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Spinal Cord Stimulation-induced Analgesia

Electrical Stimulation of Dorsal Column and Dorsal Roots Attenuates Dorsal Horn Neuronal Excitability in Neuropathic Rats

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ABSTRACT

Background: The sites of action and cellular mechanisms by which spinal cord stimulation reduces neuropathic pain remain unclear.

Methods: We examined the effect of bipolar electrical-conditioning stimulation (50 Hz, 0.2 ms, 5 min) of the dorsal column and lumbar dorsal roots on the response properties of spinal wide dynamic range (WDR) neurons in rats after L5 spinal nerve injury. The conditioning stimulation intensity was set at the lowest current that evoked a peak antidromic sciatic Aα/β-compound action potential without inducing an Aδ- or C-compound action potential.

Results: Within 15 min of the dorsal column or root conditioning stimulation, the spontaneous activity rate of WDR neurons was significantly reduced in nerve-injured rats. Conditioning stimulation also significantly attenuated WDR neuronal responses to mechanical stimuli in nerve-injured rats and inhibited the C-component of the neuronal response to graded intracutaneous electrical stimuli applied to the receptive field in nerve-injured and sham-operated rats. It is noteworthy that dorsal column stimulation blocked windup of WDR neuronal response to repetitive intracutaneous electrical stimulation (0.5 Hz) in nerve-injured and sham-operated rats, whereas dorsal root stimulation inhibited windup only in sham-operated rats. Therefore, stimulation of putative spinal substrates at A-fiber intensities with parameters similar to those used by patients with spinal cord stimulators attenuated established WDR neuronal hyperexcitability in the neuropathic condition and counteracted activity-dependent increase in neuronal excitability (*i.e.*, windup).

Conclusions: These results suggest a potential cellular mechanism underlying spinal cord stimulation–induced pain relief. This *in vivo* model allows the neurophysiologic basis for spinal cord stimulation–induced analgesia to be studied.

What We Already Know about This Topic

- Spinal cord stimulation is frequently applied to treat neuropathic pain, but its site and mechanisms of action are unclear.

What This Article Tells Us That Is New

- Bipolar electrical stimulation at the dorsal column or lumbar dorsal roots attenuated dorsal horn neuronal hyperexcitability in nerve-injured rats and inhibited short-term neuronal sensitization.

SPINAL cord stimulation is an effective neuromodulatory technique for managing a variety of chronic pain conditions, particularly neuropathic pain, which is often refractory to current pharmacotherapies.[1–3] Yet, the biologic basis for the effectiveness of spinal cord stimulation in treating neuropathic pain is unclear. Differences in lead design, stimulation mode, and intensity-selecting criteria also present barriers to correlating previous findings in experimental animals with mechanisms underlying therapeutic effects in patients.

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For example, bipolar stimulation that induces paresthesia that covers the painful areas is commonly used in patients, whereas monopolar stimulation at 60–90% of muscle twitching intensity (*i.e.*, motor threshold) is often employed in experimental animals.[4,5] It is unclear how paresthesia intensity correlates with the motor threshold. The class and number of nerve fibers that are activated under each circumstance are also unknown. Because the electrical field of epidural stimulation may spread to nearby tissues *via* highly conductive cerebrospinal fluid, many action sites for spinal cord stimulation–induced pain relief may exist, but they have not been clearly defined.

Spinal cord stimulation was developed as a therapeutic modality based on the gate-control theory in which activation of afferent A fibers is postulated to attenuate spinal pain transmission.[6] Wide dynamic range (WDR) neurons in the dorsal horn are important for spinal pain processing and are candidates for the "transmission" cells in the gate theory.[7–9] The action potential (AP) windup phenomenon in WDR neurons reflects an activity-dependent short-term increase in neuronal excitability.[10–12] Although windup is different from the longer lasting central sensitization, it is a useful experimental model for studying mechanisms that may contribute to initiating persistent pain.[10–13] Electrophysiologic studies in preclinical neuropathic pain models represent an important approach to studying the neurophysiologic mechanisms of spinal cord stimulation. Here, we applied a bipolar electrical stimulus to the thoracic dorsal column and the lumbar roots to compare how conditioning stimulation at a site that is rostral (dorsal column) or caudal (dorsal root) to the area where epidural spinal cord stimulation leads are usually placed in patients may differently affect lumbar WDR neuronal activity. This experimental paradigm allowed us to examine the respective effects of antidromic and orthodromic activation of large afferent fibers on spontaneous activity and the evoked responses of WDR neurons to mechanical stimuli, graded intracutaneous electrical stimuli, and windup-inducing electrical stimulation. Because of the evolving nature of anatomic and functional changes in the nervous system and changes in the efficacy of analgesics after nerve injury,[14–20] we examined rats both at the peak of neuropathic pain (14–16 days postinjury) and at a later maintenance-recovery phase (45–75 days postinjury).[18] For the first time, antidromic compound APs in the sciatic nerve were recorded to standardize conditioning stimulation intensities (*i.e.*, selective activation of $A\alpha/\beta$-fibers). Our observations suggest that dorsal column and root stimulation both attenuate the established WDR neuronal hyperexcitability in nerve-injured rats and suppress the short-term spinal neuronal sensitization in sham-operated rats.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (300–400 g; Harlan Bioproducts for Science, Madison, WI) were used for all animal experiments. 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 Care and Use of Laboratory Animals* to ensure minimal animal use and discomfort.

L5 Spinal Nerve Ligation Surgery

After rats were anesthetized, the left transverse process of the L6 vertebra was removed and spinal nerve ligation (SNL) was performed on the left L5 spinal nerve, which was tightly ligated with a 6-0 silk suture and cut distally.[18,21] In the sham-operated control group, the L6 transverse process was not removed, and the L5 spinal nerve was not ligated or cut.

Animal Behavioral Tests

Hypersensitivity to 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) as described previously.[18,22] 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. If a positive response (*e.g.*, abrupt paw withdrawal, licking, shaking) occurred, 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 paw withdrawal threshold (PWT), or (2) the upper or lower end of the von Frey hair set was reached before a positive/negative response had been obtained. The PWT was determined according to the formula provided by Dixon.[23]

Tracheotomy and Mechanical Ventilation

Animals were anesthetized intraperitoneally with 45–50 mg/kg pentobarbital and a tracheotomy was performed. Rats were ventilated mechanically (50–70 cycles/min, inspiratory pressure, 10–14 cm H_2O; Kent Scientific Corporation, Torrington, CT). During neurophysiologic experiments, rats were anesthetized intraperitoneally with 1.5% isoflurane and paralyzed with pancuronium bromide (1–2 mg/kg; Elkins-Sinn, Inc., Cherry Hill, NJ) *via* intermittent intraperitoneal injections given as needed (1 mg · kg · h). Sufficient depth of anesthesia 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 of heart rate (300–350 beats/min) during paralysis. Core body temperature was kept in the normal range (36.0–37.0°C).

Spinal Dorsal Horn Recordings

The experimental setup and procedure are illustrated in the schematic diagram (figs. 1A and B). A long T10–L3 laminectomy was performed, and the dura mater was incised and retracted. Extracellular recordings of single dorsal horn neuron activity were obtained with microelectrodes as described previously.[9] Analog data were collected with a real-time computer-based data acquisition and processing system (DAPSYS 6; Brian Turnquist, Johns Hopkins University, Baltimore, MD). To avoid potential pitfalls in data interpretation

