Surround Inhibition Mediates Pain Relief by Low Amplitude Spinal Cord Stimulation: Modeling and Measurement

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Abstract

Low-frequency (<200 Hz), subperception spinal cord stimulation (SCS) is a novel modality demonstrating therapeutic efficacy for treating chronic neuropathic pain. When stimulation parameters were carefully titrated, patients experienced rapid onset (seconds–minutes) pain relief without paresthesia, but the mechanisms of action are unknown. Using an integrated computational model and in vivo measurements in urethane-anesthetized rats, we quantified how stimulation parameters (placement, pulse width, frequency, and amplitude) influenced dorsal column (DC) axon activation and neural responses in the dorsal horn (DH). Both modeled and recorded DC axons responded with irregular spiking patterns in response to low-amplitude SCS. Maximum inhibition of DH neurons occurred at ~80% of the predicted sensory threshold in both modeled and recorded neurons, and responses were strongly dependent on spatially targeting of stimulation, i.e., the complement of DC axons activated, and on stimulation parameters. Intrathecal administration of bicuculline shifted neural responses to low-amplitude stimulation in both the model and experiment, suggesting that analgesia is dependent on segmental GABAergic mechanisms. Our results support the hypothesis that low-frequency subperception SCS generates rapid analgesia by activating a small number of DC axons which inhibit DH neuron activity via surround inhibition.

Key words: computational modeling; in vivo recording; neuropathic pain; spinal cord stimulation

Significance Statement

Spinal cord stimulation (SCS) is an effective treatment from chronic pain, but conventional stimulation generates paresthesias, a buzzing sensation that some patients find uncomfortable. Recent studies have demonstrated substantial pain relief using low-frequency SCS that does not generate paresthesia; however, it is unclear how this form of stimulation works. In this study, we used computational models and recordings of dorsal horn (DH) neurons and dorsal column (DC) axons to study low-frequency, low-amplitude SCS and proposed a novel mechanism of action. The mechanism of action we proposed may help design future parameter selection and drive the development of SCS as a therapy.

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**Introduction**

Spinal cord stimulation (SCS) is an established treatment for chronic pain. SCS was developed based on the gate-control theory, which posits that activation of large diameter (Aβ) afferents leads to inhibition of pain transmitting neurons in the dorsal horn (DH; Melzack and Wall, 1965). Conventional SCS uses low-stimulation frequencies (<200 Hz) at amplitudes above perception threshold (PT) to activate dorsal column (DC) axons and inhibit pain (Parker et al., 2012). Stimulation above PT generates parasthesias, artificial sensations that some patients find undesirable. Some subperception modalities of SCS – using higher stimulation frequencies (1–10 kHz) – demonstrated at least equivalent pain relief without evoking parasthesias, but the timescale for pain-relief is generally slower than with conventional SCS, suggesting a different mechanism of action (Al-Kaisy et al., 2014). A notable exception was low-frequency stimulation delivered below PT that resulted in rapid onset of pain relief, and efficacy appeared to require specific stimulation parameters and precise spatial targeting of stimulation (Metzger et al., 2021).

Although significant and sustained pain relief was reported by patients, the mechanism(s) of action for low-frequency subperception SCS remained unclear. We surmised that the mechanism was based on activation of DC axons since it is applied at conventional frequencies and requires that electrodes are configured to achieve pain-parasthesia overlap with above-perception stimulation amplitudes during device programming. Further, the precise spatial targeting suggested that the mechanism may depend on activation of specific axons based on the somatotopic organization within the dorsal columns (Smith and Bennett, 1987; Feirabend et al., 2002).

Therefore, we tested the hypothesis that surround inhibition contributed to the mechanisms of low-frequency subperception SCS. Surround inhibition refers to sensory networks where spatial selectivity is amplified by the contrast of excitation from inputs within the central receptive field and inhibition from inputs in the surround receptive fields (Blakemore et al., 1970; Beck and Hallett, 2011). The importance of surround inhibition to sensation and pain is exemplified by the expansion of receptive field areas following disinhibition and inhibition of DH neurons from electrical stimulation, including by SCS, of sensory fibers from adjacent receptive fields (Hillman and Wall, 1969; Menétrey et al., 1977; Kato et al., 2009, 2011; Luz et al., 2010; Lee et al., 2019; Fan and Sdrulla, 2020).

We combined validated computational models and in vivo recordings from DC axons and DH neurons to study the mechanisms underlying low-frequency subperception SCS. DC axons responded to stimulation at amplitudes below putative sensory threshold, but patterns of activation were asynchronous at amplitudes close to the activation threshold (AT) both in silico and in vivo. Models predicted that such DC axon activity produced inhibitory effects in the DH network, and that maximum suppression of DH neurons occurred when low amplitude electrical stimulation was delivered to the surround receptive field. Experimental measurements of the effect of Aβ electrical stimulation (Aβ-ES) on activity of DH neurons corroborated model predictions, and partial abolition of inhibitory effects by the local application of bicuculline further supported the presence of a segmental inhibitory mechanism consistent with depictions of surround inhibition (Hillman and Wall, 1969). These results support that the pain-relieving effects of low-frequency, fast-acting subperception SCS are mediated by segmental surround inhibition and raise the possibility that surround inhibition can be exploited to optimize SCS.

**Materials and Methods**

**Code accessibility**

Code required to reproduce the figures in this manuscript will be made available. Model code is posted on ModelDB.

