Burst and Tonic Spinal Cord Stimulation Differentially Activate GABAergic Mechanisms to Attenuate Pain in a Rat Model of Cervical Radiculopathy

Nathan D. Crosby, Christine L. Weisshaar, Jenell R. Smith, Martha E. Zeeman, Melanie D. Goodman-Keiser, and Beth A. Winkelstein*

Abstract*—*Objective: **Spinal cord stimulation (SCS) is widely used to treat neuropathic pain. Burst SCS, an alternative mode of stimulation, reduces neuropathic pain without paresthesia. However, the effects and mechanisms of burst SCS have not been compared to conventional tonic SCS in controlled investigations. This study compares the attenuation of spinal neuronal activity and tactile allodynia, and the role of γ-aminobutyric acid (GABA) signaling during burst or tonic SCS in a rat model of cervical radiculopathy.** ***Methods:*** **The effects of burst and tonic SCS were compared by recording neuronal firing before and after each mode of stimulation at day 7 following a painful cervical nerve root compression. Neuronal firing was also recorded before and after burst and tonic SCS in the presence of the $GABA_B$ receptor antagonist, CGP35348.** ***Results:*** **Burst and tonic SCS both reduce neuronal firing. The effect of tonic SCS, but not burst SCS, is blocked by CGP35348. In a separate study, spinal cord stimulators were implanted to deliver burst or tonic SCS beginning on day 4 after painful nerve root compression; allodynia and serum GABA concentration were measured through day 14. Burst and tonic SCS both reduce allodynia. Tonic SCS attenuates injury-induced decreases in serum GABA, but GABA remains decreased from baseline during burst SCS.** ***Conclusion and Significance:*** **Together, these studies suggest that burst SCS does not act via spinal GABAergic mechanisms, despite its attenuation of spinal hyperexcitability and allodynia similar to that of tonic SCS; understanding other potential spinal inhibitory mechanisms may lead to enhanced analgesia during burst stimulation.**

***Index Terms*—Burst stimulation, γ-aminobutyric acid (GABA), neuropathic pain, radiculopathy, spinal cord stimulation (SCS).**

I. Introduction

SPINAL cord stimulation (SCS) has been used for decades to treat a wide range of chronic pain conditions [1]. In particular, cervical radiculopathy and cervical brachialgia are neuropathic conditions that cause neck, shoulder, and arm pain from spinal nerve and/or nerve root compression, and are commonly treated with SCS [2], [3]. Conventional tonic SCS administers continuous pulses of electrical stimulation with varying frequencies, pulse widths, and intensities to the dorsal columns from electrodes implanted in the epidural space outside of the spinal cord [1], [2], [4], [5]. Tonic stimulation also often induces tingling paresthesias in the dermatomal regions correlated with the area of the spinal cord where the stimulation is applied, which is used to guide electrode placement and SCS programming [3], [6].

Despite many positive reports of SCS being effective for treating chronic pain, the reported success rate of SCS in clinical studies has improved little [7]. Better understanding the neurophysiology of chronic pain and defining the mechanisms that underlie SCS-induced analgesia can help to more effectively design and administer SCS therapies.

In an effort to improve pain relief and to reduce side effects such as paresthesia, alternative modes of stimulation have been developed. Burst SCS is one such mode that uses periodic bursts of electrical pulses for pain management rather than the continuous pulses used in tonic SCS [3], [6], [8], [9]. In 2010, De Ridder *et al.* [3] reported that burst SCS may suppress neuropathic pain as well or potentially even better than tonic SCS, possibly because burst stimulation delivers more charge per second than tonic SCS to activate different neuronal populations while remaining below the threshold for paresthesia. Additional clinical studies, including placebo controlled studies, have also found that burst SCS decreases pain intensity more effectively, while inducing significantly less paresthesias, than tonic SCS [8], [9]. It has also been proposed that the differences between burst and tonic stimulation may be due to the modulation of the medial pain pathway by burst SCS, which could alter attention to pain rather than more effectively suppressing pain signals [6]. Since animal models provide valuable platforms for studying the mechanisms of SCS [10], initial comparisons of burst and tonic SCS were conducted in a rat model of visceral nociception [11]. However, no study has directly investigated the relative effectiveness of those modes of SCS in modulating neuronal activity, pain symptoms, and relevant mechanisms involved in nociception in the context of radicular pain.

Many neurophysiological and neurochemical mechanisms have been proposed as responsible for the analgesic effects of tonic SCS, including modulation of γ-aminobutyric acid (GABA) signaling in the dorsal horn. GABA is the major inhibitory neurotransmitter in the spinal cord, contributing to the

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inhibition of nociception in the spinal dorsal horn [12]. Mechanical allodynia after nerve injury is often accompanied by a decrease of GABA in the dorsal horn [13], [14], probably leading to a disinhibition of neuronal firing. Tonic SCS has been shown to stimulate GABA release in the dorsal horn that inhibits neuronal excitability and also attenuates the release of excitatory neurotransmitters, including glutamate [15], [16]. The analgesic effects of tonic SCS have been attributed specifically to $\mathrm{GABA_B}$ receptor activation, since analgesia can be augmented with $\mathrm{GABA_B}$ agonists [17] or abolished by spinal perfusion with $\mathrm{GABA_B}$ receptor antagonists [15]. Based on these findings, $\mathrm{GABA_B}$ agonists like baclofen have been used clinically to enhance the effects of spinal cord stimulation, especially in patients that do not experience sufficient pain relief from SCS alone [17], [18]. The role that GABA signaling through the $\mathrm{GABA_B}$ receptors plays in the analgesic effects of burst SCS remains unknown.

Cervical nerve root compression in the rat produces hallmarks of chronic neuropathic pain, including sustained behavioral hypersensitivity [19]–[21], sustained tissue compression and late axonal degeneration [21], [22], spinal neuroimmune activation [19], [22], [23], and spinal neuronal hyperexcitability [19], [24]. As such, this study compares burst and tonic SCS for reducing neuropathic pain signals and for modulating nociceptive mechanisms in a rat model of cervical nerve root compression. In two separate studies, burst and tonic SCS were compared in the context of GABAergic mechanisms for the attenuation of neuropathic pain. In the first study, electrophysiological recordings of dorsal horn neurons were acquired on day 7 after a painful nerve root compression, immediately before and at several times after acute burst or tonic SCS in order to compare the degree of attenuation of neuronal firing induced by each mode of stimulation. In order to compare the role of GABA signaling in burst and tonic SCS, the spinal cord was superfused with a $\mathrm{GABA_B}$ receptor antagonist prior to the application of SCS and spinal neuronal activity recordings. In order to relate the findings from that electrophysiological study in anesthetized rats to SCS-induced analgesia, a separate group of rats underwent implantation of spinal cord stimulators to deliver burst or tonic SCS and allodynia was measured to compare the effects of each mode of SCS on behavioral sensitivity. Because GABA is transported across the blood–brain barrier and serum concentrations can serve as a marker of central nervous system function [25]–[27], temporal serum GABA levels were also quantified to evaluate the stimulation of GABA release by burst and tonic SCS.