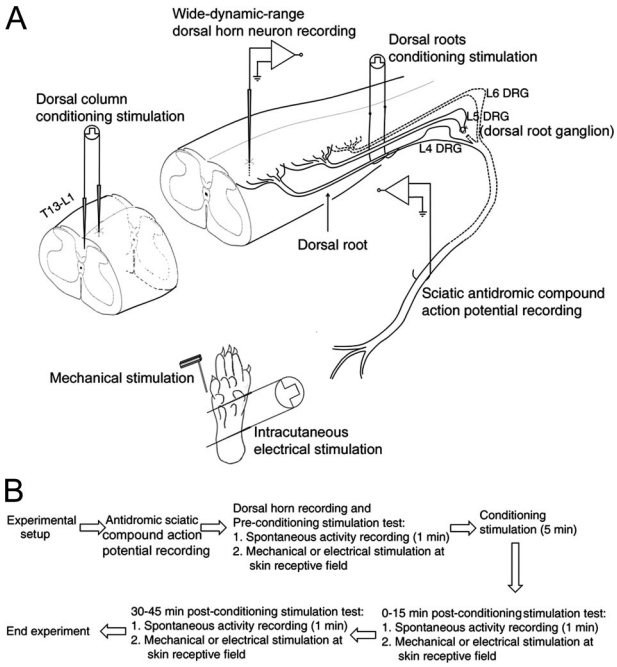

Fig. 1. Schematic diagram illustrating experimental setup and procedures. (*A*) The antidromic compound action potentials evoked by bipolar electrical stimulation (0.2 ms, 0.01–3.0 mA) at the dorsal column (T13–L1 spinal level) and the lumbar dorsal roots were recorded at the sciatic nerve with a monopolar recording electrode. Extracellular recordings of dorsal horn neurons were obtained with a microelectrode inserted within the L4 spinal segment. Mechanical and intracutaneous electrical test stimuli were applied to the skin receptive field of the dorsal horn neuron. (*B*) Schematic of the experimental paradigm used in neurophysiologic studies.

from different neurophysiologic properties of WDR cells in superficial *versus* deep dorsal horn and in injured *versus* noninjured spinal segments,[9,11,24] we examined WDR neurons located at deep laminae (III–V, 400–1200 μm below dorsal surface) in the noninjured 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.[9,25] Mechanical search stimuli consisted of stroking the plantar skin with a cotton swab, mild pinching with the experimenter's fingers, and pinching with serrated forceps. Only WDR neurons with defined receptive fields (RFs) in the plantar region of the hind paw were studied. Rats were euthanized (100–300 mg, intraperitoneal sodium pentobarbital) at the end of the experiment.

Recording of Sciatic Compound APs Evoked by Dorsal Column and Root Stimulation

The left sciatic nerve and its branches were exposed and dissected from surrounding tissue. A monopolar silver hook electrode was placed on the sciatic nerve at the mid-thigh level for recording compound APs. The reference electrode was placed in the nearby muscle. For dorsal column stimulation, two tungsten needle electrodes (insulated except for the most distal 0.3–0.5 mm) were inserted into the ipsilateral dorsal column at the T13–L1 level (*i.e.*, tip 0.5 mm below spinal cord surface). The dorsal root stimulation was applied through a pair of platinum hook electrodes placed underneath the L4 and L5 dorsal roots.

Experimental Design

Given that there are possible differences in WDR neuronal excitability changes associated with "allodynic" *versus* "nonallodynic" animals,[26] nerve-injured rats that did not show mechanical hypersensitivity (*i.e.*, PWT decrease of more than 50% from the preinjury level at day 5 postinjury and at 2–3 days before planned electrophysiologic recordings) were excluded from electrophysiologic studies to prevent potential pitfalls in data analysis.[18]

Study 1: To Examine Antidromic Sciatic Compound APs Evoked in Response to Graded Electrical Stimulation Applied to Ipsilateral Dorsal Column or Lumbar Dorsal Roots. To standardize the intensities for selectively activating Aα/β-fibers without activating Aδ-fibers for each stimulation site, we took advantage of the fact that AP initiation at a point along the axon leads to AP propagation both antidromically and orthodromically, and that the area under the Aα/β-compound AP waveform at the sciatic nerve is proportional to the number of afferent fibers activated by electrical stimulation. Therefore, we recorded antidromic sciatic compound APs evoked by graded electrical stimulation (0.01–3.0 mA, 0.2 ms) applied to the two sites. Different compound AP waveforms corresponding to Aα/β- and Aδ-fiber activation were distinguished on the basis of the activation threshold and the conduction velocity (CV). For each site, we determined online the Aα/β-plateau intensity (lowest intensity to evoke a peak Aα/β-compound AP without inducing an Aδ- or C-fiber component, fig. 2A) for later use as conditioning stimulus. In off-line analysis, the areas under the Aα/β- and Aδ-compound AP waveforms generated by graded electrical stimulation were measured to establish the stimulus-response (S-R) functions (see Supplemental Digital Content 1, which is the figure for this experiment, http://links.lww.com/ALN/A648). For each stimulation site, we compared plateau intensities and S-R functions of the Aα/β-compound APs between different experimental groups. The Aα/β-plateau intensities of the two stimulation sites were also compared within an experimental group.

Study 2: To Examine the Effects of Conditioning Stimulation on Spontaneous Activity of WDR Neurons. An increased spontaneous activity rate in WDR neurons may underlie spontaneous pain after nerve injury and contribute to central sensitization.[3,8,27] We investigated whether conditioning stimulation attenuates increased spontaneous firing of WDR cells in nerve-injured rats. The spontaneous activity of WDR neurons was recorded for 1 min, followed by the preconditioning stimulation test in studies 3 and 4. Then, bipolar conditioning stimulation was applied to the dorsal column or lumbar dorsal roots. Dorsal horn recording was stopped during conditioning stimulation because of significant stimulation artifacts. At 0–15 min and 30–45 min after cessation of conditioning stimulus, spontaneous