**Model of dorsal column axon activation**

We modeled DC axons using cable models of mammalian axons modified to account for dorsal column axon membrane dynamics and responses to stimulation (Fig. 1A; McIntyre et al., 2002; Titus et al., 2022). We coupled the DC axon models to a previously described finite element model (FEM) of the rat spinal cord (Pelot et al., 2018) to quantify responses to a range of stimulation amplitudes, pulse durations and pulse repetition frequencies (Fig. 1B). In brief, the FEM was built using transverse MRIs at the thoracic level (T10) and consisted of 10 vertebrae positioned within a box of 20 × 20 × 60 mm with outer boundaries grounded. Mesh resolution was doubled until activation thresholds changed by <1% (~3.14 × 10^6 elements). We positioned in the epidural space a model of our in vivo electrode: two 1 × 2 mm platinum contacts spaced 2 mm apart center-to-center along the length of the spine. The conductivities of the tissues and materials were selected from literature (Pelot et al., 2018). The potentials generated by SCS were extracted from the FEM and applied to the compartments of the validated DC axon model. Axon positions were selected to sample the range of positions within the DC, and axon diameters ranged from 2.2 to 6 μm with an increment of 0.2 μm and from 6 to 8 μm with an increment of 0.5 μm. The stimulation geometry was a simple bipolar configuration, with one contact set as the cathode and the other contact set to an equal anode; a single point current source was...
placed inside each electrode contact, and the outer boundary surfaces of the model were set to ground. All stimulation pulses were symmetric, biphasic, and rectangular. Simulated amplitudes ranged from 20 to 150 mA, except for 10-kHz stimulation (Extended Data Fig. 2-1), and simulated amplitudes for 10-kHz stimulation were 150 and 300 mA based on a predicted MT of 300 mA. Only activated axons are shown and the color of each axon represents its firing rate in response to symmetric biphasic rectangular 90-Hz, 275-μs stimulation.

Network models of the dorsal horn

We developed a biophysical network model of DH neurons based on a prior model and features of the model were described previously (Fig. 2A1; Zhang et al., 2014). The model was adapted to account for distributed center-surround network dynamics. The model had three types of neurons, an inhibitory (IN) interneuron, an excitatory (EX) interneuron, and a wide-dynamic range (WDR) projection neuron, and each model neuron contains four compartments: a dendrite, soma, axon hillock, and axon (see Table 1 for neuron geometries). We modeled ionic currents in each type of model neuron using Hodgkin–Huxley-like membrane models replicated from the prior model (Zhang et al., 2014). Each model node contained one of each neuron, 15 Aβ fiber inputs, 15 Aδ fiber inputs, and 3 C-fiber inputs. We modeled neuropathic pain inputs to the model as spike times along these afferents with mean firing rates (A-fiber μ = 2.2 Hz, C-fiber μ = 1.5 Hz) drawn from recordings of afferents from a neurona (Wall and Gutnick, 1974). We also added bursting (μsrais_interburst = 30 ms, μsrais_interburst = 551 ms, μspikes_per_burst = 6) in one third of the A-fibers (Kajander and Bennett, 1992; Liu et al., 2000). In the model, each zone was made up of an individual node (Fig. 2A2) with distinct inhibitory and excitatory connections between nodes (Fig. 2A3), and each zone in the model represented a corresponding peripheral receptive field area (Fig. 2A4) and DC somatotopy (Fig. 2A5). One zone was designated the “center” zone (“zone 1”), and flanking zones (“zone 2” and “zone 3”) representing the “surround” were added and reciprocally interconnected via excitatory and inhibitory connections (Fig. 2A3). Connections between zones were from the excitatory or inhibitory interneurons in one node (e.g., zone 2) to the WDR neuron in another zone (e.g., zone 1). Synaptic connections between individual neurons replicated the connections from the previous model and Table 2 displays the synaptic connections between neurons. Synaptic properties also matched the prior model (Table 3). We validated the DH network model by comparing zone 1 model WDR neuron responses to experimental recordings of Lamina V WDR neurons in response to increasing amplitude of peripheral electrical stimulation (Hillman and Wall, 1969). The activity of the zone 1 model WDR neuron matched the magnitude and pattern of responses of experimentally recorded neurons during simulated peripheral nerve stimulation (Fig. 2C), indicating that the model architecture replicated realistic center-surround receptive field dynamics. All simulations were conducted in the NEURON simulation environment (v7.5 and v7.6) using second-order implicit Crank–Nicholson integration and a timestep of 0.0125 ms (Hines and Carnevale, 1997). Each simulation was run for 18 s with stimulation on for 10 s at the end.

We compiled a library of DC axon responses for each stimulation configuration with the responses of all possible axon position and diameter combinations. As dorsal column fibers are generally collaterals of large myelinated primary afferents (Melzack and Wall, 1965; Niu et al., 2013), individual DC axon responses were sampled from
the library of responses to generate spike time inputs to the DH network model via the model’s Aβ inputs. Each individual fiber was assigned as an input to a node of the network model based on its medial-lateral and dorsal-ventral position within the DCs. According to anatomic tracings of DC axons, fibers reach their most dorsal and medial point approximately two levels rostral to their entry point into the spinal cord (Smith and Bennett, 1987; Niu et

Table 1: Neuron geometries for individual compartments

| Neuron | Dendrite (cylinder [diameter, length]) | Soma (sphere [diameter]) | Axon hillock (cone [initial diameter, final diameter, length]) | Axon (cylinder [diameter, length]) |
|--------|--------------------------------------|--------------------------|---------------------------------------------------------------|-----------------------------------|
| EX     | [3 μm, 300 μm]                       | [25 μm]                  | [2 μm, 1 μm, 9 μm]                                           | [1 μm, 1000 μm]                   |
| IN     | [3 μm, 400 μm]                       | [10 μm]                  | [1 μm, 0.5 μm, 30 μm]                                        | [1 μm, 1000 μm]                   |
| WDR    | [2.5 μm, 350 μm]                     | [20 μm]                  | [2 μm, 1 μm, 9 μm]                                           | [1 μm, 1000 μm]                   |
Table 2: Synaptic connections and conductances between neurons