II. Methods

A. Experimental Design

Two complementary experiments compared the effectiveness of burst and tonic SCS for modulating pain and nociceptive mechanisms in an established model of painful cervical nerve root compression [19], [28]. Both studies used male Holtzman rats (322–438 g) that were housed under conditions compliant with the U.S. Department of Agriculture and the Association for Assessment and Accreditation of Laboratory Animal Care. All experimental procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and carried out under the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain [29].

In the first study, burst and tonic SCS were assessed for attenuating dorsal horn neuronal hyperexcitability. Rats ($\mathrm{n} = 8$) underwent painful C7 dorsal nerve root compression [19], [28]. Hyperalgesia was assessed before injury and at day 7 prior to making the electrophysiological recordings. Dorsal horn neuronal firing was quantified during mechanical stimulation of the forepaw before and after the application of either burst or tonic SCS on day 7. Since SCS has been shown to attenuate hyperexcitability of wide dynamic range (WDR) neurons in the dorsal horn [30]–[32], the effects of burst and tonic SCS were specifically evaluated in WDR neurons. In a separate group of rats, the $\mathrm{GABA_B}$ receptor antagonist, CGP35348 ($\mathrm{n} = 7$), was applied to the spinal cord during application of burst and tonic SCS on day 7 after painful nerve root compression. Dorsal horn neuronal firing was quantified before and after stimulation to evaluate the role of $\mathrm{GABA_B}$ receptor activation in the effects of burst SCS relative to tonic SCS.

In the second study, burst and tonic SCS were evaluated for attenuating allodynia in separate groups of rats that underwent painful cervical nerve root compression and implantation of a spinal cord stimulation system. Allodynia was measured in the forepaws before nerve root compression (baseline) and at days 1, 3, 4, 5, 7, 10, and 14 after injury. The stimulator was implanted at the time of nerve root compression on day 0 but was not turned on until after behavioral testing on day 4 in order to ensure the development of radiculopathy. At that time, burst ($\mathrm{n} = 5$ rats) or tonic ($\mathrm{n} = 7$ rats) SCS was initiated and was applied continuously through day 14. In addition, blood samples were collected from each rat before nerve root compression, and during the application of SCS on days 7 and 14 to quantify serum GABA levels by ELISA. In a separate group, rats ($\mathrm{n} = 3$) underwent painful nerve root compression with no stimulator implanted; allodynia and serum GABA levels were evaluated at baseline, day 7, and day 14 to provide control responses from nerve root compression alone for comparison with responses from rats that received burst or tonic SCS after injury.

B. Painful Cervical Nerve Root Compression

Compression of the right C7 dorsal nerve root was performed using procedures described previously [19], [20]. Briefly, rats were anesthetized with inhaled isoflurane (4% for induction, 2–3% for maintenance), and placed in a prone position. A midline incision was made along the back of the neck from the base of the skull to the T2 vertebra. The C6 and C7 vertebrae were exposed by separating the paraspinal musculature overlying the vertebrae. A C6/C7 hemilaminectomy and partial facetectomy on the right side were performed to expose the C7 dorsal nerve root between the dorsal root ganglion (DRG) and the spinal cord. The nerve root was compressed for 15 min with a 10-gf microvascular clip (World Precision Instruments; Sarasota, FL, USA) inserted through a small incision in the dura. After the

surgical procedure, incisions were closed using 3–0 polyester suture and surgical staples. Rats were monitored during recovery in room air before being returned to normal housing conditions.

C. Assessment of Behavioral Sensitivity

Mechanical hyperalgesia was measured in the ipsilateral and contralateral forepaws of each rat that underwent painful nerve root compression and electrophysiological recordings on day 7. Hyperalgesia was measured by applying a series of weighted von Frey filaments (1.4, 2, 4, 6, 8, 10, 15, and 26 g) to the plantar surface of the paw to identify the paw withdrawal threshold [19], [22]. Each filament was applied five times to determine if the rat displayed a positive response, exhibited by shaking, licking, or withdrawing the forepaw. If the rat responded to two consecutive filaments, the lesser of the two filament weights was recorded as the paw withdrawal threshold. Rats not responding to any of the filaments were assigned the maximum threshold of 26 g. Testing was repeated in three rounds separated by at least 10 min, and the average threshold from the three rounds was calculated for each forepaw of each rat. For rats that underwent implantation of the spinal cord stimulators, allodynia was measured separately in the ipsilateral and contralateral forepaws by applying a 4-g von Frey filament ten times, in three separate rounds of testing, with 10 min of rest between rounds [20]. The total number of paw withdrawals for each forepaw of each rat was summed over the three rounds. A repeated-measures ANOVA compared paw withdrawal thresholds or the number of paw withdrawals to baseline at each time point.

D. Electrophysiological Recordings and SCS

Extracellular electrophysiological recordings were acquired in the dorsal horn on day 7 after painful nerve root compression to assess neuronal firing before and after burst and tonic SCS. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and given supplementary doses (5–10 mg/kg, i.p.) as needed based on toe pinch reflexes. With the rat in a prone position, an incision was made along the back of the neck from the base of the skull to the T2 vertebra. A bilateral laminectomy and dural resection were performed to expose the spinal cord from C3 to C7. A tracheotomy was performed and rats were connected to a ventilator and CO_2 monitor to control respiration throughout the recording session (CWE; Ardmore, PA). A lateral thoracotomy was also performed to alleviate intrathoracic pressure and to isolate the spinal cord from any movement of the rat during ventilation. Rats were immobilized on a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA) using ear bars and a vertebral clamp at T2 to stabilize the cervical spine. The spinal cord was bathed in 37 °C mineral oil for the duration of recording, and core temperature was maintained at 35–37 °C using a temperature controller with a rectal probe (Physitemp; Clifton, NJ, USA).