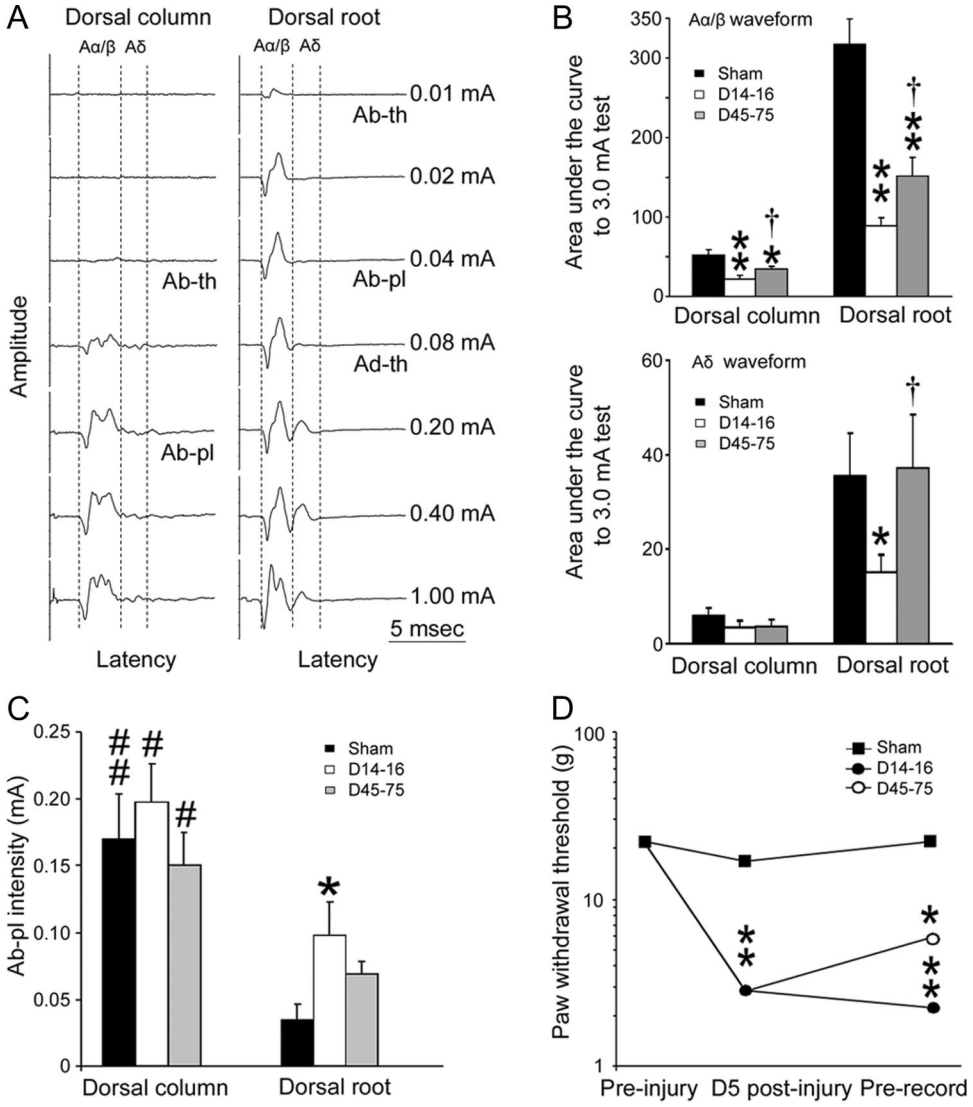

Fig. 2. Conditioning stimulation intensity was determined by recording the antidromic sciatic compound action potential. (*A*) Sciatic compound action potentials evoked by dorsal root stimulation usually revealed two distinct groups of waves corresponding to Aα/β and Aδ fiber activation. The Aδ component to dorsal column stimulation is often hard to differentiate or missing. (*B*) The areas under the Aα/β and Aδ curves/waveforms in response to 3.0-mA stimulation at the dorsal column and the dorsal roots were plotted. Data are expressed as mean ± SEM. *$P < 0.05$. **$P < 0.01$ *vs.* sham-operated group. †$P < 0.05$ *versus* day 14–16 post–spinal nerve ligation. (*C*) The Aα/β plateau for each stimulation site was plotted. Data are expressed as mean ± SEM. *$P < 0.05$ *versus* sham-surgery group. #$P < 0.05$. ##$P < 0.01$ *versus* dorsal root stimulation. (*D*) The ipsilateral paw withdrawal threshold was significantly decreased from preinjury baseline at day 5 postinjury and 2–3 days before the electrophysiologic recording dates (prerecord). Data are expressed as median.*$P < 0.05$. **$P < 0.01$ *versus* corresponding preinjury baseline. Ab-pl = Aα/β plateau, the lowest stimulus intensity that evokes a peak Aα/β component without inducing an Aδ component; Ab-th = Aα/β threshold; Ad-th = Aδ threshold.

activity was recorded for 1 min followed by postconditioning stimulation tests (fig. 1B). A small group of sham-operated rats was also included to blind the experimenter to animal group assignments.

Study 3: To Examine the Effects of Conditioning Stimulation on WDR Neuronal Response to Mechanical Stimulation Applied to the Skin Receptive Field. Mechanical hypersensitivity is an important and characteristic manifestation of neuropathic pain, but its underlying mechanisms remain undefined.[3,27,28] Brushing may elicit dynamic allodynia, and von Frey filaments may elicit punctate hyperalgesia in patients. Therefore, we studied WDR neurons in the ipsilateral L4 spinal segment that had a defined RF in the plantar region of the hind paw. We briefly mapped the RF with a 10-g von Frey monofilament. A "sensitive site" in the RF was identified for application of von Frey stimulation. We recorded the evoked neuronal responses to a series of mechanical stimuli consisting of brushing across the RF with a small camel hair brush (five applications at 1 Hz) and indentation of the plantar skin with increasing forces of von Frey monofilaments (0.2–26 g, 5 s). The same test module was applied before conditioning stimulation and at 0–15 min and 30–45 min after conditioning stimulation.

Study 4: To Examine the Effects of Conditioning Stimulation on WDR Neuronal Responses to Graded Intracutaneous Electrical Stimuli. The A- and C-fiber–mediated responses to mechanical stimuli are not readily differentiated in WDR neurons. In contrast, the WDR neuronal response to a suprathreshold electrical stimulus consists of an early A-fiber component and a later C-fiber component.[12] This unique feature of WDR neuronal response to an electrical stimulus allows us to examine the effects of conditioning stimulation on both A- and C-fiber-mediated activities in the same neuron. The intensity of a constant current electrical stimulus is also easier to quantify and more highly repeatable than natural stimuli. A pair of fine stimulating electrodes was inserted subcutaneously in the RF at the plantar area of the hind paw and positioned orthogonal to the paw axis (fig. 1A). The evoked responses to graded intracutaneous electrical stimuli (0.1–10.0 mA, 2.0 ms, 15-s intervals) were examined in both nerve-injured and sham-operated rats. The S-R functions of the A- and C-components of the WDR neuronal response were then determined. The electrical thresholds for activation of the A- and C-components were defined as the lowest milliampere stimulus current to evoke an AP firing within the range of the A- and C-fiber latencies, respectively. If the threshold after the conditioning stimulation was greater than the maximum stimulator power (10 mA), the value of 15 mA was assigned as the cut-off threshold. The same test module was repeated at 0–15 min and 30–45 min after conditioning stimulus. This study was conducted in a separate group of animals from that used in study 3.

Study 5: To Examine the Effect of Conditioning Stimulation on Windup of WDR Neuronal Response to Repetitive Electrical Stimulation of the Receptive Field. The C-fiber-mediated AP windup phenomenon is prominent and highly repeatable in WDR neurons. We examined the effects of conditioning stimulation at the dorsal column and lumbar dorsal roots on windup of WDR neuronal response to a train of 16 intracutaneous electrical pulses (supra-C-fiber threshold, 2.0 ms) applied at 0.5 Hz.[9,11] At 30 s after 0.5-Hz stimulation, when the after-discharges of WDR neurons had mostly diminished, another 12 pulses at 0.1 Hz were delivered. Because 0.1-Hz stimulation rarely induces windup under physiologic conditions, it was used as a negative control for 0.5-Hz stimulation. The same windup test was also repeated at 0–15 min and 30–45 min after conditioning stimulus. Studies 4 and 5 were carried out in the same animals with the same intracutaneous electrodes.

Data Analysis

The S-R functions of WDR neurons to graded mechanical and electrical stimuli were compared between the precondition and postcondition stimulation conditions in each group using a two-way repeated measures analysis of variance (ANOVA). A one-way repeated measures ANOVA was used to compare spontaneous activity, total responses to mechanical and graded electrical stimuli between the preconditioning and postconditioning stimulation conditions in each group. The S-R functions of sciatic compound APs were compared between different experimental groups with a two-way mixed model ANOVA. The Tukey test was used to compare specific data points. Because the PWT data and C-threshold to graded electrical stimulation were discrete data points with cut-off values, the data were not normally distributed. Accordingly, data were presented as medians, and nonparametric ANOVA (Friedman and Kruskal-Wallis tests) was used to analyze the threshold data with Wilcoxon signed rank and Mann-Whitney tests.

The number of APs in the C-component evoked by each stimulus in the train was used to plot windup curves/functions against the stimulation number of the train. Absolute windup was the total number of APs in C fiber-component evoked by the 0.5-Hz train at 16× input. Input was defined as the number of APs evoked by the first stimulus of the 0.5-Hz train. For each group, a two-way repeated measures ANOVA with Tukey test was used to compare differences in windup response between preconditioning and postconditioning stimulation conditions. A one-way repeated-measures ANOVA with Tukey test was used to compare the absolute windup between the preconditioning and postconditioning stimulation conditions. When a Student *t* test was used for specific analysis, all comparisons were made with Bonferroni adjustments. STATISTICA 6.0 software (StatSoft, Inc., Tulsa, OK) was used to conduct all statistical analyses. Unless otherwise specified, two-tailed tests were performed, data are expressed as mean ± SEM, and $P < 0.05$ was considered statistically significant in all tests.

Results

Characterization of the Antidromic Sciatic Compound AP Evoked by Stimulation of the Dorsal Column and Root in Sham-operated and Nerve-injured Rats

At suprathreshold intensity, the sciatic compound AP evoked by dorsal root stimulation often revealed two distinct groups of waves (fig. 2A). The fast component corresponds to the Aα/β-fiber activation (CV, 15.6 ± 0.2 to 49.9 ± 1.5 m/s). The slower component, referred to as the Aδ-compound AP, usually had a smaller amplitude than the fast Aα/β component, and could be distinguished by a higher threshold and slower CV (9.21 ± 0.2 to 15.6 ± 0.2 m/s) than the Aα/β component. We used similar CV ranges to separate different compound APs evoked by dorsal column stimulation (Aα/β, 15.2 ± 0.3 to 34.0 ± 1.5 m/s; Aδ, 10.1 ± 0.2 to 15.2 ± 0.3 m/s). These CVs are comparable to those reported previously.[29] The area under the waveform of each component was measured off-line and plotted against the stimulus intensity to establish the S-R function (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648).