| Source | Target | Synapse type | Maximum conductance (g_{max}, nS) |
|--------|--------|--------------|----------------------------------|
| Aβ     | IN     | AMPA         | 14.6                             |
| Aβ     | WDR    | AMPA         | 24                               |
| Aβ     | WDR    | NMDA         | 0.1                              |
| Aδ     | WDR    | AMPA         | 24                               |
| Aδ     | WDR    | NMDA         | 0.1                              |
| C      | EX     | AMPA         | 8                                |
| C      | EX     | NMDA         | 4                                |
| C      | EX     | NK1          | 0.02                             |
| C      | WDR    | NK1          | 0.014                            |
| IN     | EX     | GABA_A       | 5.3                              |
| IN2**  | EX1*   | GABA_A       | 3.66                             |
| IN3*   | EX1*   | GABA_A       | 3.66                             |
| IN     | WDR    | GABA_A       | 5.3                              |
| IN     | WDR    | Glycine      | 5.3                              |
| IN2*   | WDR1*  | GABA_A       | 4.5                              |
| IN2*   | WDR1*  | Glycine      | 4.5                              |
| IN3*   | WDR1*  | GABA_A       | 4.5                              |
| IN3*   | WDR1*  | Glycine      | 4.5                              |
| IN     | EX     | NMDA         | 0.21                             |
| EX     | WDR    | AMPA         | 0.29                             |
| EX2*   | WDR1*  | NMDA         | 0.014                            |
| EX2*   | WDR1*  | AMPA         | 0.14                             |

Starred neurons(*) show connections between nodes and these connections are reciprocal and repeated for each node (see Fig. 2).

From there, fibers are pushed laterally as new fibers enter the cord and preserve their relative somatotopic organization up to the DC nuclei (Loutit et al., 2021; Smith and Bennett, 1987). For center fibers, we selected the 20 most medial and dorsal fiber positions by minimizing the equation $Z = X^{0.15} + Y^{0.45}$, where $X$ was the medial-lateral position and $Y$ was the rostral-caudal position. We randomly selected 15 of these positions for each simulation as the primary target of stimulation. When stimulation targeted the center of the receptive field in the model, all 15 axons were inputs to zone 1. When stimulation targeted the surround, all 15 axons were inputs to zone 2. When stimulation targeted a mix of center and surround, eight fibers were inputs to zone 1 and seven fibers were inputs to zone 2. We repeated this process and 15 of the 20 next most medial and dorsal fiber positions were assigned to the secondary target of stimulation. When stimulation targeted the center receptive field area, these fibers corresponded to zone 2 inputs. Finally, we assigned 15 of the next 20 fibers as inputs to zone 3, representing the distant surround. For each fiber position, we randomly selected a fiber diameter from a range of diameters commonly found within the rat DCs ($\mu = 4.4 \mu m, \sigma = 1.0 \mu m$).

Table 3: Synaptic time constants and reversal potentials

| Synapse | Rise time constant, $\tau_1$ (ms) | Fall time constant, $\tau_2$ (ms) | Reversal potential, $E_{syn}$ (mV) |
|---------|----------------------------------|----------------------------------|-----------------------------------|
| AMPA    | 0.1                              | 5                                | 0                                 |
| NMDA    | 20                               | 100                              | 0                                 |
| NK1     | 100                              | 3000                             | 0                                 |
| GABA_A  | 0.1                              | 20                               | $-70$                             |
| Glycine | 0.1                              | 10                               | $-70$                             |

Using the procedure described above, we generated 25 maps of activated DC axons to test stimulation inputs while accounting for biological variability with different fiber diameters and positions. We quantified the response of each specific axon across amplitudes for each combination of frequency and pulse width (Fig. 2D) and assigned each DC fiber as an input to the DH network based on the fiber position and spatial targeting condition (Fig. 2D). Across all 25 maps of DC axons, the mean firing rate of the sampled axons decreased for axons further away from the electrode (Fig. 2D3), and axons in the most dorsal positions close to the electrode were most likely to be activated, especially at low-stimulation amplitudes (Fig. 2D4).

For model states representing neuropathic pain, we randomly varied the values of several parameters related to disinhibition in the network. First, we reduced the GABAergic conductance by up to 50% of the initial value (Moore et al., 2002). Second, the reversal potentials of inhibitory synapses were shifted in 4-mV increments by up to 16 mV (Coull et al., 2003). Third, the number of Aβ and C fibers that were active was increased by up to 50% in surround nodes to represent an expanded pain area. Finally, the conductance of Aβ fiber inputs to inhibitory interneurons was reduced by up to 50% (Zhang et al., 2014). Each of these changes was implemented separately for each node, and the degree of disinhibition from each model variant was selected using Latin hypercube sampling with 30 different simulations.

Animal preparation

Recordings from DC axons and DH neurons were conducted in separate experiments on male Sprague Dawley rats (300–500 g). DH neuron responses were recorded from eight rats and DC responses were recorded from six rats. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at Duke University. Rats were housed in pairs and were initially anesthetized with isoflurane (3.0%, inhaled, Abbott Laboratories) and then urethane (1.2 g/kg, s.c.; Sigma-Aldrich). Anesthesia was supplemented with a second dose of urethane (0.4 g/kg, i.p.) and a third dose (0.1 g/kg, i.p.) if pinching the hindpaw evoked a withdrawal reflex. A tracheotomy was performed to allow intubation to maintain respiration during paralysis. Respiration, heart rate and SpO2 were monitored throughout the surgical procedure (PhysioSuite; Kent Scientific). Temperature was monitored using a rectal probe and maintained between 35°C and 37°C using a heating blanket (Gaymar T/Pump). Rats were attached to a stereotaxic frame using ear bars and vertebral clamps attached to T12 and L2 (Kopf instruments). An incision was made over the left hindlimb to expose the sciatic nerve and its distal branches. A laminectomy was performed to expose the spinal cord between T13 and L1. For DC recordings, a second laminectomy was performed to expose the cervical (C6) segment. The dura was resected over the exposed spinal cord segments. Following data collection animals were euthanized with an overdose of Euthasol followed by bilateral thoracotomy.
**DC axon unit recording**

