2013). Yet our understanding of the specific mechanisms by which SCS and PNS produce analgesia remains incomplete.

Analgesia by SCS and PNS in part relies on sufficient activation of large myelinated A β -fibres to trigger inhibitory pain modulation. Thus, determining the optimal stimulation intensity is important for success. It has been shown that the dorsal horn is an important site for SCS modulation of nociceptive transmission (Guan et al., 2010b). Further, dorsal horn

wide-dynamic range (WDR) neurons play an important role in spinal nociceptive transmission and exhibit a progressive increase in responses (windup) to repeated electrical stimulation of C-fibres. This action potential (AP) windup phenomenon represents a useful cellular model for studying mechanisms that underlie the development of short-term neuronal sensitization involved in nociceptive processing (Li et al., 1999; Guan et al., 2006).

A significant portion of SCS-induced analgesia is mediated through the dorsal column (Shealy, 1977; Wester, 1987), and dorsal column stimulation at clinical SCS parameters inhibits WDR neuronal activity in nerve-injured rats (Guan et al., 2010b; Shechter et al., 2013). However, it is unclear whether SCS and PNS at low and high intensities differentially affect spinal nociceptive transmission under neuropathic pain conditions. In fact, different intensities of electrical stimulation may exert differential, sometimes opposing, effects on neuronal activity and pain modulation (Ren et al., 1989; Zhuo and Gebhart, 1997; Grande et al., 2004). Because SCS is used clinically for alleviating chronic neuropathic pain conditions in patients, animal models of mononeuropathy are particularly suitable for mechanistic studies of SCS analgesia (Smits et al., 2006; Maeda et al., 2008; Song et al., 2011; Barchini et al., 2012). Accordingly, we examined the intensity-dependent features of SCS and PNS analgesia by means of *in vivo* extracellular recordings from WDR neurons in rats after L5 spinal nerve ligation (SNL). Using various stimulation intensities, we also compared the effects of dorsal column stimulation and tibial nerve stimulation (representing SCS and PNS, respectively) on WDR neuronal responses to C-fibre-mediated noxious inputs and to windup phenomenon.

2. Methods

2.1 Animals and L5 SNL surgery

Adult male Sprague-Dawley rats (300–400 g, Harlan, Indianapolis, IN, USA) were anaesthetized with isoflurane (2%, Abbott Laboratories, North Chicago, IL, USA). Under aseptic conditions, the left L5 spinal nerve was tightly ligated with a 6-0 silk suture and cut distally (Chung et al., 2004; Guan et al., 2008). The muscle layer was approximated with 4-0 chromic gut suture and the skin closed with metal clips. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee (Baltimore, MD, USA) as consistent with the National Institutes of Health Guide for the Use of Experimental Animals to ensure minimal animal use and discomfort.

2.2 Animal preparation and maintenance of anaesthesia during neurophysiological studies

Rats were anaesthetized with pentobarbital (45–50 mg/kg, intraperitoneal) and tracheotomy was performed. The rats were ventilated mechanically by a pressure-controlled small-animal ventilator (Kent Scientific Corporation, Litchfield, CT, USA) at a respiratory rate of 50–70 cycles/min and inspiratory pressures of 10–14 cm H₂O. Sufficient depth of anaesthesia was judged from areflexia to sensory stimuli (e.g., no withdrawal reflexes, corneal reflex) when rats were in the unparalyzed state and by the absence of gross fluctuations in heart rate. The animals were monitored by electrocardiography throughout the experiment, and heart rate was maintained at 300–350 beats/min. During the neurophysiological experiments, the rats were anaesthetized with 1.5% isoflurane carried in medical air and were paralyzed with

pancuronium bromide (1–2 mg/kg, intraperitoneal, Elkins-Sinn Inc., Cherry Hill, NJ, USA). A circulating hot-water pad was used to maintain core body temperature in the normal range (36.0–37.0 °C).

2.3 Dorsal column and tibial nerve conditioning stimuli

2.3.1 Dorsal column conditioning stimulus—Since the dura was removed to allow insertion of the fine-tip microelectrode for recording WDR neuronal activity, two tungsten needle electrodes (insulated except for the most distal 0.3–0.5 mm) were inserted into the ipsilateral dorsal column at T13–L1 level to provide localized bipolar dorsal column stimulation (Fig. 1A). The electrical fields produced by bipolar electrical stimulation through penetrating needle electrodes activates the dorsal column more accurately and efficiently than stimulation from surface electrodes, thus minimizing nonselective activation of nearby structures (e.g., dorsal root, grey matter and other tracts) that might also influence neuronal activity. This method was used in our previous studies (Guan et al., 2010b; Shechter et al., 2013).