Effects of Dorsal Column Stimulation. The S-R functions (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648) and peak Aα/β-compound APs to 3.0 mA stimulation were significantly lower in the nerve-injured groups (day 14–16, 21.4 ± 5.1, $P < 0.01$; day 45–75, 34.1 ± 3.6, $P < 0.05$) than in the sham-operated group (52.2 ± 7.6, fig. 2B); both values

were significantly greater at 45–75 days after SNL than at 14–16 days post-SNL ($P < 0.05$). However, the size of the Aα/β-compound AP reached a plateau near 0.5 mA in both sham-operated and nerve-injured groups (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648). The Aα/β-plateau intensities measured online were comparable among the three groups (sham, 0.170 ± 0.033 mA, n = 14; day 14–16, 0.197 ± 0.028 mA, n = 24; day 45–75, 0.150 ± 0.024 mA, n = 18; fig. 2C). The Aδ component was often missing or hard to differentiate, likely because of the small number of Aδ fibers that travel in the dorsal column (figs. 2A and B). At any given postinjury time point, Aα/β-plateau intensity was significantly greater for dorsal column stimulation than for dorsal root stimulation (sham, $P = 0.002$; day 14–16, $P = 0.024$, and day 45–75, $P = 0.031$; fig. 2C).

Effects of Dorsal Root Stimulation. The Aα/β-compound AP remained at a plateau level in response to stimulation intensities between 0.03–0.4 mA in sham-operated and nerve-injured groups (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648). Yet, the size of the Aα/β-compound AP increased again with additional increases in the stimulus intensity until it reached the next higher plateau level at 1.0 mA in all groups. The S-R functions (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648) and peak area under the curve (3.0 mA, fig. 2B) of the Aα/β-compound AP were significantly higher in the sham-operated group (317.4. ± 31.6, n = 16) than in the nerve-injured groups (day 14–16, 88.4 ± 10.3, $P < 0.01$, n = 22; day 45–75, 151.9 ± 23.4, $P < 0.01$, n = 16). The Aα/β-plateau intensity measured online was significantly higher 14–16 days post-SNL (0.098 ± 0.024 mA) than in the sham-operated group (0.035 ± 0.012 mA, $P = 0.021$, fig. 2C). There was a significant recovery in S-R function (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648) and peak area under the curve ($P < 0.05$, fig. 2B) of the Aα/β-compound AP 45–75 days post-SNL as compared with day 14–16 post-SNL. An Aδ-compound AP can often be observed in response to dorsal root stimulation at higher intensities. According to S-R functions, the Aδ-compound AP gradually increased from the baseline with stimulus intensity greater than 0.5 mA, and reached a plateau at a stimulus intensity of 1.0 mA. The S-R functions (see Supplemental Digital Content 1, http://links.lww.com/ALN/A648) and peak area under the curve (fig. 2B) of the Aδ component were significantly higher in the sham-operated group (35.6 ± 8.9) than at day 14–16 post-SNL (15.3 ± 3.6, $P = 0.034$), but were comparable to those observed at day 45–75 post-SNL (37.2 ± 11.2).

The ipsilateral PWT of nerve-injured rats included in the current study was significantly decreased from the preinjury baseline at day 5 postinjury and 2–3 days before electrophysiologic recording dates (fig. 2D).

Stimulation of Dorsal Column and Root Attenuated the Increased Spontaneous Discharges of WDR Neurons in Nerve-injured Rats

Effects of Dorsal Column Stimulation. Before conditioning stimulation, the spontaneous activity rate (APs/min) of

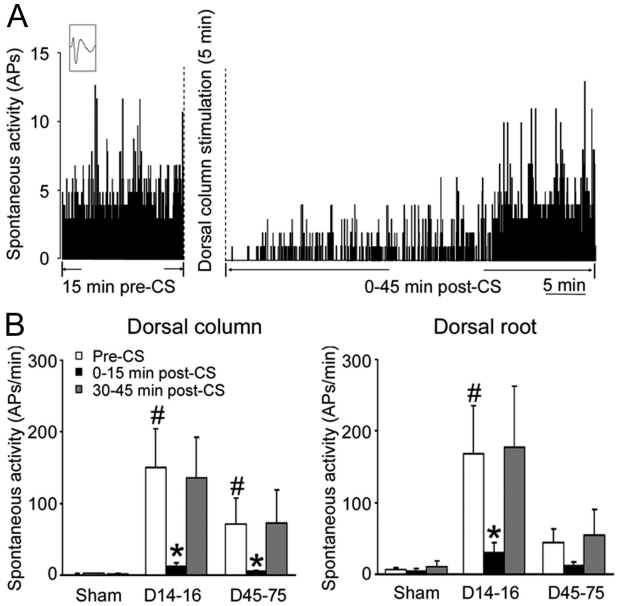

Fig. 3. Stimulation of the dorsal column and dorsal root inhibited the spontaneous activity of wide dynamic range (WDR) neurons in nerve-injured rats. (*A*) Peristimulus time histogram shows the spontaneous WDR neuronal activity before and 0–45 min after dorsal column stimulation (5 min, 0.2 ms, Aα/β plateau, bin size: 2.0 s). (*B*) The spontaneous activity rates decreased significantly 0–15 min after conditioning stimulation (CS) at the dorsal column (day 14–16, n = 25; day 45–75, n = 19) or root (day 14–16, n = 22). All data are presented as mean ± SEM unless otherwise specified. *$P < 0.05$ *versus* corresponding pre-CS value. #$P < 0.05$ *versus* sham-operated group (n = 8–9) pre-CS. AP = action potential.

WDR neurons was significantly higher at 14–16 days (150.3 ± 53.2 APs/min, $P = 0.011$, n = 25) and at 45–75 days post-SNL (71.6 ± 36.0 APs/min, $P = 0.034$, n = 19), compared with that in sham-operated rats (2.9 ± 26 APs/min, n = 9, figs. 3 A and B). At 0–15 min poststimulation, spontaneous activity rates were significantly decreased to 12.0 ± 5.0 APs/min (day 14–16, $P = 0.016$) and 4.7 ± 2.3 APs/min (day 45–75, $P < 0.047$) from the respective prestimulation baseline, but gradually returned to the prestimulation level 30–45 min poststimulation (fig. 3B).

Effects of Dorsal Root Stimulation. Before conditioning stimulation, the spontaneous activity rate was significantly higher in rats 14–16 days post-SNL (167.3 ± 67.6 APs/min, $P = 0.021$, n = 22) than in sham-operated rats (6.0 ± 3.3 APs/min, n = 8, fig. 3B). At 0–15 min after conditioning stimulation of the ipsilateral L4 and L5 dorsal roots, the spontaneous activity rate decreased significantly to 30.2 ± 13.7 APs/min in the day14–16 post-SNL group ($P = 0.036$); however, the decrease in spontaneous activity rate in the day 45–75 post-SNL group (11.1 ± 6.0 APs/min) was not significant, as compared to the prestimulation value (43.4 ± 19.5 APs/min, $P = 0.060$, n = 22).