We recorded responses of 24 individual DC axons from six animals to epidural SCS (Fig. 3A) using methods described previously (Crosby et al., 2017). Briefly, DC axons were stimulated using a custom bipolar paddle consisting of two 1.5 × 1 mm platinum contacts spaced 2 mm apart and insulated dorsally using silicone. The electrode was inserted into the epidural space underneath the T10–T11 vertebrae. Motor thresholds (MTs) for DC axon recordings were determined by identifying the lowest amplitude that evoked a visible twitch using 90 Hz/225 μs. Axons were recorded using a bipolar tungsten microelectrode (impedance 8–10 MΩ, tip spacing 190 μm; FHC) inserted into the DC medially at an angle of 30–70° relative to the sagittal plane. Signals were bandpass filtered from 500 Hz to 5 kHz, amplified by 1000 (XCel13; FHC), further amplified to a total gain of 10,000 (SR560; Stanford Research Systems), and sampled at 20 kHz (PowerLab; ADInstruments). Once a single unit was acquired, it was confirmed to be a single projecting axon using the following criteria: invariant latency to stimulation, faithful response to a short train of 200-Hz stimulation, and the amplitude/waveform matched between activation at the cervical and thoracic locations. The responses of individual DC axons were assessed in response to 5 s of SCS at amplitudes near their individual ATs (Fig. 3B). Each stimulation block for DC axon recordings lasted ~4 min, and units were reconfirmed between stimulation blocks. After identifying units, we filtered the signal and used a custom algorithm (MATLAB R2021a; MathWorks) to find spike times (Fig. 3C). The stimulation artifact is ~0.5 ms wide and occurs ~1 ms before the start of each action potential; the artifact cannot be distinguished from individual action potential shapes in Figure 3C and was removed from the zoomed in window.

**DH unit recording, classification, and sorting**

We recorded responses of 215 DH neurons from eight rats to Aβ-ES (Fig. 3A). Although the dorsal columns follow a somatotopic organization based on fiber entry level (Smith and Bennett, 1987), precisely targeting groups of dorsal column fibers in a rodent spinal cord is difficult, as rodents cannot directly indicate where sensations are generated by SCS. However, since peripheral nerves correspond to well-defined receptive field areas in accordance with the somatotopic organization (Swett and Woolf, 1985; Kambiz et al., 2014), for in vivo DH neuron recordings, we used peripheral Aβ-ES rather than dorsal column stimulation to stimulate selectively center and surround receptive field areas. For DH recording experiments, MTs were measured by stimulating the tibial branch of the sciatic nerve using a bipolar cuff electrode with a 0.5- to 1.0-mm internal diameter (Microprobes for Life Science) with 50-Hz/200-μs stimuli in all animals. MTs were also measured with 90-Hz/225-μs and/or 50-Hz/300-μs stimuli in four animals, and MTs were not significantly different between parameters. Following MT testing, rats were paralyzed with gallamine triethiodiide (1 ml/h at 0.2 g/ml) injected through an intraperitoneal catheter. To record single units, 16- or 32-contact recording electrodes (NeuroNexus) were slowly inserted into the lumbar spinal cord exposed by the T13-L1 laminectomy; 16-contact electrodes had a recording span of 375 μm (Neuronexus electrode A1x16-Poly2-5mm-50s-177-A16) and 32-contact electrodes had a recording span of 275 μm (Neuronexus electrode A1x32-Poly3-5mm-25s-177). Electrodes were inserted at a 30–45° rostral-caudal and medial-lateral angle just medial to the dorsal root entry zone. Electrode depths spanned from ~50 to 800 μm with an average depth of 400 μm. The rostral-caudal position of each electrode was identified with respect to the vertebral level. Signals were amplified, filtered, and sampled on a 32-channel recording system (MAP System, Plexon). Brushing was used as a search stimulus to identify units within the receptive field. Individual single units were identified online and then confirmed offline and sorted using feature analysis (Offline Sorter V3, Plexon). The primary features used for sorting were the first three principal components, waveform energy and nonlinear energy, and waveform amplitude.

After identifying individual DH units, we quantified responses to 90-Hz/225-μs peripheral electrical stimulation of the tibial or peroneal branch of the sciatic nerve at different stimulation amplitudes (20%, 40%, 60%, and 80% MT; Fig. 3D). Responses to peripheral electrical stimulation were also evaluated during ongoing stimulation of the sciatic nerve with a bipolar cuff electrode (Microprobes for Life Science) at 1 Hz and an amplitude 50–60 times MT to drive C-fiber inputs to the DH. In one group of rats (n = 2), bicuculline methiodide (Alfa Aesar) was administered following at least one block of baseline recordings (Fig. 3D). A total of 10 μl of 0.3 μg/μl bicuculline in 0.9% saline solution was administered intrathecally at the recording site with a Hamilton syringe. A subsequent block of recordings was initiated after recording spontaneous activity for at least 20 min. Each stimulation block lasted 11 min with ~20–30 min before each block to record spontaneous activity and responses to mechanical stimulation. Units were confirmed through online sorting before each stimulation block.