A monopolar platinum ball electrode was placed over the C4 dorsal columns [see Fig. 1(a)], and a grounding electrode was attached to the incised skin on the neck. The ball electrode was spring loaded to avoid compression of the dorsal columns [33]. Constant current stimulation was applied using a Grass

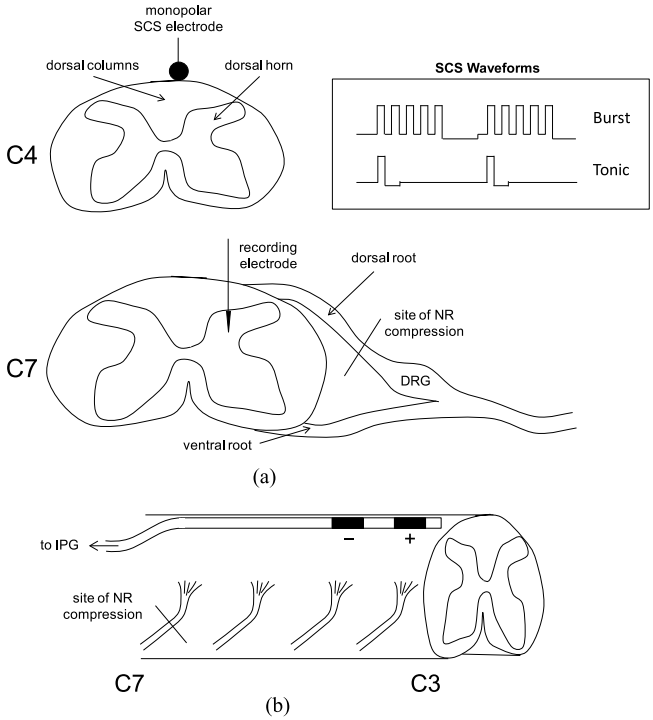

Fig. 1. Schematic of the application of SCS and recording of dorsal horn neurons. (a) Painful nerve root (NR) compression was applied to the right C7 dorsal nerve root proximal to the dorsal root ganglion (DRG). On day 7 after NR compression, monopolar burst and tonic SCS waveforms (inset) were applied to the dorsal columns of the C4 spinal cord using a monopolar ball electrode. Evoked neuronal firing was recorded in the C7 dorsal horn ipsilateral to the site of NR compression during mechanical stimulation of the forepaw. (b) In a separate study, a stimulator lead with planar electrodes and implantable pulse generator (IPG) delivered bipolar burst or tonic SCS to the C3/C4 dorsal columns beginning on day 4 after painful nerve root compression. The lead included an anode (+) and a cathode (−) oriented in the rostral-caudal direction at the midline of the spinal cord.

stimulator with a photoelectric stimulus isolation unit (S48 Stimulator, Grass Technologies; Warwick, RI, USA). The motor thresholds (MT) for burst and tonic SCS were identified separately in each rat to be the stimulation intensities at which small contractions were first observed in the paraspinal musculature or forelimbs. Burst and tonic SCS parameters were chosen to match those parameters that have been used previously in preclinical and clinical studies, to enable comparison of our findings with published reports [3], [6], [11], [30]. As such, burst SCS was applied at 40 Hz at an amplitude of 90% MT, with each burst containing five pulses at a frequency of 500 Hz with a pulsewidth of 1 ms. Tonic stimulation delivered pulses at a frequency of 40 Hz with a pulsewidth of 0.3 ms and amplitude of 90% MT.

A carbon fiber electrode (Kation Scientific; Minneapolis, MN, USA) was lowered into the C7 spinal dorsal horn ipsilateral to the compressed nerve root using a micropositioner [see Fig. 1(a)] (Narishige; Tokyo, Japan). Extracellular potentials were amplified with a gain of 10^3 and conditioned using a bandpass filter between 0.3 and 3 kHz (World Precision Instruments; Sarasota, FL, USA). The signal was processed with a 60-Hz HumBug adaptive filter (Quest Scientific; North Vancouver, BC, Canada), digitally sampled at 25 kHz (Micro1401,

CED; Cambridge, U.K.), then monitored with a speaker for audio feedback (A-M Systems; Carlsborg, WA, USA) and recorded using Spike2 software (CED; Cambridge).

As the electrode was lowered into the spinal cord, light brushing and noxious pinch of the ipsilateral forepaw were used to identify mechanically sensitive wide dynamic range (WDR) neurons with receptive fields on the plantar surface of the forepaw, based on their characteristic responses to the nonnoxious and noxious mechanical stimuli, as has been previously reported by others [31]. Once a neuron was identified, baseline evoked neuronal firing was recorded during mechanical stimulation of the forepaw consisting of five consecutive 1-s applications of the nonnoxious 1.4-g and noxious 26-g von Frey filaments, and 10 s of noxious pinch with forceps [34], [35]. A baseline period of 2 s was recorded before each stimulus to quantify spontaneous firing. Burst SCS was then applied for 5 min, during which time the evoked firing could not be recorded because artifacts from the stimulation masked neuronal activity. After SCS, evoked activity was immediately (0 min) recorded during 1.4-g von Frey, 26-g von Frey, and pinch stimulation and recording were repeated again at 2, 5, 10, and 15 min post-SCS. The stimulation protocol was then repeated in the same neuron using tonic SCS after neuronal firing returned to pre-SCS baseline. The protocol was reversed to apply tonic SCS before burst SCS in alternating neurons in order to avoid any effects of the order of burst and tonic SCS application. Recording continued for up to 6 h for each rat, or until 4–6 neurons were recorded for each mode of stimulation.

Voltage potentials from each recorded neuron were spike-sorted and counted using Spike2 software (CED; Cambridge, U.K.). Pre- and post-SCS firing were determined by counting the spikes evoked separately by the 1.4-g filament, 26-g filament, and noxious pinch in the baseline period prior to SCS, and at each time point after SCS. Spontaneous firing was subtracted from the spike counts for each mechanical stimulus in order to isolate firing evoked by the forepaw stimulus. For each of the 1.4-g filament, 26-g filament, and pinch stimuli, a repeated-measures ANOVA was used to compare the number of evoked spikes between the pre-SCS baseline and evoked firing at each post-SCS time point. Changes in neuronal firing immediately (0 min) after SCS were calculated as the percent change in spikes relative to the pre-SCS baseline values [30]. For neurons in which both burst and tonic stimulation protocols were completed without losing the signal from the recorded neuron, paired Student's *t*-tests compared the percent changes in evoked firing immediately after burst SCS to the changes in firing after tonic SCS.