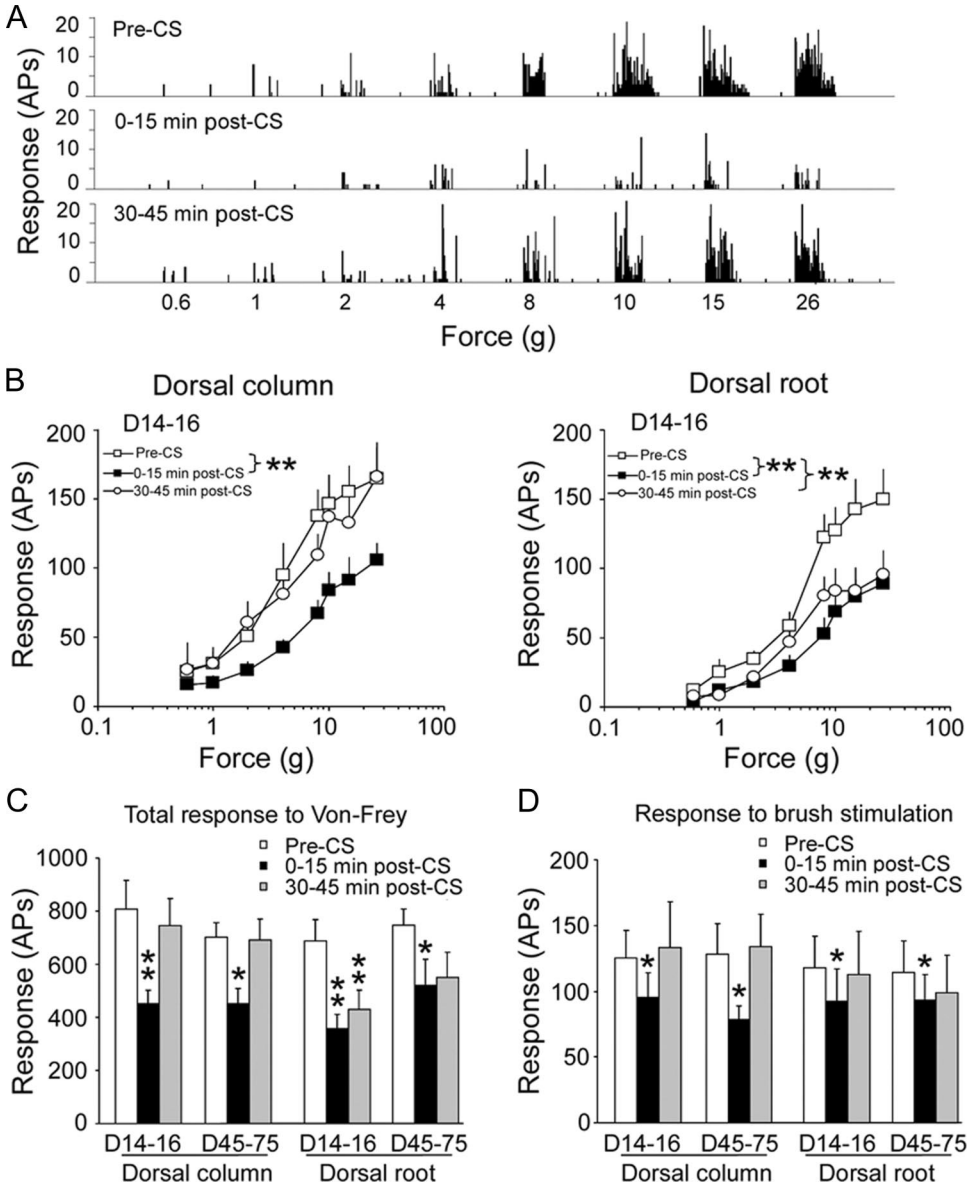

Fig. 4. Stimulation of the dorsal column and dorsal root attenuated the evoked mechanical responses of wide dynamic range (WDR) neurons in nerve-injured rats. (*A*) Peristimulus time histograms (bin size: 0.2 s) show an example of WDR neuronal response to punctate mechanical stimuli (0.6–26.0 g von Frey probe, 5 s) applied to the skin receptive field before and after dorsal column conditioning stimulation (CS). (*B*) At 14–16 days postinjury (n = 10), the stimulus-response functions of WDR neuronal response to mechanical stimuli were significantly attenuated 0–15 min after CS at the dorsal column and roots. The total responses of WDR neurons to graded mechanical (*C*) and brushing (*D*) stimuli were plotted for each group. All data are presented as mean ± SEM unless otherwise specified. $^{*}P < 0.05$. $^{**}P < 0.01$ *versus* prestimulation value. AP = action potential.

Stimulation of the Dorsal Column and Root Significantly Inhibited the Evoked Responses of WDR Neurons to Mechanical Stimuli in Nerve-injured Rats

Effects of Dorsal Column Stimulation. The conditioning stimulation inhibited WDR neuronal response to punctate mechanical stimuli (0.6–26.0 g von Frey probe, 5 s; fig. 4A). The S-R functions at 0–15 min poststimulation were significantly lower than those at the prestimulation level at both postinjury time points (day 14–16, $P = 0.006$, n = 10, fig. 4B; day 45–75, $P = 0.013$, n = 7, data not shown). The same was true for total responses of WDR neurons to graded punctate mechanical stimuli (0.6–26.0 g von Frey probe, 5 s; total number of APs prestimulation *vs.* poststimulation: day 14–16, 808 ± 109 *vs.* 450 ± 52 APs, $P = 0.009$; day 45–75, 703 ± 54 *vs.* 451 ± 58 APs, $P = 0.019$, fig. 4C). The response to brushing stimuli was also significantly reduced in the day 14–16 post-SNL group from 125 ± 21 to 95 ± 19 APs ($P = 0.037$) and in the day 45–75 post-SNL group from 128 ± 24 to 78 ± 10 APs ($P = 0.025$, fig. 4D). The inhibition largely diminished 30–45 min after conditioning stimulation.

Effects of Dorsal Root Stimulation. The dorsal root conditioning stimulation also significantly attenuated S-R functions at both postinjury time points (day 14–16, $P = 0.002$,

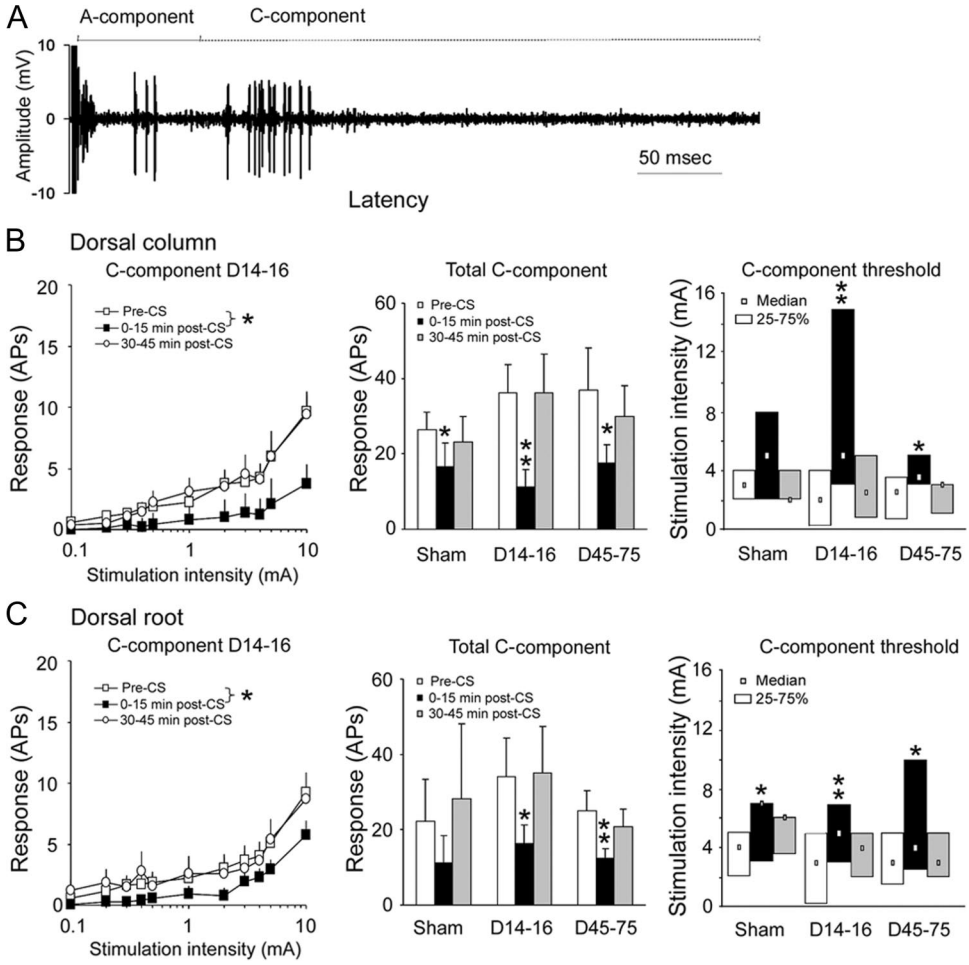

Fig. 5. Dorsal column and root conditioning stimulation (CS) inhibited the C-component of wide dynamic range (WDR) neuronal response to graded intracutaneous electrical stimuli. (*A*) Analog recordings of WDR neuronal responses displaying A- and C-components to an intracutaneous electrical stimulus (supra–C-fiber activation threshold, 2.0 ms). (*B, C*) At day 14–16 postinjury, the stimulus-response function and total number of action potentials (APs) in the C-component were significantly attenuated 0–15 min after dorsal column (n = 17) and root (n = 15) CS. Dorsal column stimulation reduced the C-component in sham-operated rats (n = 6) and at 45–75 days postinjury (n = 11). Dorsal root stimulation reduced the C-component only at 45–75 days postinjury (n = 16). All data are presented as mean ± SEM unless otherwise specified. The box-and-whisker plot shows the median threshold intensity for activation of the C-component. $^{*}P < 0.05$. $^{**}P < 0.01$ *versus* prestimulation value.

n = 8, fig. 4B; day 45–75, $P = 0.003$, n = 6, data not shown). The inhibition remained significant at 30–45 min after stimulation at the 14–16 day post-SNL time point ($P = 0.007$, fig. 4B). At 0–15 min poststimulation, the number of total responses to punctate mechanical stimuli (prestimulation *vs.* poststimulation: day 14–16, 673 ± 76 *vs.* 355 ± 56 APs, $P = 0.002$; day 45–75, 745 ± 62 *vs.* 521 ± 95 APs, $P = 0.019$, fig. 4C) and to brush stimulus (day 14–16, 118 ± 24 *vs.* 92 ± 24 APs, $P = 0.029$; day 45–75, 114 ± 23 *vs.* 93 ± 19 APs, $P = 0.031$, fig. 4D) was significantly reduced in both groups.