2.3.2 Tibial nerve conditioning stimulus—The left tibial nerve was exposed and a pair of platinum hook electrodes was placed beneath it (Fig. 1B). We chose the tibial nerve because (1) it is a continuation of the common sciatic nerve, which provides almost all of the sensory innervation to the plantar and ventral aspects of the hind paw, where the test stimulation is applied; (2) it contains a greater proportion of sensory fibres than does the sciatic nerve; and (3) the distance between the stimulating site and compound AP recording site (L4 dorsal root) is longer than it would be if the sciatic nerve were used, thus providing greater separation between the A α / β and A δ waveforms of the compound AP.

2.4 Recording of compound APs evoked by dorsal column and tibial nerve stimulation

To standardize the conditioning stimulus intensity and selectively activate A α / β -fibres at each stimulation site, we recorded compound APs evoked by graded electrical stimulation (0.01–3.0 mA, 0.2 ms) as described. Different compound AP waveforms corresponding to A α / β - and A δ -fibre activation were distinguished on the basis of the conduction velocity (CV) and the activation threshold (Guan et al., 2010b; Shechter et al., 2013). We determined the threshold intensity that evoked an A α / β waveform (A β -threshold or Ab0) and the intensity that produced the A α / β -plateau [A β -plateau or Ab1; defined as the lowest intensity to evoke a peak A α / β -compound AP without inducing an A δ - or C-fibre component (Fig. 1A and B)]. To record the antidromic compound APs evoked by dorsal column stimulation, we placed a monopolar silver hook electrode on the sciatic nerve at mid-thigh level. To record the orthodromic compound APs evoked by tibial nerve stimulation, we exposed the left L4 dorsal root and recorded it with a monopolar silver hook electrode. The reference electrode was placed in the nearby muscle. We used compound APs as subjective measurements to titrate the volume of A α / β -fibre activation at each stimulation site. However, it should be noted that the antidromic compound AP to dorsal column stimulation may not be equivalent to the orthodromic compound AP to tibial nerve stimulation.

2.5 Extracellular recording of WDR neurons

We obtained extracellular recordings of the activity of individual dorsal horn neurons with microelectrodes as described previously (Guan et al., 2010b). Analogue data were collected with a real-time, computer-based data acquisition and processing system (DAPSYS 6; Brian Turnquist, The Johns Hopkins University, Baltimore, MD, USA). To avoid potential pitfalls in data interpretation that stem from WDR cells having different neurophysiological properties depending on whether they are in superficial or deep dorsal horn, or in injured or uninjured spinal segments (Herrero et al., 2000; Guan et al., 2006), we examined WDR neurons located at deep laminae (III–V, 400–1200 μm below the dorsal surface) in the uninjured spinal segment L4. The spinal segment was identified by the respective dorsal root and dorsal root entry zone and WDR cells were identified by their characteristic responses (Homma et al., 1983; Guan et al., 2010b). Only WDR neurons with defined receptive fields in the plantar region of the hind paw were studied.

2.6 Experimental procedure

After recording compound APs to calibrate the amplitude of conditioning stimulation, we examined WDR neuronal responses to the electrical test stimuli applied through a pair of fine stimulating electrodes inserted subcutaneously into the receptive field (Fig. 1A). The WDR neuronal response to a suprathreshold electrical stimulus consists of an early A-fibre component (0–75 ms) and a later C-fibre component (75–500 ms) (Li et al., 1999; Guan et al., 2010a) (Fig. 1C). The stimulus-response (S-R) functions of the C-components to graded intracutaneous electrical stimuli (0.1–10 mA, 2.0 ms, 15-s interval) were determined first. The thresholds for activation of C-components were defined as the lowest stimulus current (mA) to evoke an AP within the range of the C-fibre latencies. The windup phenomenon was then examined by recording the WDR neuronal response to a train of 16 intracutaneous electrical pulses (supra-C-fibre threshold, 2.0 ms) applied at 0.5 Hz (Guan et al., 2010b). At 30 s after the 0.5 Hz stimulation, when the after-discharges of WDR neurons had mostly diminished, another 12 pulses were delivered at 0.1 Hz. Because 0.1 Hz stimulation rarely induces windup under physiological conditions, it was used as a negative control for 0.5 Hz stimulation. At 20 min after baseline testing, the conditioning stimulus (5 min, 0.2 ms, biphasic pulse) was applied at the dorsal column or tibial nerve. The same test module (S-R function and windup) was repeated in the same neuron at 0–15 min and 30–45 min after the conditioning stimulus.