We classified individual units by their waveform shape because prior studies found a functional difference between units with monophasic and biphasic waveforms (Lee et al., 2019), and both monophasic and biphasic neurons exhibited a variety of responses to peripheral nerve stimulation (Fig. 3E). In prior studies, monophasic neurons were putatively excitatory (pEX) based on their firing patterns and receptive field areas while biphasic neurons were putatively inhibitory (pIN; Lee et al., 2019). Units were classified as pEX, pIN, or unclassified using a custom classification algorithm (MATLAB; MathWorks). The algorithm modeled each average unit waveform as a double exponential (Eq. 1), then extracted key features from the fitted double exponential and its first derivative (Snyder et al., 2016). Single units were classified as pEX or pIN if the confidence that they belonged to the respective class was higher than 60%. We also classified neurons based on responses to mechanical stimulation (brush, press, pinch crush) on the ipsilateral hindpaw. Among neurons classified as pEX that we identified as mechanically responsive, 12% were classified as high threshold (only responded to
Figure 3. Experiment recording setup. A, Individual DC axons and multiple DH neurons were recorded in separate animals. DC axons were recorded from the lumbar spinal cord using a bipolar tungsten microelectrode. Multiple single units were recorded from the lumbar dorsal horn using a 16- or 32-contact silicon microelectrode. The sciatic nerve was exposed to stimulate individual branches or the entire sciatic nerve. DH neurons were evaluated for their response to receptive field targeted peripheral nerve stimulation and stimulation of the full sciatic nerve. B, Timeline of evaluation of DC axon responses to bipolar stimulation at T10–T11 at different amplitudes. Amplitudes were randomized across trial blocks. C, Example DC axon recording during dorsal column stimulation (DCS). Black trace is raw recording, gray lines indicate detected spikes. The gray box shows the waveforms of the recorded unit. D, Timeline of DH recordings. After identifying units from the receptive field area using mechanical stimulation of the ipsilateral rat hindpaw, spontaneous activity was recorded for at least 10 min. Next, multiple single units were recorded with different (randomized) amplitudes of tibial nerve stimulation with and without concomitant stimulation of the sciatic nerve at C-fiber amplitude. In some animals, neurons were also recorded after applying bicuculline (BICU) intrathecally at the recording site. See Extended Data Figure 3-1 for responses of DH neurons to mechanical stimulation before and after application of BICU. E, Individual units were classified based on their waveform shape (Snyder et al., 2016; Lee et al., 2019). Plots left of raster show the sorted waveforms of individual units and the mean waveform. Raster plots show 10 s of monophasic putatively excitatory (pEX) and biphasic putatively inhibitory (pIN) unit responses to receptive-field targeted stimulation at 60% MT. Raster color represents the change in firing rate over the full 30-s window of stimulation. F, Example Z-scores calculated for one neuron excited by Aβ-ES and one neuron inhibited by Aβ-ES. Neurons were classified as responders if three or more consecutive bins exhibited $|z| \geq 1.96$ (Montgomery, 2006).
crush), 76% were wide-dynamic range neurons (increasing response rates with increasing stimulus strength), and 12% were low-threshold (only responded to brush and/or press). Among neurons classified as pIN that were mechanically responsive, 18% were high threshold, 55% were wide-dynamic range, and 27% were low-threshold:

\[ V = a_1 \cdot \exp \left( -\frac{(x - b_1)^2}{c_1} \right) + a_2 \cdot \exp \left( -\frac{(x - b_2)^2}{c_2} \right). \]

We constructed peristimulus time histograms (PSTHs) of neural activity to identify neurons that were responsive to stimulation (Fig. 3F). We expected that application of bicuculline would differentially affect pEX and pIN neuron responses to mechanical stimulation. Bicuculline substantially increased the percentage of pEX neurons that responded to mechanical brush and crush inputs (from 40% to 70% for brush and from 43% to 50% for crush) but did not change the response rate of pIN neurons that responded to mechanical inputs (Extended Data Fig. 3-1). However, bicuculline unmasked a significant change in brush responses for both pEX and pIN neurons (\( p < 0.001; \) Extended Data Fig. 3-1), but the change in brush responses were significantly larger in pEX neurons [two-sample Kolmogorov–Smirnov test (kstest2), \( p < 0.05 \)]. Bicuculline also unmasked a significant change in responses to crush inputs in both neuron classes (t test, \( p < 0.001 \)), but the changes did not differ between pEX and pIN neurons. Further, while application of bicuculline increased the activity of neurons both in the presence and absence of mechanical stimulation, pIN neurons showed a significant increase in spontaneous activity compared with pEX neurons (kstest2, \( p < 0.01 \)). Taken together, these differential effects of bicuculline on AP morphology-classified pEX and pIN neurons support our waveform-based classification and justify independently considering the effects of A\( \beta \)-ES separately on each subclass of neuron.

We compared single unit activity during SCS to spontaneous activity before stimulation to determine responders to stimulation following established criteria (Zhang et al., 2015). First, we objectively determined the optimal bin width for each neuron and each stimulation frequency by evaluating the spike count per bin at each frequency (Shimazaki and Shinomoto, 2007), and we compared activity during SCS to spontaneous activity before stimulation with the same bin widths. We identified bins with the stimulation artifact present in the stimulation condition according to the bin width, and to enable a direct bin-to-bin comparison, we omitted activity in these corresponding bins from spontaneous activity. Neurons were classified as responders if stimulation activity compared with baseline spontaneous activity was significantly different (\( z > 1.96 \)) in three or more consecutive bins. The responses of all neurons are shown, but neurons had to have a firing rate of at least 1.5 Hz and respond to at least one amplitude to be included in the clustering analyses.

Individual neuron responses were normalized to their greatest change in firing rate at each amplitude such that the largest change corresponded to +1 for excitatory responses or −1 for inhibitory responses (Lemay and Grill, 2004). We clustered normalized neurons responses using fuzzy c-means clustering (\( m = 2.0, \) max iterations = 500, minimum improvement = 1e-5) based on the first two principal components of their response to all amplitudes. We identified the major cluster groups with between two and six clusters and used the silhouette evaluation or Davies–Bouldin evaluation to determine the optimal number of clusters for each group (Davies and Bouldin, 1979; Roussiee u, 1987).