E. Spinal Superfusion of $\mathrm{GABA_B}$ *Receptor Antagonist*

A separate group of rats underwent the same surgical and electrophysiological recording procedures described previously, but received spinal application of the $\mathrm{GABA_B}$ receptor antagonist, CGP35348, for the duration of the SCS and dorsal horn electrophysiological recordings at day 7 after nerve root compression. Rats were mounted on the stereotaxic frame and the burst and tonic motor thresholds were determined. The spinal cord was then covered with agar (1 g agar/40 mL 0.9% saline) to form a well directly over the C7 spinal cord for superfusion of the drug solutions. A separate well was formed over the C4 dorsal columns to allow contact of the stimulating electrode with the spinal cord. CGP35348 (Abcam; Cambridge, MA, USA) was solubilized in artificial cerebrospinal fluid (Harvard Apparatus; Holliston, MA). The concentration of CGP35348 (10 mM) was chosen based on previous studies applying it to the dorsal spinal cord with effective modulation of spinal GABA signaling [36], [37]. Based on the fact that peak dorsal horn drug concentrations occur after 15–30 min of drug superfusion [38], CGP35348 was applied to the C7 spinal cord for 20 min before the stimulation and recording protocols were initiated; drug solutions were refreshed every 20–30 min throughout the recording session.

F. Implantation and Application of SCS

To evaluate the effects of burst and tonic SCS on behavioral sensitivity after painful nerve root compression, spinal cord stimulators were implanted in rats to deliver dorsal column stimulation. A laminectomy was performed to expose the spinal cord from C3-T1 with a facetectomy exposing the right C7 dorsal nerve root. The nerve root was compressed as described earlier [19], [20]. After removal of the compression clip, a customized bipolar lead with two planar platinum/iridium electrodes (each with a 3 $\mathrm{mm^2}$ surface area, 400-Ω impedance) was placed on the spinal cord, and secured with sutures to the C2 and T2 vertebrae. The anode and cathode were separated by 1 mm, arranged rostrocaudally at the midline over the C3/C4 dorsal columns [see Fig. 1(b)]. The lead was tunneled subcutaneously and connected to an implantable pulse generator (IPG) (Eon Mini Pulse Generator, St. Jude Medical; Plano, TX, USA), which was located rostral to the left hip of the rat. Incisions were closed using 3–0 polyester sutures and surgical staples, and rats were monitored during recovery, before being returned to normal housing conditions. Bipolar stimulation was applied continuously beginning after behavioral assessment on day 4. Burst SCS was applied with 5 pulses per burst at 500 Hz with a pulse width of 1 ms, a burst frequency of 40 Hz, and amplitude of 80% MT. Tonic SCS was applied at 50 Hz with a pulsewidth of 0.25 ms and amplitude of 80% MT. These parameters were different from those delivered by the Grass stimulator in the first study due to a lack of resolution in the parameter settings of the IPG. However, because previous studies have demonstrated tonic SCS to reduce pain and induce GABA release at amplitudes as low as 60% MT [11], [15], these IPG settings are sufficient to evaluate the analgesic effects of SCS. The location of the stimulator lead was confirmed to be over the C3/C4 dorsal columns at the termination of the study by visual inspection. Rats were removed from the study if they displayed lethargy and/or paralysis after IPG implantation, which was infrequent.

G. Quantification of Serum GABA Concentrations by ELISA

Blood samples were collected before nerve root compression (baseline) and on days 7 and 14 in rats undergoing painful nerve root compression alone or nerve root compression with IPG implantation and SCS. Rats were anesthetized with isoflurane

TABLE I
SUMMARY OF GROUP INFORMATION, BEHAVIORAL THRESHOLDS, MOTOR THRESHOLDS, AND NEURON ACTIVITY DURING SCS MODES FOR GROUPS AFTER PAINFUL NERVE ROOT COMPRESSION AND/WITH THE $GABA_B$ RECEPTOR ANTAGONIST

			Day 7 Paw Withdrawal Threshold (% of BL)		Motor Threshold (μA)		Neurons Recorded per Rat	
Group	Rats	Weight (g)	Ipsilateral	Contralateral	Burst	Tonic	Burst	Tonic
NR Compression	n = 8	412 ± 36	33 ± 11%*	76 ± 26%	182 ± 60	324 ± 68#	6.1 ± 1.5	6.4 ± 2.3
NR Compression + CGP	n = 7	396 ± 18	25 ± 10%*	105 ± 34%	183 ± 23	319 ± 58#	4.1 ± 1.3	4.3 ± 1.7

NRC = nerve root compression, CGP = CGP35348, BL = baseline, SD = standard deviation, $^*p < 0.0001$, Day 7 versus BL; $^\#p < 0.0002$, Tonic versus Burst.