Five intensities of dorsal column stimulation were tested: 0.5Ab0 and Ab0 represent two lower intensities that induce minimal or no activation of A α / β fibres; 0.5(Ab0 + Ab1) and Ab1 are medium and high intensities, respectively, that evoke a partial and peak A α / β -compound AP without inducing an A δ - or C-fibre component; Ab2 is twice the amplitude of Ab1 and was tested to determine if it induces a greater effect than Ab1 or if the effect from the conditioning stimulus has reached a plateau or maximum level at Ab1. Three intensities of tibial nerve stimulation were tested: Ab0, Ab1 and Ab2.

Because of the potential for dorsal horn neurons to become sensitized after repetitive test stimulation and possible carryover effects with multiple conditioning stimuli, only two intensities of conditioning stimulation (same site) were tested in each experiment. We used a

crossover design in which the order of the two intensities was altered from experiment to experiment and a 60–90-min interval between intensities to minimize any order or time effect. Baseline was always measured before applying the conditioning stimulus. Dorsal column stimulation and tibial nerve stimulation were examined in different animals. Animals were randomly selected (by animal number) for each experiment. All experiments were conducted in rats during the maintenance phase of neuropathic pain (4–5 weeks post-SNL) (Guan et al., 2008).

2.7 Data analysis

The number of APs in the C-component of the WDR neuronal response to graded electrical stimuli was used to plot the S-R function, and the number in the response to the train of windup-inducing stimuli was used to plot the windup function. The area under the S-R function and windup function curves (i.e., total response) were compared between the pre- and post-conditioning stimulation conditions in each group by using a one-way repeated measures analysis of variance (ANOVA). For each conditioning stimulation, a two-way mixed model ANOVA was used to compare data between different intensity groups. Data at 0–15 min and 30–45 min post-stimulation were normalized to the respective prestimulation values. To compare data between dorsal column and tibial nerve stimulation, we normalized the total number of APs in the C-component response to graded electrical stimuli and the area under the windup function at 0–15 min post-stimulation (the peak effect) to the respective prestimulation values and compared data between groups by using two-way ANOVA. STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK, USA) was used to conduct all statistical analyses. The Tukey's honestly significant difference *post hoc* test was used to compare specific data points. Two-tailed tests were performed and data are expressed as mean $\pm$ standard error of the mean; $p < 0.05$ was considered significant in all tests.

3. Results

3.1 Characterization of the compound APs evoked by stimulation of the dorsal column and tibial nerve

At suprathreshold intensity, the dorsal root compound APs evoked by tibial nerve stimulation often revealed two distinct groups of wave forms that correspond to A α / β - and A δ -fibre activation, which can be distinguished on the basis of the activation threshold and CV. The fast component corresponds to the A α / β -fibre activation (CV, 17.2 ± 0.6 to 47.1 ± 1.2 m/s). The slower component, referred to as the A δ -compound AP, usually had a smaller amplitude than the fast A α / β component, and could be distinguished by its higher threshold and slower CV (11.1 ± 0.4 to 17.2 ± 0.6 m/s). We used similar CV ranges to separate different compound APs evoked by dorsal column stimulation (A α / β : 15.1 ± 0.5 to 42.2 ± 0.4 m/s; A δ : 10.5 ± 0.3 to 15.1 ± 0.5 m/s). The CVs were comparable to those reported previously (Pinto et al., 2008; Guan et al., 2010b).