Stimulation of the Dorsal Column and Root Significantly Decreased the C-component of the WDR Neuronal Response to Graded Intracutaneous Electrical Stimuli in Sham-operated and Nerve-injured Rats

Effects of Dorsal Column Stimulation. WDR neuronal responses showed an early A-fiber component (0–75 ms) and a later C-fiber component (75–400 ms) to an intracutaneous electrical stimulus (fig. 5A). At 0–15 min after conditioning stimulation, S-R functions (fig. 5B, sham and day 45–75 post-SNL, data not shown) and the total number of APs in the C-component to graded intracutaneous stimuli (0.1–10 mA, 2 ms) were significantly decreased in all groups (prestimulation *vs.* poststimulation: sham, 26.3 ± 4.7 *vs.* 16.5 ± 6.3 APs, $P = 0.038$, n = 6; day 14–16, 36.2 ± 7.5 *vs.* 11.3 ± 4.6 APs, $P = 0.001$, n = 17; day 45–75, 37.0 ± 10.9 *vs.* 17.5 ± 4.7 APs, $P = 0.021$, n = 11, fig. 5B). The median threshold intensity for activation of the C-component was significantly increased from the prestimulation value at day 14–16 ($P = 0.004$) and at day 45–75 post-SNL ($P = 0.011$, fig. 5B), but not in the sham-operated group ($P = 0.14$). Dorsal column stimulation did not significantly affect the A-component or its activation threshold in any group (data not shown).

Effects of Dorsal Root Stimulation. S-R functions were significantly attenuated (fig. 5C, sham and day 45–75 post-

SNL data not shown) and the median thresholds of the C-component were significantly increased from prestimulation values (sham, $P = 0.027$, n = 7; day 14–16, $P = 0.017$, n = 15; day 45–75, $P = 0.02$, n = 16; fig. 5C) in all three groups 0–15 min after conditioning stimulation. In addition, the total number of APs in the C-component (fig. 5C), but not in the A-component (data not shown), was significantly decreased from the respective prestimulation level at both post-SNL time points (prestimulation *vs.* poststimulation: day 14–16, 34.2 ± 10.1 *vs.* 16.2 ± 4.8 APs, $P = 0.025$; day 45–75, 24.8 ± 5.5 *vs.* 12.3 ± 2.6 APs, $P < 0.001$; sham, 22.3 ± 11.2 *vs.* 11.6 ± 7.1 APs, $P = 0.078$, fig. 5C).

Dorsal Column Stimulation, but not Dorsal Root Stimulation, Significantly Inhibited Windup in Nerve-injured Rats

Windup was induced by 0.5-Hz stimulation (fig. 6A), but not by 0.1-Hz stimulation applied 30 s later, in both sham-operated and nerve-injured rats before dorsal column stimulation. At 0–15 min poststimulation, windup functions to 0.5-Hz stimulation were significantly depressed in all groups (sham, $P = 0.021$, n = 6, fig. 6B; day 14–16, $P = 0.012$, n = 17, fig. 6B; day 45–75, $P = 0.03$, n = 11, data not shown). The absolute windup was also significantly decreased from the respective prestimulation value in each group (sham, $P = 0.035$; day 14–16, $P = 0.022$; day 14–16, $P = 0.037$, fig. 6D) but was partially recovered at 30–45 min poststimulation. In a separate study, dorsal root stimulation significantly attenuated the windup function ($P = 0.010$) and reduced the absolute windup ($P = 0.016$) to 0.5-Hz stimulation in the sham-operated group 0–15 min poststimulation (n = 7, figs. 6C and D). However, it did not significantly decrease windup in the nerve-injured rats (day 14–16, n = 15; day 45–75, n = 16, figs. 6C and D).

Comparison of Peak Inhibitory Effects Produced by Dorsal Column and Root Stimulation

The inhibitory effects of dorsal column *versus* dorsal root conditioning stimulation on the spontaneous activity rate of WDR neurons at 0–15 min poststimulation were not significantly different at either postinjury time point tested (fig. 7A). To compare their peak inhibitory effects on the mechanical response, the total response to graded von Frey mechanical stimuli at 0–15 min poststimulation was normalized by the corresponding prestimulation values. At day 14–16 post-SNL, relative responses (% prestimulation) were 62.2 ± 6.9% and 52.7 ± 7.9% after stimulation of the dorsal column and dorsal root, respectively (fig. 7B). Relative responses were also comparable between the two sites of stimulation at day 45–75 post-SNL (66.5 ± 8.9 *vs.* 67.9 ± 10.0%). To compare inhibition of the C-component caused by dorsal column or dorsal root stimulation, the C-component response at 0–15 min poststimulation was normalized by the respective prestimulation value. The relative responses were not significantly different between the two stimulation sites in the sham-operated group (dorsal column *vs.* dorsal root: 56.6 ± 18.9 *vs.* 49.7 ± 10.7%) or at day 45–75 post-SNL (51.1 ± 9.2 *vs.* 65.6 ± 17.2%). However, at day 14–16 post-SNL, the relative response after dorsal column stimulation (29.4 ± 7.4%) was significantly less than that after dorsal root stimulation (52.1 ± 8.2%, $P = 0.048$, fig. 7C). The relative decreases in absolute windup at 0–15 min poststimulation were comparable between dorsal column (−68.5 ± 18.2%) and root (−66.8 ± 19.4%) stimulation in the sham-operated group. However, in nerve-injured rats, this value was significantly greater after dorsal column (day 14–16, −84.9 ± 36.5%, $P = 0.011$; day 45–75, −68.9 ± 16.5%, $P = 0.041$) than root (day 14–16, −4.3 ± 15.6%; day 45–75, −26.8 ± 11.3%, fig. 7D) stimulation. Relative change of absolute windup was calculated as follows: poststimulation value − prestimulation value/prestimulation value ×100%.

Discussion

Both dorsal column and root conditioning stimulation (1) attenuated the nerve injury–induced elevation in WDR neuron spontaneous activity rate during the acute phase of neuropathic pain at day 14–16 post-SNL, (2) inhibited the evoked responses of WDR neurons to mechanical stimuli during the acute and chronic (day 45–75 post-SNL) phases of neuropathic pain, (3) inhibited the C-fiber–mediated response of WDR neurons to graded intracutaneous electrical stimuli in nerve-injured and sham-operated rats, and (4) blocked windup in sham-operated rats. Dorsal column stimulation also significantly inhibited windup in nerve-injured rats.