Results

We quantified the effect of A\( \beta \) electrical stimulation on the activity of DC axons and DH neurons in computational models and in vivo experiments. We initially used stimulation parameters (90 Hz, 225 \( \mu \)s/phase) consistent with those of clinically effective low-frequency subperception SCS, 90-Hz SCS at amplitudes just above the activation threshold for individual DC axons drove asynchronous firing in both model DC axons and individual DC axons recorded in vivo. When applied as the A\( \beta \) fiber inputs to a computational model of the DH network, these irregular spiking patterns led to greater suppression of model WDR neurons than did regular spiking patterns from DC axon responses evoked by higher stimulation amplitudes. We quantified the effects of electrical stimulation on DH neurons in vivo across functional classes (putatively excitatory or inhibitory neurons), recording location, and stimulation parameters. Since identifying receptive fields using SCS via pain-paresthesia overlap mapping is impractical in rodents, we used peripheral A\( \beta \)-ES, rather than DC stimulation, to stimulate selectively center and surround receptive field areas. Stimulation parameters and recording location both had strong effects on neural responses, but neuron functional class did not have a significant effect. Importantly, data from both the DH model and in vivo experiments indicated that the rostral-caudal location, rather than the identity of the neural targets, was responsible for generating substantial intersegmental inhibition in the spinal dorsal horn. In a subset of animals, we applied the GABAA receptor antagonist bicuculline to represent the loss of inhibition that occurs in chronic pain states (Moore et al., 2002; Braz et al., 2012). Disinhibition following bicuculline unmasked activity in putatively excitatory neurons and revealed differences in neuron responses to A\( \beta \)-ES, further supporting a spinal GABAergic inhibitory mechanism mediating the effects of stimulation. Finally, we compared model network responses to different SCS paradigms in network states both before and after impairing inhibition, and from these results, proposed possible methods to optimize stimulation parameters, even following GABAergic disinhibition, by exploiting surround inhibition.

Low-amplitude SCS drove irregular spiking in DC axons

We quantified the response of DC axons to different amplitudes of 90-Hz SCS. The spiking patterns did not always match the stimulation patterns, and both model

\[ V = a_1 \cdot \exp \left( -\frac{(x - b_1)^2}{c_1} \right) + a_2 \cdot \exp \left( -\frac{(x - b_2)^2}{c_2} \right). \]
axons (Fig. 4A) and axons recorded in vivo (Fig. 4B) exhibited bursting when stimulated at 100–140% of activation threshold (AT). Although the experimental responses showed greater variability in spiking patterns, both model and experimental axons exhibited bursts dominated by 90-Hz spiking that increased in length with stimulation amplitude (Fig. 4C), and immediate entrainment to 90 Hz at 100% AT was never observed in either model or experiment. Furthermore, both model and experimental axons exhibited spiking asynchronous with the stimulation pulses at low-stimulation amplitudes (Fig. 4D), and only 25% of axons fired reliably in response to any single specific stimulation pulse at 100% of their AT. However, the majority (72% of model and 86% of experimental) of axons began firing synchronously with the stimulation pulses as stimulation amplitude was increased to ≥120% AT. Thus, DC axons exhibited variable patterns of activation strongly dependent on the amplitude of stimulation, and, in particular, low amplitude stimulation evoked asynchronous bursting activity.

Irregular spiking in DC axons drove inhibition in dorsal horn network model

Both model-calculated and experimentally recorded patterns of DC axon activity were applied as the Aβ fiber
inputs to the biophysically based DH network model (Fig. 2), and changes in model WDR neuron activity were quantified. Interestingly, inhibition of model WDR neurons was greatest by model-generated DC axon spiking patterns evoked at the lowest amplitude (100–110% AT), and patterns from higher amplitudes resulted in less inhibition of WDR neurons (Fig. 4E). Similarly, with DC axon inputs from in vivo recordings, the greatest inhibition of model WDR neurons also occurred with patterns evoked at lower stimulation amplitudes, and the average reduction in WDR model neuron spiking was similar by patterns evoked at the lowest amplitude (100–110% AT) than at 120–130% AT. Nevertheless, the model predicted that irregular spiking inputs from Aβ fibers (Fig. 2A1), and the excitatory effect of higher stimulation amplitudes was because of increased direct excitation of WDR neurons. In contrast to effects on model WDR neurons by model-derived patterns of DC axon activity, model WDR neurons were more inhibited by patterns of DC axon activity derived from in vivo recordings during stimulation at 130–140% AT than at 120–130% AT. Nevertheless, the model predicted that irregular spiking inputs from DC axons led to stronger net inhibition of excitatory neurons in the DH network than regular spiking inputs from higher amplitude stimulation. Collectively, this indicated that both the pattern of DC axon activity and the relative balance of direct excitation from Aβ fibers and indirect (interneuron-mediated) inhibition altered WDR neuron activity.

**Inhibition of model WDR neuron activity was maximized by engaging surround inhibition and selecting specific SCS parameters**

The prior simulations predicted the effect of stimulation amplitude on DC activity, and we also quantified how the population of activated DC axons altered responses of DH neurons by altering the balance of direct excitation and indirect (interneuron-mediated) inhibition of WDR neurons. Surround inhibition in the DH is hypothesized to depend on precise topography of Aβ fiber stimulation (Hillman and Wall, 1969; Lee et al., 2019; Fan and Sdrulla, 2020) and involve segmental GABAergic mechanisms (Zhang et al., 2014, 2015). Therefore, we compared changes in model WDR neuron activity evoked by stimulation delivered to different receptive field areas by altering the proportion of center versus surround model DC fibers that were activated (Fig. 5A). This shift in the proportion of activated center versus surround fibers is analogous to shifting the contacts that are active during SCS to target different fiber groups, as fibers from relative surround receptive field areas are positioned more dorsally and medially in more rostral positions along the cord (Smith and Bennett, 1987; Niu et al., 2013). In general, stimulus suppression the activity of model WDR neurons relative to model WDR neuron activity during a modeled pain input, but model WDR neuron responses were dependent on the origin of activated DC axons (ANOVA, p < 0.05, post hoc Tukey’s test, p < 0.05), i.e., the proportion of activated DC axons that arose from the surround receptive field area versus the center receptive field area. Activating DC fibers from the surround receptive field was necessary for maximal suppression of the model WDR neuron at lower stimulation amplitudes (Fig. 5B). Maximizing WDR suppression at a putatively subperception amplitude (40% MT) was achieved when we assumed SCS activated axons that originated primarily from the surround receptive field (Fig. 5C,D). Furthermore, model WDR suppression exhibited a nonmonotonic relationship with stimulation amplitude, which is explained by the shift from indirect (interneuron-mediated) inhibition to direct activation as stimulation amplitude was increased and more center model DC axons originating from the center were activated. Finally, the effects on WDR activity were rapid. Consistent with clinical observations, models predicted that inhibition of WDR neuron activity occurred within seconds of the start of stimulation in response to low rate but not high rate SCS (Extended Data Fig. 2-1A).