3.2 Dorsal column stimulation attenuated WDR neuronal response in an intensity-dependent manner

Windup function was created by plotting the C-component of WDR neurons to successive electrical stimuli applied at a frequency of 0.5 Hz (16 pulses, supra-C-fibre activation

threshold) and then at 0.1 Hz (12 pulses) against the stimulation trial number (Fig. 2A, Fig. S1A). Dorsal column stimulation caused intensity-dependent attenuation of windup (Fig. 1C, Fig. 2A–C, Fig. S1A). Compared with the respective prestimulation baseline, the areas under the windup function (i.e., total number of APs in the C-component to 0.5-Hz stimulation) were significantly decreased at 0–15 min after dorsal column stimulation at intensities of 0.5(Ab0 + Ab1), Ab1 and Ab2 (Fig. 2B). However, stimulation at the lower intensities of 0.5Ab0 and Ab0 were not effective. To compare the inhibitory effects across different intensity groups, we normalized the areas under the windup function of the 0.5 Hz train at 0–15 min and 30–45 min after dorsal column stimulation to the prestimulation baseline. Only stimulation at intensities at or above 0.5(Ab0 + Ab1) induced significant inhibition at 0–15 min post-stimulation (Fig. 2C).

Dorsal column stimulation also caused intensity-dependent attenuation of S-R functions of the C-component response to graded electrical stimuli (Fig. 2D–F, Fig. S1B). Compared with the respective prestimulation baseline value, the areas under the S-R function (i.e., total number of APs in the C-component evoked by graded intracutaneous stimuli) were significantly decreased at 0–15 min after dorsal column stimulation at intensities of 0.5(Ab0 + Ab1), Ab1 and Ab2 (Fig. 2E). Similarly when data were normalized by the respective prestimulation values, only stimulation at an intensity at or above 0.5(Ab0 + Ab1) could induce significant inhibition (Fig. 2F). The C-fibre component threshold at 0–15 min after stimulation was significantly increased in the 0.5(Ab0 + Ab1), Ab1 and Ab2 groups (Fig. 2G).

3.3 Tibial nerve stimulation inhibited windup, but not acute responses, to graded intracutaneous electrical stimulation

The windup response of WDR neurons was also attenuated by the tibial nerve stimulation in an intensity-dependent fashion (Fig. 3A–C, Fig. S2A). Compared with the respective prestimulation baseline values, the areas under the windup function were significantly decreased at 0–15 min after stimulation at Ab1 and Ab2 intensities, and the inhibition remained at 30–45 min after stimulation at both intensities (Fig. 3B and C). Except for the response to the highest intensity intracutaneous stimulation applied to the receptive field (10 mA), the acute responses of WDR neurons to graded intracutaneous stimuli were largely unchanged by tibial nerve stimulation at all intensities tested (Fig. 3D, Fig. S2B). Furthermore, stimulation did not significantly change the areas under the S-R function, as compared with the prestimulation baseline (Fig. 3E and F).

3.4 Dorsal column stimulation induced greater inhibition of the acute response in WDR neurons than did tibial nerve stimulation

The peak inhibition of windup at each stimulus intensity was comparable between dorsal column stimulation and tibial nerve stimulation (Fig. 4A). However, stimulation of the dorsal column produced significantly greater inhibition of the S-R function in WDR neurons than did stimulation of the tibial nerve at the same intensity (Fig. 4B).

4. Discussion

Tibial nerve and dorsal column stimulation-induced inhibition of WDR neurons may be an important neuromodulatory mechanism for both PNS and SCS. In SNL rats, conditioning electrical stimuli applied to either the dorsal column or tibial nerve inhibited the windup response of WDR neurons to repetitive noxious inputs in an intensity-dependent fashion. However, dorsal column stimulation, but not tibial nerve stimulation, caused an intensity-dependent attenuation of acute C-fibre-mediated responses to graded intracutaneous electrical stimuli in WDR neurons.