Features of Conditioning Stimulation–induced Inhibition of WDR Neuronal Activity Mimic Spinal Cord Stimulation–induced Pain Relief

Ongoing pain and allodynia in patients are components of neuropathic pain attenuated by spinal cord stimulation.[4,30,31] In animal models of neuropathic pain, increased spontaneous discharges in peripheral and dorsal horn neurons may underlie ongoing pain and the prolongation of neuropathic pain.[3,27,32] Bipolar conditioning electrical stimuli applied to the ipsilateral dorsal column and lumbar dorsal roots inhibited both spontaneous discharges and the evoked mechanical responses of WDR neurons in nerve-injured rats. These findings are in line with previous studies showing that monopolar electrical stimulation applied to the dorsal aspect of the cord suppressed the responses of spinothalamic tract neurons to noxious somatic stimuli and normalized the hyperexcitability of WDR neurons in neuropathic rats.[26,33] Similar to previous observations,[26] the duration of neuronal inhibition exceeded the short conditioning stimulation period. This scenario is likely because of the slow release and sustained actions of inhibitory neurotransmitters and changes in gene expression.[34,35] These features of dorsal column stimulation–induced inhibition of WDR neuronal activity are consistent with actions of spinal cord stimulation in patients and those predicted by computer models.[4,36] Because the dorsal column is in close proximity to the epidural lead in patients, the dorsal column stimulation–induced in-

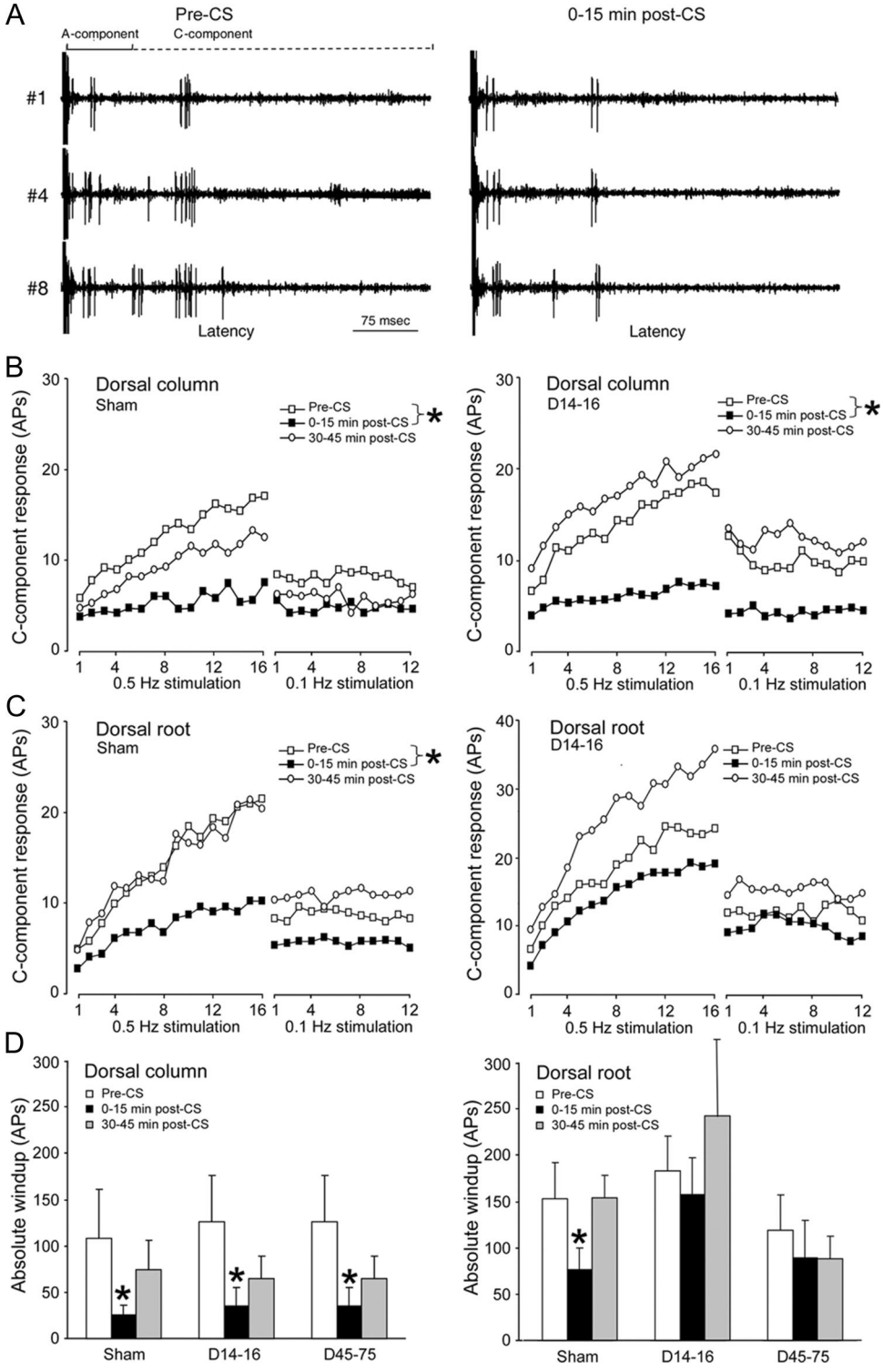

Fig. 6. Conditioning stimulation (CS) at the dorsal column, but not at the dorsal roots, significantly inhibited windup in nerve-injured rats. (*A*) An analog recording of wide dynamic range neuronal response to the first, fourth, and eighth stimulus of a train of intracutaneous electrical stimuli (0.5 Hz, 16 pulses, 2.0 ms) before and 0–15 min after dorsal column CS. (*B, C*) The C-component to 0.5-Hz stimulation and 0.1-Hz stimulation were plotted against the stimulation sequence number of each trial. The dorsal column stimulation significantly attenuated the windup functions in sham-operated (n = 6) and day 14–16 postinjury groups (n = 17). The dorsal root stimulation attenuated windup function in the sham-operated group (n = 7), but not at day 14–16 postinjury (n = 15). For clarity, error bars are not shown. (*D*) The absolute windup was significantly decreased in each experimental group 0–15 min after dorsal column stimulation (day 45–75, n = 11); it was significantly decreased only in the sham-operated group after dorsal root stimulation (day 45–75, n = 16). All data are presented as mean ± SEM unless otherwise specified. *$P < 0.05$ *versus* prestimulation value. AP = action potential.

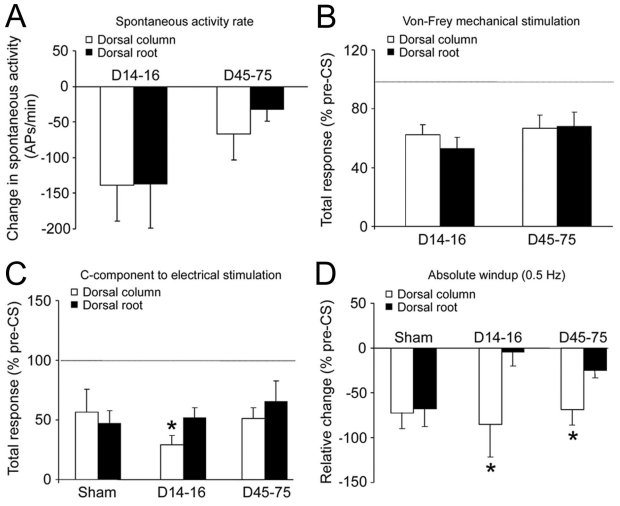

Fig. 7. Comparison of peak inhibitory effects (0–15 min) produced by dorsal column and root stimulation. (*A, B*) The decreases in spontaneous activity rate and total mechanical response of wide dynamic range neurons were comparable between the two sites of conditioning stimulation (CS) in nerve-injured rats. (*C*) Compared to dorsal root stimulation, dorsal column stimulation induced a significantly greater inhibition of the C-component response to graded intracutaneous electrical stimuli (0.1–10 mA, 2 ms) at 14–16 days postinjury. (*D*) The decrease in absolute windup was significantly greater after dorsal column stimulation than after dorsal root stimulation at the two postinjury time points. All data are presented as mean ± SEM unless otherwise specified. *$P <$ 0.05 *versus* dorsal root stimulation. AP = action potential.

hibition of WDR neuronal activity that we observed could be directly relevant to spinal cord stimulation–induced pain relief. Together, these findings suggest an important spinal cellular mechanism that may account, at least partially, for the efficacy and prolonged action of spinal cord stimulation in neuropathic pain patients.