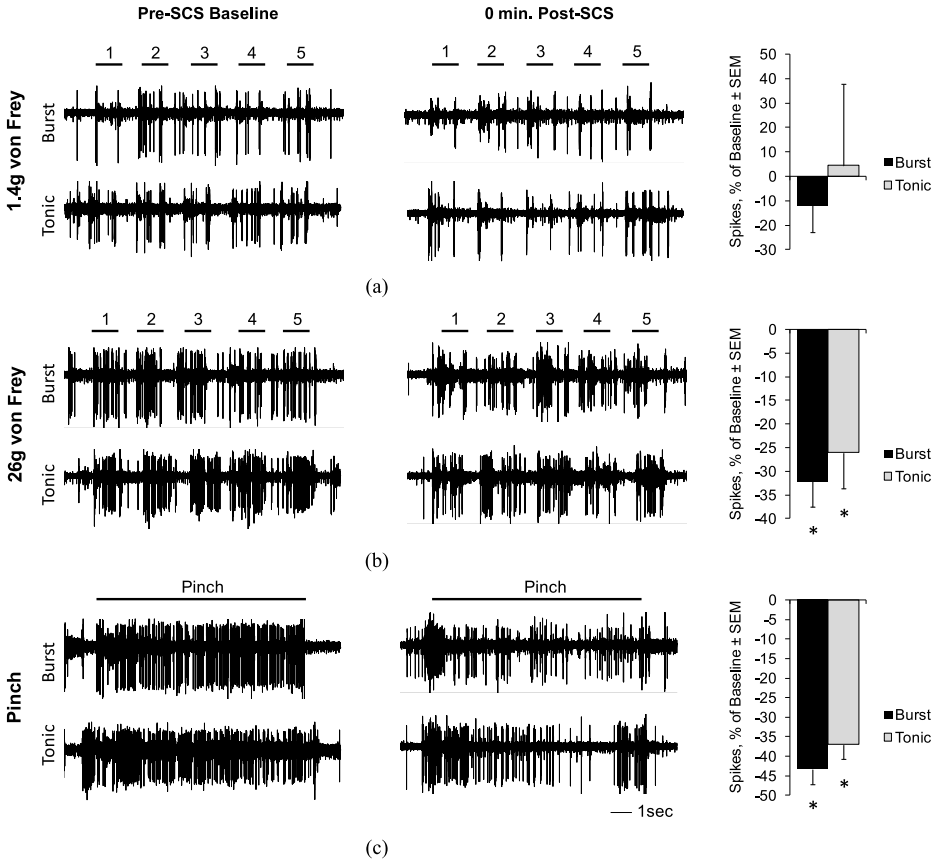

Fig. 2. Representative firing and quantification of neuronal activity in the C7 spinal cord before and immediately after burst and tonic SCS at day 7 following painful nerve root compression in response to forepaw stimulation. (a) No change in neuronal firing is observed during stimulation of the ipsilateral forepaw by a 1.4-g von Frey filament after burst or tonic SCS. Firing is significantly reduced after burst or tonic SCS during (b) 26-g von Frey filament ($^*p \leq 0.0014$ versus pre-SCS baseline), and (c) noxious pinch stimulation ($^*p < 0.0001$ versus pre-SCS baseline).

(4%) and the tail was sterilized with alcohol. A 25-G needle with a 1 mL syringe was used to collect 0.5 mL of blood from the tail artery. Blood samples were allowed to clot for 20–40 min at room temperature, and then, centrifuged for 15 min at 1000 g at 4 °C. The supernatant was collected and centrifuged again for ten minutes at 10 000 g at 4 °C. Serum was collected in a clean Eppendorf tube and frozen at −80 °C. Serum GABA concentrations were measured by competitive ELISA (MyBioSource; San Diego, CA), according to the manufacturer's instructions. Each sample was compared to the standard curve from the assay kit to determine the GABA concentration. All samples and standards were run in duplicate. Data are reported as the fold change in concentration relative to the preinjury baseline; serum GABA concentrations on each day were compared to baseline by repeated-measures ANOVA with a *post hoc* Tukey's HSD test.

III. RESULTS

A. Burst and Tonic SCS Similarly Attenuate Dorsal Horn Neuronal Hyperexcitability

The paw withdrawal threshold (PWT) in the ipsilateral forepaw is significantly reduced from baseline at day 7 after painful nerve root compression ($p < 0.0001$), but the PWT in the contralateral forepaw is unchanged from baseline [see Table I]. The average motor threshold is significantly higher

for tonic stimulation relative to burst stimulation in rats undergoing electrophysiological recordings at day 7 after painful nerve root compression ($p \leq 0.0002$) [see Table I]. A total of 49 neurons were recorded after burst SCS, and 51 neurons were recorded after tonic SCS [see Table I]. In 39 of those neurons, paired firing data were collected after both burst and tonic SCS. Firing evoked by 26-g von Frey filament stimulation is significantly reduced by $32 \pm 5\%$ from baseline immediately after burst stimulation ($p < 0.0001$), and significantly reduced by $26 \pm 7\%$ from baseline after tonic stimulation ($p < 0.0001$) [see Fig. 2]. Likewise, firing during noxious pinch is reduced by $43 \pm 4\%$ immediately after burst SCS ($p < 0.0001$), and by $37 \pm 4\%$ after tonic SCS ($p < 0.0001$) [see Fig. 2]. The percent reductions in firing after burst and tonic SCS are not different from each other during either the 26-g filament or noxious pinch stimulation [see Fig. 2]. Neuronal firing evoked by 1.4-g filament stimulation of the ipsilateral forepaw is not changed from baseline for either mode of stimulation [see Fig. 2].

The suppressive effects of burst and tonic SCS on dorsal horn neuronal firing evoked by noxious stimulation are sustained for up to 10 min after the cessation of SCS [see Fig. 3]. Compared to pre-SCS baseline firing, the number of spikes evoked by 26-g von Frey filament stimulation of the ipsilateral forepaw decreases immediately (at 0 min) and 2 min after burst SCS ($p \leq 0.0004$). However, after tonic SCS the evoked firing remains decreased from pre-SCS baseline levels during 26-g filament stimulation at these times and also at 5 min and 10 min ($p \leq 0.035$) [see Fig. 3]. The number of spikes evoked during noxious pinch stimulation decreases from pre-SCS baseline levels at all of the time points measured up to 10 min after burst SCS ($p \leq 0.034$), while pinch-evoked firing decreases after tonic SCS from baseline only at 0, 2, and 5 min after stimulation ($p \leq 0.0016$) [see Fig. 3]. The number of spikes evoked by forepaw stimulation with the nonnoxious 1.4-g filament is not different from baseline at any recorded time point after burst or tonic stimulation [see Fig. 3]. Of note, evoked firing is not different after burst SCS compared to tonic SCS for any mechanical stimulus at any pre- or post-SCS time point.

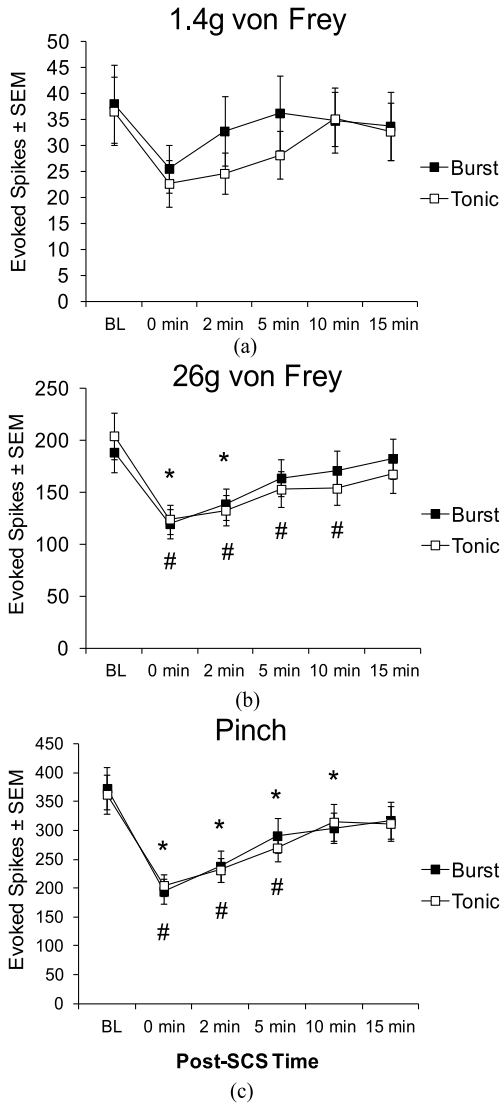

Fig. 3. Evoked spike counts for up to 15 min after the cessation of burst and tonic SCS application. (a) Burst and tonic SCS do not change firing evoked by 1.4-g von Frey filament stimulation of the ipsilateral forepaw. (b) 26-g von Frey filament evokes fewer spikes than at baseline for up to 2 min following the application of burst SCS ($^*p \leq 0.0004$ versus burst SCS baseline), and up to 10 min after tonic SCS ($\#p \leq 0.035$ versus tonic SCS baseline). (c) Noxious pinch evokes fewer spikes than at baseline for up to ten minutes after burst SCS ($^*p \leq 0.034$ versus burst SCS baseline), and up to 5 min after tonic SCS ($\#p \leq 0.0016$ versus tonic SCS baseline).