Increasing evidence suggests that SCS analgesia is intricately linked with spinal segmental mechanisms (Meyerson and Linderöth, 2000; Guan et al., 2010b). Although the dorsal column system may also be involved in the processing of altered somatosensory information during neuropathic conditions (Sun et al., 2001; Saade et al., 2002), lesion studies have shown that a large portion of the analgesic effect produced by SCS is mediated through the dorsal column. Therefore, the primary goal of SCS is to activate the dorsal column, which contains axons that originate in the large-diameter afferent sensory neurons (e.g., A β -fibres). Dorsal horn WDR neurons, many of which are projection neurons, play an important role in pain transmission. Here, dorsal column stimulation significantly inhibited both the S-R function and windup in WDR neurons at intensities equal to and above 0.5(Ab0 + Ab1). However, the inhibitory effects at the lower stimulus intensities (e.g., 0.5Ab0, Ab0) were not significant. These findings are in line with our previous study in which a similar setup showed that dorsal column stimulation at Ab1 intensity induced immediate and strong inhibition of WDR neuronal activity in nerve-injured rats (Guan et al., 2010b). The dorsal column stimulation at Ab2 intensity, which is twice the amplitude of Ab1, did not induce a greater inhibition on the S-R function and windup response than did Ab1, suggesting the effect from the conditioning stimulus may reach a plateau at Ab1 intensity.

Previous studies suggested that PNS also leads to inhibition of nociceptive pathways at the spinal level (Long, 1978; Hanai, 2000). The current finding that conditioning electrical stimuli to the tibial nerve inhibit windup in WDR neurons in an intensity-dependent manner supports this notion. Although PNS may use spinal segmental mechanisms (e.g., gate-control) that are similar to those that underlie SCS analgesia (Foreman and Linderöth, 2012; Guan, 2012), details regarding the biological basis for PNS analgesia remain unclear. Our *in vivo* electrophysiological evidence indicates that both antidromic (dorsal column stimulation) and orthodromic (tibial nerve stimulation) activation of afferent A α / β -fibres inhibits C-fibre-mediated windup in WDR neurons of rats in the maintenance phase of neuropathic pain (4–5 weeks post-SNL). However, only dorsal column stimulation, not tibial nerve stimulation, significantly inhibited the acute responses of WDR neurons to graded electrical stimulation. The reasons for the differing effects of the two treatments in our experimental setting are unclear but may be due to several factors. First, even the highest intensity tested in this study (i.e., Ab2) may not be strong enough for tibial nerve stimulation to achieve its maximal inhibitory effect. Transcutaneous electrical nerve stimulation that activates A δ -fibres produces stronger inhibition of the flexor reflex mediated by C-fibres in rats than stimulation that activates A β -fibres (Sjolund, 1985). Furthermore, conditioning stimulation of peripheral nerves strongly inhibits the C-fibre-mediated response in WDR

neurons at A δ -fibre intensity (Hanai, 2000). Another relevant study showed that low-frequency stimulation of primary afferent A δ -fibres could induce long-term depression of synaptic transmission in substantia gelatinosa neurons (Liu et al., 1998). Thus, it might be necessary for PNS to be applied at higher intensities to achieve significant spinal neuronal inhibition. Second, current spreading from dorsal column stimulation may also activate other dorsal tracts that are in close proximity to the lead (e.g., dorsolateral funiculus) and also may contribute to the pain inhibition. Third, besides the spinal segmental mechanisms, relief of neuropathic pain by SCS may also result from activation of supraspinal structures (Song et al., 2011, 2013; Barchini et al., 2012). Yet it remains to be examined if PNS and SCS differentially activate descending inhibitory pathways and promote distinct supraspinal mechanisms (Salibi et al., 1980; Ren et al., 1996; El-Khoury et al., 2002; Barchini et al., 2012). Finally, A α / β -fibre recruitment may differ depending on whether dorsal column stimulation or tibial nerve stimulation is used. Dorsal column stimulation activates ascending (orthodromic) and antidromic fibres. Yet only the A α / β -fibres from the peripheral nerve that travel in the dorsal column and send collateral branches to spinal cord will be activated by dorsal column stimulation. On the other hand, tibial nerve stimulation would activate most A α / β -fibres within the nerve, including those that connect in the dorsal column and others that terminate only at the spinal level. It may be that the two populations of A α / β -fibres interact with different neuronal components in the spinal cord (e.g., interneurons) and activate different nociceptive modulatory mechanisms. However, this remains to be shown.