To identify the optimal parameter settings for fast-acting subperception SCS in the computational model, we quantified the effects of a broad range of stimulation frequencies and pulse widths (selected to deliver a similar neural dose by adjusting both the frequency and pulse width of stimulation; Paz-Solis et al., 2022) on inhibition of model WDR neurons (Fig. 5E). Increasing the frequency of SCS targeted to surround receptive fields increased the maximum reduction in WDR firing rate but decreased the range of amplitudes that produced a reduction in WDR firing rate. For example, the range of stimulation amplitudes that reduced the firing rate of model WDR neurons by at least 50% was 35–60% of MT for 50-Hz/300-μs stimulation, but only 35–40% for 200-Hz/200-μs stimulation. Higher stimulation frequencies also excited WDR neurons at higher amplitudes of SCS (Fig. 5F). For each of the 25 random selections of DC axons (Fig. 2D), we determined the stimulation parameters that produced the maximum reduction in WDR firing rate for amplitudes between 30% and 60% of MT (amplitudes within the therapeutic range). 50 Hz/300 μs produced the greatest inhibition in 16% (4/25) cases, 90 Hz/225 μs was optimal in 44% cases, and 90 Hz/275 μs was optimal in the remaining 40% cases. The fact that 90-Hz stimulation was optimal in most model conditions and the use of 90-Hz stimulation during the clinical study using fast-acting subperception SCS (Metzger et al., 2021) justified the use of 90-Hz stimulation for our in vivo experiments.

We also simulated changes in model WDR activity after multiple impairments to inhibition to represent changes observed with chronic pain progression (see Materials and Methods; Fig. 5G). These simulations of diverse pain states exhibited more variability in the model WDR responses to stimulation than the healthy model simulations but showed similar trends (Fig. 5H). Following pain state induction, 90 Hz/275 μs was the most effective parameter setting in 43% (13/30) of pain states, and parameter settings from 30 to 200 Hz were optimal for at least one pain state (Fig. 5F). Nonetheless, these results still indicated that 90-Hz stimulation was optimal for our in vivo experiments, even with disrupted dorsal horn inhibitory mechanisms.
Figure 5. Model responses to low-rate, low-amplitude SCS depend on spatial targeting, stimulation parameters (amplitude, pulse width, rate), and pain state. A, Spatial targeting in the model was simulated by altering the population of DC axons that was activated at each amplitude. We changed the peripheral origin of model DC axons based on the rostral-caudal position of stimulation. For stimulation in the most caudal position (brown), axons from the center of the peripheral receptive field were positioned in the most medial and dorsal positions within the DCs (see Fig. 1; Smith and Bennett, 1987). Conversely, for stimulation in the most rostral position (purple), axons from the surround were in the most medial and dorsal positions. Targeting in between (green) mixed...
Inhibition of DH neurons by low-amplitude Aβ-ES was disrupted by bicuculline

We recorded responses of multiple single units in the DH of anesthetized rats to quantify the effects of stimulation location and stimulation parameters on neural activity. The effects of stimulation on DH neurons were heterogeneous (Zhang et al., 2015), and we classified neurons into two functional classes: putatively excitatory (pEX) or putatively inhibitory (pIN) according to the shape of the recorded AP (see Materials and Methods; Lee et al., 2019). We used peripheral Aβ-ES rather than DC Aβ-ES to study the effects of targeting stimulation to specific receptive field areas. We focused on short-term effects of stimulation, and quantified changes in neural activity over a 30-s window following stimulation onset. We plotted the changes in activity of all neurons during stimulation and detected no differences in the distribution of inhibitory versus excitatory neuron responses to Aβ-ES between the pEX and pIN neuron classes (Fig. 6A,B, ANOVA, p = 0.9). The responses of both pEX and pIN were dependent on stimulation amplitude, and consistent with the effects on model neurons, stimulation at 40 and 60% MT significantly reduced pEX and pIN activity compared with 20% MT for both normalized and raw responses (Fig. 6C, mANOVA, p < 0.001, post hoc Tukey’s test, p < 0.001). Similarly, there was a significant effect of stimulation amplitude (mANOVA, p < 0.001), but not neuron class (p = 0.57) on DH neuron responses to Aβ-ES delivered during coincident high-amplitude (above C-fiber threshold) sciatic nerve stimulation that increased the mean firing rate of recorded neurons before application of Aβ-ES (Extended Data Fig. 6-1). Taken together, these results are the first to demonstrate that putatively subperception (40% MT or below; Crosby et al., 2017) Aβ-ES is sufficient to produce effects in DH neurons and corroborate our hypothesis that low-level Aβ-fiber activation drives these effects.