Spinal cord–stimulating electrodes are usually placed several levels rostral to the affected spinal segment in patients. Therefore, the electrical field does not directly activate distal nerve roots. Yet, in our study, dorsal root stimulation inhibited spontaneous discharges and evoked mechanical responses of WDR neurons in nerve-injured rats to a similar degree as dorsal column stimulation. Accordingly, distal nerve roots that receive inputs from the affected painful area may also be useful targets for neuromodulatory control of pain. The dorsal root stimulation–induced neuronal inhibition may underlie, at least partially, the analgesia induced by peripheral nerve stimulation. The efficacy of spinal stimulation for pain relief may be improved by optimizing the lead configuration and incorporating stimulation of the distal roots. However, which dorsal root (*i.e.*, injured *vs.* uninjured) is important for the observed inhibitory actions is yet to be determined. By recording antidromic sciatic compound APs, we confirmed that conditioning stimulation at both sites selectively activated Aα/β-fibers. Recently, antidromic activation of large fibers was also recorded in lower limbs of patients during spinal cord stimulation.[37] This technique may provide an objective method to estimate the percentage of fibers that are activated during spinal cord stimulation. Compared to that observed in sham-operated rats, the Aα/β-compound AP was substantially smaller in rats at 14–16 days after axotomy-induced nerve injury. The mechanisms for the significant recovery of the Aα/β- and Aδ-compound APs observed 45–75 days postinjury remain unclear, but may involve nerve regeneration or collateral sprouting in the peripheral nerve distal to the ligation site.[38–40]

Inhibitory Actions of Conditioning Stimulation Support Gate-control Theory of Pain

According to the gate-control theory,[6] large fiber inputs should inhibit not only allodynia/hyperalgesia but also nociceptive pain. Here, we present direct *in vivo* electrophysiologic evidence for the predictions of the gate-control theory of pain under physiologic and neuropathic pain conditions. First, in sham-operated rats, both S-R function and windup of C-fiber–mediated responses in WDR neurons were significantly inhibited by stimulating large fibers either antidromically from the dorsal column or orthodromically from dorsal roots, indicating that spinal nociceptive transmission and pain sensitization are inhibited under normal conditions. Second, in rats at acute (day 14–16) and chronic (day 45–75) phases of neuropathic pain, both antidromic and orthodromic stimulation remained effective in attenuating the S-R function of C-fiber–mediated responses to graded electrical stimulation. However, unlike dorsal column stimulation, dorsal root stimulation failed to inhibit windup in neuropathic conditions. These findings indicate that the dorsal column may be a more effective treatment target than the dorsal roots under neuropathic conditions. However, how nerve injury decreases the susceptibility of windup to the inhibitory actions of the dorsal root stimulation remains to be determined.

Inhibition of the C-component, likely including inputs from low threshold unmyelinated afferents expressing the vesicular glutamate transporter 3,[41] may also contribute to a reduced mechanical response by the conditioning stimuli in nerve-injured rats. Indeed, dorsal column stimulation inhibited the nociceptive withdrawal flexion reflexes and attenuated C-fiber–mediated heat response in humans.[42,43] Yet, this notion of nonselective pain inhibition contradicts an earlier clinical observation that epidural spinal cord stimulation preferentially attenuates pathologic chronic pain, but does not affect, or only moderately affects, acute nociceptive pain.[44] In addition, monopolar electrical stimulation applied at the dorsal aspect of the cord normalized the neuronal hyperexcitability in "allodynic" rats after nerve injury, but did not suppress WDR neuronal activity in "nonallodynic" and control rats.[26] The same stimulation also increased the spontaneous discharge in approximately one-third of WDR neurons studied.[26] We postulate that these discrepancies may be partially related to differences in stimulation mode, site, and intensity used in these studies. Compared to the stimulation from a monopolar plate electrode placed over the cord, electrical fields produced by bipolar stimulation

through needle electrodes inserted into the dorsal column may be more focal. Therefore, bipolar stimulation in the current study may activate the ipsilateral dorsal column more efficiently (*i.e.*, at lower intensities) and also induce minimal activation of nearby structures (*e.g.*, gray matter and other tracts) that may also influence neuronal activity. The number of Aα/β-fibers activated by our conditioning stimulation at the Aα/β-plateau intensity may be greater than that excited by epidural stimulation at the motor threshold.[26] The muscle contraction at motor threshold 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 in the lower limbs.[45] So far, how motor thresholds in previous studies compare with Aα/β-plateau intensity remains unclear. However, the use of a miniature bipolar electrode (Medtronic, Inc., Minneapolis, MN) inserted into the epidural space of rats[31] in our preliminary work indicated that conditioning stimulation at the motor threshold activates only a small fraction of the Aα/β-fiber afferent population activated by stimulation at Aα/β-plateau intensity (unpublished data, Y. Guan, M.D., Ph.D., February 2010). Based on the gate-control theory, we postulate that activation of a greater number of Aα/β-fibers may lead to a stronger suppression of WDR neuronal excitability.

Potential Mechanisms Underlying the Neuronal Inhibitory Actions of Conditioning Stimulation

Spinal segmental mechanisms likely play an important role in conditioning stimulation–induced neuronal inhibition. A synchronized antidromic dorsal column volley could directly induce inhibitory postsynaptic potentials in dorsal horn neurons[33,46] and facilitate primary afferent depolarization to elicit presynaptic inhibition of incoming afferent inputs.[47] Neurons in superficial laminae, where most C-fibers terminate, have been thought to play an important role in inhibitory sensory modulation by spinal cord stimulation.[48,49] It is noteworthy that some superficial laminae interneurons that express γ-aminobutyric acid are activated by convergent Aβ-fiber inputs and may suppress the activity of nociceptive projection neurons.[50,51] Therefore, the conditioning stimulation may initiate a feed-forward activation of endogenous inhibition to restore the segmental pain inhibition that is compromised after injury.[52–55] Protein kinase C-γ–expressing interneurons that populate inner lamina II are also activated by large afferent fibers and may contribute to neuropathic pain.[56,57] Their roles in spinal cord stimulation–induced analgesia warrant further investigation. The intracellular mechanisms and neurochemistry of spinal cord stimulation–induced neuropathic pain relief remain unclear[4,58] but may involve enhanced release of inhibitory neuromodulators (*e.g.*, γ-aminobutyric acid, glycine, β-endorphin, acetylcholine) and reduced release of excitatory neurotransmitters in the spinal cord.[30,35,59,60] Although the primary action site of spinal cord stimulation may exist at the superficial dorsal horn, our study suggests that its inhibitory action could affect deep dorsal horn neuronal activity, in part because deep WDR neurons are functionally connected with superficial cells.[61,62]

In addition to the gate theory, other mechanisms may also contribute to conditioning stimulation–induced pain relief. For example, antidromic activity evoked by synchronized high-frequency dorsal-column stimulation may also reduce the afferent conduction safety factor in Aα/β-fibers.[63,64] This conduction blockade likely occurs where afferents in the dorsal column branch to the dorsal horn and may contribute to decreased mechanical response. Surprisingly, the same conditioning stimulation did not significantly reduce the A-component of WDR neurons in response to intracutaneous electrical stimulation. This apparent discrepancy may result from the conduction block on the barrage of activity induced by mechanical stimulation having a greater effect than a single AP evoked by the short electrical pulse. Although some large A fibers are nociceptors and the dysfunction of fibers in the dorsal column may contribute to neuropathic pain,[65,66] spinal cord stimulation does not induce pain in patients or in experimental animals; conditioning stimulation also rarely increased WDR neuronal excitability in the current study. We are also aware that the mechanisms of conditioning stimulation–induced inhibition of WDR neuronal activity may involve a complex set of interactions at several levels of the nervous system. For example, in addition to the dorsal column, conditioning stimulation may also activate other dorsal tracts that are in close proximity to the lead (*e.g.*, dorsolateral funiculus), and roles for supraspinal mechanisms in spinal cord stimulation–induced neuronal inhibition remain a topic of debate.[67–69] Furthermore, the prolonged "carry over" effect after spinal cord stimulation may involve long-term plastic change and remodeling in both spinal and supraspinal structures, in addition to the immediate and short-term actions predicated by the gate theory.

Previously, it was shown that monopolar electrical stimulation could inhibit established long-term potentiation,[70] a phenomenon that may share similar mechanisms with hyperalgesia.[71,72] Here, pretreatment with either dorsal column or root stimulation blocked C-fiber–mediated windup in sham-operated rats. Windup in WDR neurons represents a useful cellular model for studying mechanisms that might trigger the development of a persistent pain state and for testing central drug actions. Our study suggests that conditioning stimulation of putative spinal substrates with parameters similar to those used by patients with spinal cord stimulators not only inhibits spinal pain transmission (*e.g.*, attenuation of S-R functions to mechanical and graded electrical stimuli) and attenuates the established WDR neuronal hyperexcitability in the neuropathic condition, but also counteracts the progress of short-term neuronal sensitization to repetitive noxious inputs.[7] Thus, our study identified a potential biologic basis for the inhibition of pain by spinal cord stimulation and provided an *in vivo* cellular model to study the actions and mechanisms by which spinal cord stimulation provides therapeutic relief for neuropathic pain.[73] Future studies are warranted to examine whether pretreatment with

conditioning stimulation prevents the development of hyperalgesia and central sensitization.

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