Clinically, common indications for SCS include failed back surgery syndrome, complex regional pain syndrome and other intractable neuropathic conditions (Waltz, 1997; Carter, 2004; Foletti et al., 2007; Olsson et al., 2008). PNS is also emerging as a promising treatment option for a growing list of clinical pain conditions. Although our current findings indicate that SCS may be more suitable than PNS for attenuating certain pain conditions, such as pain evoked by acute noxious inputs, this interpretation remains to be tested in the patients. Further, SCS and PNS are often applied at an intensity that elicits paresthesia over the painful area and titrated to the highest comfortable level in patients. Previous studies also suggested that a correlation may exist between the intensity of SCS, including subthreshold SCS, and the duration of pain relief (Meyerson et al., 1995; Meyerson and Linderöth, 2006; Wolter et al., 2012). In line with these findings, the current electrophysiological data support the predictions of the gate-control theory that a higher stimulus intensity (e.g., Ab1), by activating a greater number of A-fibres, may lead to a stronger suppression of spinal nociceptive transmission than can be achieved with the lower intensities (e.g., Ab0). Experimentally SCS is usually tested in animals at an intensity slightly below the motor threshold (MoT), which is considered to be the tolerance threshold (Meyerson and Linderöth, 2006; Song et al., 2009). By examining the antidromic compound AP that results from stimulation applied through the SCS lead in rats, we found evidence that SCS at the MoT may activate only a small fraction of the afferent A-fibre population that is activated at the A α / β -plateau intensity (Yang et al., 2011). Behaviourally 50 Hz SCS near the MoT induced only moderate inhibition of neuropathic mechanical hypersensitivity in rats (Yang et al., 2011). Similarly, even at high intensity, conventional SCS applied clinically usually produces satisfactory but only partial pain relief (Carter, 2004; Meyerson and Linderöth, 2006). Thus, the efficacy of SCS analgesia needs to be improved.

Preclinical models of mononeuropathy are particularly suitable for the exploration of mechanisms underlying SCS inhibition of clinical neuropathic pain states (Linderroth and Meyerson, 2010; Foreman and Linderroth, 2012). It is widely understood that SCS preferentially attenuates exaggerated pain sensitivity under pathological conditions (Gybels and Kupers, 1987; Linderroth and Meyerson, 2010; Foreman and Linderroth, 2012). Yet stimulation of dorsal column and dorsal root also inhibited the windup of C-fibre-mediated response of WDR neurons in sham-operated rats (Guan et al., 2010b). So far, the clinical evidence of the effectiveness of SCS and PNS on inhibiting acute nociceptive pain appears to be controversial (Saade et al., 1985; Marchand et al., 1991; Meyerson et al., 1995; Cogiamanian et al., 2011). The reason for these conflicting results remains unclear, but it may be partially due to use of different stimulation parameters (e.g., intensity), experimental conditions and outcome measures between studies. Future studies may help determine if SCS and PNS also induces intensity-dependent neuronal modulation of nociceptive pain in sham-operated animals.

In summary, we found that the inhibition of acute responses of WDR neurons to peripheral noxious input and their short-term neuronal sensitization (windup) by dorsal column stimulation are both dependent on stimulation intensity. The inhibition from dorsal column stimulation was monophasic. PNS, such as tibial nerve stimulation, can also attenuate short-term neuronal sensitization in an intensity-dependent fashion. Effects of changing stimulation intensity on the efficacy of SCS- and PNS-induced pain relief need to be determined. These findings may provide a rationale for physicians to determine the therapeutic stimulation intensity and select the suitable treatment site and modality (e.g., SCS or PNS) for better management of chronic pain in their patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What's already known about this topic?

- Spinal cord stimulation and peripheral nerve stimulation are alternative methods for pain management.
- Analgesia by spinal cord and peripheral nerve stimulation relies on sufficient activation of A β -fibres to trigger spinal nociceptive inhibitory mechanisms.

What does this study add?

- Dorsal column and tibial nerve stimulation inhibited wide-dynamic range neuronal activity in an intensity-dependent manner in nerve-injured rats.
- These findings begin to differentiate peripheral-from spinal-targeted neuromodulation therapies and may help to select the optimum target and therapeutic intensity for neuropathic pain treatment.

Figure 1.