B. $GABA_B$ *Receptor Antagonist Differentially Modulates the Effects of Burst and Tonic SCS*

The $GABA_B$ receptor antagonist, CGP35348, differentially modulates the effects of burst and tonic SCS in the dorsal horn. In the presence of CGP35348, burst SCS significantly attenuates dorsal horn neuronal firing by $36 \pm 4\%$ ($p < 0.0001$) and by $44 \pm 4\%$ ($p < 0.0001$) during 26-g filament and noxious pinch stimulation of the ipsilateral forepaw, respectively [see Fig. 4(a)]. That suppression of evoked firing is not different from the attenuation of neuronal activity after burst SCS with no GABA receptor antagonist [see Fig. 4(a)]. However, CGP35348 abolishes the effects of tonic SCS, with no change from baseline firing during the 26-g filament stimulation ($4 \pm 8\%$ change from baseline) or the noxious pinch application ($0.2 \pm 7.3\%$) [see Fig. 4(b)]. The reduction in firing after tonic SCS with CGP35348 is significantly less than the attenuation of firing after tonic SCS with no $GABA_B$ receptor antagonist for both the 26-g von Frey filament and pinch stimuli ($p \leq 0.049$) [see Fig. 4(b)].

C. Burst and Tonic SCS Reduce Tactile Allodynia but Differentially Stimulate GABA Release

Tactile allodynia develops after painful cervical nerve root compression [see Fig. 5(a)]. In rats undergoing nerve root compression alone, the number of paw withdrawals in the ipsilateral forepaw is elevated at days 1, 7, and 14 in response to 4-g von Frey filament stimulation ($p \leq 0.008$) [see Fig. 5(a)]. For rats with the implanted IPGs prior to SCS application, the number of paw withdrawals also increases over baseline responses on

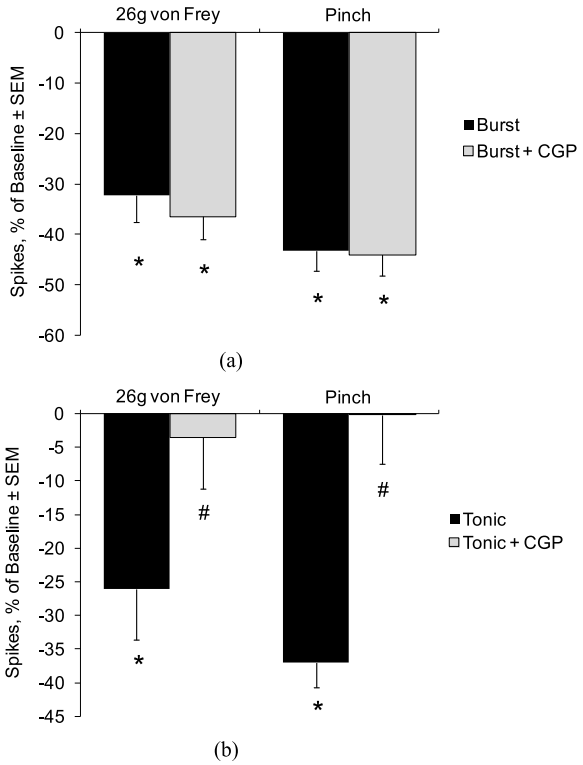

Fig. 4. Effects of $\mathrm{GABA_B}$ receptor antagonist on the attenuation of dorsal horn neuronal firing by burst or tonic SCS. (a) Burst SCS alone and burst SCS with CGP35348 (Burst + CGP) attenuate neuronal firing in response to noxious ipsilateral forepaw stimulation by both the 26-g von Frey filament and pinch ($^{*}p < 0.0001$ versus pre-SCS baseline). (b) Tonic SCS alone attenuates neuronal firing evoked by both 26 g and pinch stimulation ($^{*}p \leq 0.001$ versus pre-SCS baseline). However, tonic SCS with CGP35348 (Tonic + CGP) does not induce any change in neuronal firing from baseline, and that percent change from baseline is significantly less than the percent change after tonic SCS alone ($\#p \leq 0.049$ versus Tonic SCS alone).

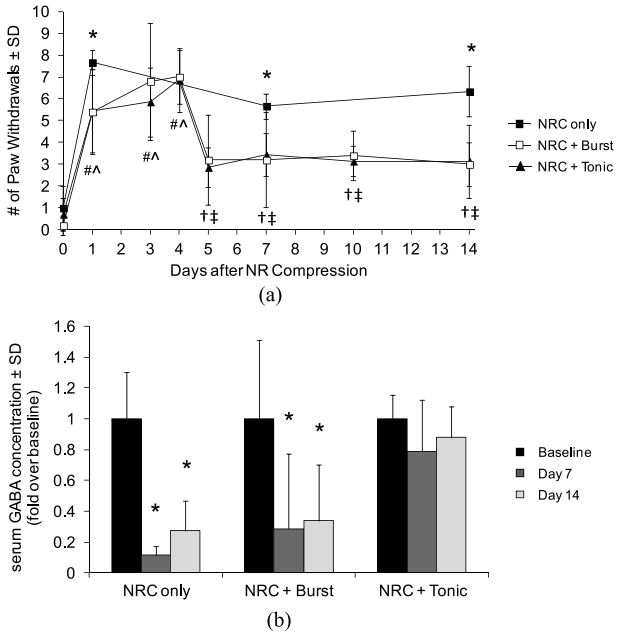

Fig. 5. Allodynia and serum GABA concentrations during burst and tonic SCS. (a) Painful nerve root compression (NRC only) induces an increase in ipsilateral paw withdrawals ($^{*}p < 0.008$, NRC only versus baseline). The groups undergoing painful NRC before the application of burst SCS (NRC + Burst) or tonic SCS (NRC + Tonic) also develop an increase in paw withdrawals after the NRC injury ($\#p < 0.0001$, NRC + Burst versus baseline; $^{\wedge}p < 0.0001$, NRC + Tonic versus baseline). Both burst and tonic SCS application started at day 4 significantly reduce the number of paw withdrawals from the number of withdrawals that are detected on day 4 before the SCS is turned ON ($^{\dagger}p < 0.023$, NRC + Burst versus day 4; $^{\ddagger}p < 0.005$, NRC + Tonic versus day 4). (b) Serum GABA levels decrease from preinjury baseline levels on days 7 and 14 after a painful nerve root compression (NRC only) and nerve root compression with burst SCS (NRC + Burst) ($^{*}p < 0.009$). However, GABA levels measured at these times after tonic SCS are not different from baseline.

days 1, 3, and 4 after nerve root compression ($p < 0.0001$) [see Fig. 5(a)]. However, for burst SCS beginning on day 4, the number of paw withdrawals is significantly reduced on days 5, 7, 10, and 14 from the corresponding levels on day 4 ($p \leq 0.023$), and the number of paw withdrawals after burst SCS is not different from baseline [see Fig. 5(a)]. Likewise, tonic SCS beginning on day 4 also significantly reduces the number of paw withdrawals on all subsequent testing days compared to day 4 ($p \leq 0.005$), also returning to baseline [see Fig. 5(a)]. The number of paw withdrawals is not different on any day for rats receiving burst SCS compared to those receiving tonic SCS. The number of paw withdrawals for the contralateral forepaw is unchanged from baseline on all days for all rats.

Following the behavioral results, serum GABA concentrations are also altered after a painful nerve root compression, but unlike the pain symptoms, the GABA levels are differentially regulated by the mode of SCS [see Fig. 5]. After painful nerve root compression, serum GABA is significantly reduced to 11 $\pm$ 6% of baseline levels on day 7 ($\mathrm{p} = 0.0007$) and 27 $\pm$ 19% of baseline on day 14 ($\mathrm{p} = 0.008$) [see Fig. 5(b)]. During the application of tonic SCS, serum GABA concentrations are 79 $\pm$ 49% of baseline on day 7, and 88 $\pm$ 36% of baseline on day 14, but are not different from baseline levels on either day [see Fig. 5(b)]. However, during the application of burst SCS, serum GABA concentrations remain significantly decreased at 29 $\pm$ 34% of baseline on day 7 ($\mathrm{p} = 0.017$), and 34 $\pm$ 20% of baseline on day 14 ($\mathrm{p} = 0.009$) [see Fig. 5(b)], similar to the responses observed with no SCS treatment.

IV. Discussion

Burst and tonic SCS similarly reduce dorsal horn neuronal firing during noxious stimulation of the forepaw on day 7 and also alter pain symptoms to the same degree after a painful compression of the cervical nerve root [see Figs. 2, 3, and 5]. Tonic SCS is believed to activate GABAergic signaling in the dorsal horn as a primary mechanism of action [16], [17], which is supported by the ineffectiveness of tonic SCS in the presence of the $\mathrm{GABA_B}$ receptor antagonist [see Fig. 4(b)]. However, the effects of burst SCS are not abolished by the $\mathrm{GABA_B}$ receptor antagonist [see Fig. 4(a)]. This difference in effectiveness is paralleled by the stimulation of GABA levels in serum, with tonic SCS restoring serum GABA concentrations to preinjury baseline levels but burst SCS not altering serum GABA levels from those that are decreased after injury [see Fig. 5(b)]. Collectively these results suggest that, despite similar attenuation of radicular pain by burst and tonic SCS, GABA signaling appears to not contribute to burst SCS-induced analgesia.

A. Effects of Burst and Tonic SCS on Neuronal Activity and Tactile Allodynia

Painful nerve root compression induces increased evoked dorsal horn neuronal firing during noxious stimulation of the forepaw by between two and three times the activity in controls [19]. Both burst and tonic SCS significantly reduce this hyperexcitability in dorsal horn WDR neurons [see Figs. 2 and 3]. This attenuation of neuronal hyperexcitability that parallels reduced behavioral sensitivity has also been reported for pharmacological interventions that attenuate pain after nerve root compression and joint injury in the rat [19], [20], [24], [39]. Tonic SCS has been previously reported to reduce allodynia after peripheral nerve injury by normalizing the responses of hyperexcitable WDR neurons [32], especially to innocuous stimuli like the 4-g von Frey filament used in the current study [see Fig. 5(a)]. Therefore, the attenuation of WDR neuronal activity in the dorsal horn is a likely contributor to the decrease in behavioral sensitivity that is also observed with burst SCS [see Figs. 2–5].

Burst and tonic SCS appear to attenuate neuronal firing for different durations after the cessation of SCS in response to the noxious 26-g von Frey filament and pinch stimuli [see Fig. 3]. However, those differences in the temporal effects of SCS modes are likely an artifact of statistical testing, because the evoked spike counts are not different *between* burst and tonic SCS for any noxious or innocuous stimulus at baseline or at any of the post-SCS times [see Fig. 3]. These results suggest that, overall, neither mode of SCS is more or less effective at attenuating spinal neuronal hyperexcitability over the duration following stimulation that was tested in this study. Furthermore, the lack of difference between the effects of tonic and burst SCS in response to noxious stimulation (26 g, pinch) of the forepaw suggests that burst SCS inhibits nociception by attenuating the hyperactivity of nociceptive neurons in the dorsal horn, similar to those effects reported for tonic SCS [32].

Similarly, the decreases in tactile allodynia are not different between burst SCS and tonic SCS [see Fig. 5(a)], further supporting that burst stimulation is as effective as tonic stimulation for reducing radicular pain in this model. Tang *et al.* [11] also reported no significant differences in the effects of burst and tonic SCS on lumbar dorsal horn neuronal firing in response to noxious pinch. However, that study found the visceromotor reflexes to be more attenuated after burst SCS than after tonic SCS [11]. That finding suggests that additional metrics, other than evoked dorsal horn neuronal firing and behavioral hypersensitivity to mechanical stimulation, may be more sensitive to potential differences between burst and tonic stimulation. However, the study by Tang *et al.* [11] assessed nociceptive circuits in naïve animals, while our studies evaluate SCS-induced analgesia after neuropathic pain is established. The extensive modifications in sensory processing that occur in chronic pain states after nerve injury complicate any direct comparisons between our findings and those of Tang *et al.*, despite both studies using matching SCS parameters.