Schematic diagram illustrating the experimental setup in rats that underwent an L5 spinal nerve ligation (SNL). (A) *Left*: The intensity of dorsal column stimulation was calibrated by recording the sciatic antidromic compound action potential (AP) evoked by bipolar electrical stimulation (0.01–3.0 mA, 0.2 ms) at the dorsal column (T13–L1 spinal level). Wide-dynamic range neuronal activity was recorded with a microelectrode inserted within the L4 spinal segment. Electrical test stimuli were applied to the skin receptive field of the dorsal horn neuron. *Right*: The intensities that produced the first detectable Aα/β waveform (Aβ-

threshold or Ab0) and the peak A α / β waveform (A β -plateau, or Ab1) were determined. (B) *Left*: The intensity of tibia nerve stimulation was calibrated by recording orthodromic compound APs at L4 dorsal root *Right*: The waves of compound APs corresponding to A α / β - and A δ -fibre activation in response to tibial stimulation. Ad0: The intensities that produced the first detectable A δ waveform. (C) An analogue recording of the wide-dynamic range neuronal response to the first, eighth and 16th stimulus of a train of windup-inducing electrical stimuli (0.5 Hz, 16 pulses, 2.0 ms, supra-C-threshold) before (i.e., baseline) and 0–15 min after dorsal column stimulation of 0.5(Ab0 + Ab1) and Ab1 intensities (5 min, 0.2 ms).

Figure 2.

Effects of dorsal column conditioning stimulation on wide-dynamic range (WDR) neuronal response in nerve-injured rats. (A) The windup function at 0–15 min after dorsal column stimulation, the time of peak inhibitory effect, is shown for each group [0.5Ab0: $n = 18$, Ab0: $n = 19$, 0.5(Ab0 + Ab1): $n = 18$, Ab1: $n = 24$, Ab2: $n = 19$]. The averaged prestimulation baseline was used for comparison. (B) The area under the windup function to a 0.5 Hz train was significantly decreased from the baseline at 0–15 min after dorsal column stimulation at intensities of 0.5(Ab0 + Ab1), Ab1 and Ab2. (C) The areas under the windup function to a 0.5 Hz train after dorsal column stimulation were normalized to the baseline value. (D) The stimulus-response functions of the C-component of WDR neurons evoked by graded electrical stimulation (0.1–10 mA, 2.0 ms) after dorsal column stimulation are shown. The averaged prestimulation baseline was used for comparison. (E) The area under the stimulus-response function was significantly decreased from baseline at 0–15 min after conditioning stimulation at intensities of 0.5(Ab0 + Ab1), Ab1 and Ab2. (F) The C-component after dorsal column stimulation was normalized to the respective prestimulation baseline value. (G) The C-threshold after dorsal column stimulation was also normalized to the respective baseline value. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus baseline. Data are expressed as mean $\pm$ standard error of the mean. Error bars in A and D are omitted to improve the clarity.

Figure 3.

Effects of tibial nerve stimulation on wide-dynamic range (WDR) neuronal response in nerve-injured rats. (A) Windup functions after tibial nerve stimulation are shown for different intensity groups (Ab0: $n = 28$, Ab1: $n = 34$, Ab2: $n = 30$). The averaged prestimulation windup function was used for comparison. (B) The area under the windup function to a 0.5 Hz train was significantly decreased from the baseline at 0–15 min after tibial nerve stimulation at intensities of Ab0, Ab1 and Ab2. (C) The area under the windup function to a 0.5 Hz train was normalized to the baseline value. (D) The stimulus-response functions of the C-component to graded electrical stimulation (0.1–10 mA, 2.0 ms) before and after tibial nerve stimulation. The averaged prestimulation baseline was used for comparison. (E) The C-component was not significantly changed after tibial nerve stimulation. (F) The C-component after tibial nerve stimulation was normalized to the respective prestimulation baseline value. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus baseline. Data are expressed as mean $\pm$ standard error of the mean. Error bars in A and D are omitted to improve the clarity.

Figure 4.

Comparison of the peak inhibitory effects between dorsal column stimulation and tibial nerve stimulation. (A) The decrease in the area under the windup function to a 0.5 Hz train after dorsal column stimulation at Ab0, Ab1 and Ab2 intensities was not significantly different from that after tibial nerve stimulation. (B) Compared with tibial nerve stimulation, dorsal column stimulation induced a significantly greater inhibition of the C-component to graded intracutaneous electrical stimuli (0.1–10 mA, 2 ms) at Ab1 and Ab2 intensities. ** $p < 0.01$, *** $p < 0.001$ versus prestimulation baseline, ### $p < 0.01$, ### $p < 0.001$ versus tibial nerve stimulation. Data are expressed as mean + standard error of the mean.