Although burst SCS reduces VAS pain scores significantly more than tonic SCS in patients with chronic pain [3], [6], [8], our study detected no differences between burst and tonic SCS for attenuating behavioral and/or neuronal sensitivity after radicular injury [see Figs. 2, 3, and 5]. The electrophysiological and behavioral metrics used in this study describe the analgesic effects of burst and tonic SCS in allodynic rats; however, patient-reported pain scores incorporate additional factors that were not explicitly studied here, including spontaneous pain, emotional perceptions of pain, and the presence or absence of paresthesia during SCS [8], [40], [41]. Indeed, De Ridder *et al.* [6] suggested that burst SCS may modulate those affective dimensions and attention to pain rather than suppressing nociception more than tonic SCS, a notion that is at least indirectly supported by our data showing similar spinal neuronal effects of burst and tonic stimulation [see Figs. 2 and 3]. Paresthesia may also play a particularly important role in determining pain outcomes during SCS because of the widely varying positive and negative experiences patients associate with paresthetic sensation [8]. Additional behavioral assessments, incorporating measures of paresthesia, spontaneous pain, and the affective dimensions of pain may better capture those effects of burst SCS that are more clinically relevant and capture all aspects of pain. In addition, while no tissue or electrode damage were observed by visual inspection, histology was not performed to evaluate the relative effects on tissue or cell health from these SCS treatments. Future studies should also evaluate the potential for tissue damage caused by the delivery of SCS.

B. GABAergic Mechanisms in Burst and Tonic SCS

Tonic SCS activates signaling through $\mathrm{GABA_B}$ receptors to inhibit nociception in the dorsal horn [15], [17]. The $\mathrm{GABA_B}$ receptor antagonist, CGP35348, abolishes the effects of tonic SCS [see Fig. 4(b)], validating the role of $\mathrm{GABA_B}$ receptor activation in tonic SCS-induced spinal inhibition. However, neuronal firing is significantly reduced from baseline after burst SCS despite the spinal superfusion of CGP35348 [see Fig. 4(a)], indicating that $\mathrm{GABA_B}$ receptor activation is not required for burst SCS-induced inhibition in the dorsal horn. Spinal superfusion of drug solutions can lead to steep drug concentration gradients in the dorsal horn, which can be greatly affected by metabolism or uptake of the drug [38]. Because CGP35348 reduces the effectiveness of tonic SCS on dorsal horn WDR neuronal firing [see Fig. 4(b)], the effective concentration in the deep dorsal horn was taken as sufficient to inhibit GABA signaling. However, other drug delivery methods, like intrathecal injection or dorsal horn microdialysis [15], [17], may produce more uniform drug concentrations and inhibition of GABA signaling. Nonetheless, based on studies with a radiolabeled neuropeptide showing that peak dorsal horn concentrations occur after 15–30 min of superfusion [38], solutions were refreshed every 20–30 min in order to maintain antagonist concentrations in the dorsal horn. Furthermore, burst and tonic SCS were applied sequentially during recording of the same dorsal horn neurons, so any differential effects of the two modes of SCS are not due to differences in drug penetration or efficacy between rats.

GABA levels in the blood may be influenced by nonneuronal endogenous sources [42], leading to a lack of correlation

between serum and central nervous system GABA concentrations [25], [43]. Quantifying intracellular and extracellular GABA concentrations in the dorsal horn would provide a more direct measurement of GABA signaling and dysfunction in the spinal cord [44], [45]. However, the changes in serum GABA levels in this study [see Fig. 5(b)] are similar to those changes in extracellular GABA in the dorsal horn after nerve injury and after nerve injury with administration of tonic SCS in the rat [14], [16]. Therefore, serum GABA levels may reflect extracellular concentrations in the dorsal horn and may also serve as a proxy indicator of the effects of SCS on spinal inhibition.

Tonic SCS restores serum GABA concentrations nearly to baseline levels, but serum GABA remains reduced during the application of burst SCS [see Fig. 5(b)]. These results suggest that burst SCS attenuates dorsal horn neuronal firing and tactile allodynia without inducing GABA release. Burst SCS has a significantly lower motor threshold than tonic SCS [see Table I] [11], and burst SCS also requires a lower amplitude than tonic SCS to relieve pain in patients [3]. These findings may be due to the fact that burst SCS has a longer pulse width than tonic SCS and requires less temporal integration to reach the activation thresholds of stimulated neurons [3], [9]. Therefore, it is possible that the burst SCS amplitudes that attenuate dorsal horn neuronal firing are too low to activate the release of GABA in the spinal dorsal horn, and instead activate other non-GABAergic mechanisms to inhibit nociception in the spinal cord. After the cessation of tonic SCS, GABA concentrations have been shown to return to pre-SCS levels over the course of several hours [14], [16]. However, burst and tonic SCS were not turned off in this study since the goal was to monitor the effects of stimulation on behavioral hypersensitivity; so, it was not possible to evaluate post-SCS GABA concentrations.

In addition to inducing GABA release, tonic SCS also stimulates release of serotonin [46], acetylcholine [47], and noradrenaline [48], each of which contribute to the inhibitory effects of SCS on dorsal horn neurons. Many of the inhibitory effects of those additional neurotransmitters are attributed to secondary activation of GABAergic interneurons in the dorsal horn [49]–[51]. However, serotonin and noradrenaline can induce direct inhibitory effects on dorsal horn projection neurons [52]–[54], so it is possible that burst SCS attenuates nociception by activating inhibitory neurotransmitter systems that may partially act independently of GABA signaling. Dorsal column stimulation can also directly inhibit dorsal horn neuronal firing, either by presynaptically depolarizing primary afferents to block excitatory neurotransmission, or producing hyperpolarizing inhibitory potentials in dorsal horn neurons [55], [56]. Further studies are required to determine what non-GABAergic neurotransmitter systems or neurophysiological mechanisms may be activated during burst SCS to inhibit nociception in the dorsal horn.

V. Conclusion

Overall, this study demonstrates that burst SCS attenuates dorsal horn neuronal firing and tactile allodynia as well as tonic SCS after painful nerve root compression. Burst and tonic SCS both reduce spinal dorsal horn WDR neuronal firing and tactile allodynia to the same degree after painful nerve root compression. However, despite these similarities between SCS modes in their suppression of dorsal horn neuronal activity, burst and tonic SCS appear to activate different cellular mechanisms. Unlike tonic SCS, burst SCS does not rely on GABA release and activation of $GABA_B$ receptors to inhibit spinal neuronal firing. Other neurochemical and neurophysiological mechanisms may provide the spinal inhibitory effects that appear to be activated by burst SCS for analgesia. This study highlights the need for continued studies to elucidate the specific mechanisms underlying burst stimulation in order to continue developing burst SCS as a treatment for chronic pain.

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