Aβ-ES also reduced the activity of pEX and pIN neurons following application of bicuculline, but, unlike prebicuculline recordings, inhibition of pEX and pIN activity increased monotonically with stimulation amplitude (Fig. 6E–H). The activity of both pEX and pIN neurons was reduced more by Aβ-ES at 40%, 60%, and 80% MT compared with 20% MT (Fig. 6G, mANOVA, post hoc Tukey’s test, p < 0.001), and again there was no effect of neuron class (p = 0.77). There was a shift from strong and persistent inhibition by Aβ-ES at 40% MT to weaker and more transient inhibition by Aβ-ES at 80% MT as seen in the PSTHs of individual neurons that were recorded simultaneously (Fig. 6I). Interestingly, the proportion of pEX but not pIN neurons responding to Aβ-ES (i.e., exhibiting a significant change in activity poststimulation compared with prestimulation) was substantially reduced by application of bicuculline compared with control (nonbicuculline) recordings. This supports a segmentally mediated but complex GABAergic mechanism as a driver for inhibition mediated by low-amplitude Aβ-ES (pEX: 53% responders to stimulation in control recordings vs 24% in bicuculline recordings, pIN: 49% control vs 52% bicuculline; Extended Data Fig. 3-1). However, the fact that stimulation remained effective despite reduced GABAergic inhibition suggests that high-amplitude Aβ-ES can recruit additional inhibitory mechanisms, such as glycineric inhibition.

Engaging surround inhibition maximized inhibition of DH neurons

Dorsal horn neurons are roughly arranged rostrocaudally by receptive field (Swett and Woolf, 1985). We segregated the individual in vivo recordings of pEX dorsal horn neurons by their rostrocaudal location along the spinal cord (Fig. 7A,B) while stimulating the same peripheral receptive field. In this way, the same peripheral inputs represented either center or surround receptive field inputs to different neurons, depending on their rostrocaudal locations, and this also enabled comparison to model neuron responses across relative positions. Mechanical brush and crush inputs were delivered on the plantar surface of the hind paw, the center receptive field area of the tibial nerve (Swett and Woolf, 1985; Kambiz et al., 2014). Since we stimulated peripherally the tibial branch of the sciatic nerve, most stimulation-affected afferents should enter through the L5 and L4 spinal roots (Swett et al., 1991) corresponding to the “center” receptive field area and recording positions 1 and 2, respectively. Position
three corresponded to the L3 spinal root, rostral to the primary entry level of afferents from the tibial nerve and therefore likely corresponding to a “surround" receptive field.

Neural responses were dependent on recording position (Fig. 7; ANOVA, *p < 0.001) and were different between position three versus both positions 1 and 2 (post hoc Tukey’s test, *p < 0.05). However, responses for pIN neurons were not dependent on position (ANOVA, *p = 0.15; Extended Data Fig. 7-1). Consistent with model results, responses of neurons in position 1 were mostly excitatory while responses in position 2 showed a mix of excitation and inhibition, and responses in position three were strongly inhibitory (Fig. 7C,D). Thus, the position-dependent responses in vivo are consistent with the center-surround architecture represented in the model, with each
zone approximately corresponding to responses in each recording position (Hillman and Wall, 1969). We only studied the effects of stimulation over 30-s windows in vivo, but as in the model, the changes in firing rates typically occurred within seconds of the onset of stimulation and persisted for the entire stimulation window (Extended Data Fig. 2-1).

The center-surround architecture was also examined by considering the proportion of pEX neurons excited at each recording location (Fig. 7A). The majority of pEX neurons in position 1 were excited by Aβ-ES, while all pEX neurons in position three were inhibited by Aβ-ES. In contrast, the percentage of pEX neurons excited by both brush and crush stimulation increased from position three to position 1, indicating that inhibition from peripheral Aβ-ES was distinct from the primary area of excitation from mechanical stimulation. These spatial relationships corroborate the center excitation-surround inhibition spatial organization of the model and support a need for precise spatial targeting of subperception Aβ-ES to maximize efficacy.

**Response clusters affected differently by 50- and 90-Hz peripheral Aβ-ES after disinhibition**

We categorized individual neurons by clustering the normalized responses to stimulation at different amplitudes. Fuzzy c-means clustering of the principal components of each response identified heterogeneous response types across the population of recorded neurons. Responses were optimally divided into two clusters (Davies–Bouldin metric; Davies and Bouldin, 1979) or four clusters (silhouette metric; Rousseeuw, 1987; Fig. 8A, boxes), and each line represents the average response of each neuron within that cluster for different stimulation amplitudes (Fig. 8A). Using two clusters divided responses into “excited” (purple) or “inhibited” (green) responses. Neurons within the “excited” cluster increased net excitation in response to stronger stimulation amplitudes, and “inhibited” responses exhibited the inverse behavior. Using four clusters divided responses into “monotonic excited” (light purple), “low-amplitude excited” (dark purple), “low-amplitude inhibited” (dark green), and “monotonic inhibited” responses (Fig. 8A, light green).

Neurons within the low-amplitude clusters exhibited strong responses to low-stimulation amplitudes (20–40% MT), but not higher stimulation amplitudes (60–80% MT). We quantified the proportion of neurons in each cluster across stimulation frequency (50- vs 90-Hz Aβ-ES; Fig. 8C,D), pain condition (none vs bicuculline), and neuron classification (pEX vs pIN; Fig. 6B).

While clustered responses to stimulation amplitude were heterogeneous across all conditions, several key differences in response clusters emerged between stimulation parameters and pain conditions. First, 50- and 90-Hz Aβ-ES had similar effects on both pEX and pIN neurons when we examined changes with two clusters (Fig. 8C). However, after application of bicuculline, 90 Hz was more likely than 50 Hz to inhibit pEX neurons and excite pIN neurons. This difference in inhibition of pEX neurons was mostly driven by a decrease (~28%) in the proportion of pEX neurons that experienced monotonic excitation with 90-Hz stimulation. The difference in inhibition of pIN neurons...
neurons was driven by a decrease in monotonic inhibition with 90-Hz stimulation (−15%). When comparing postbicuculline recordings to control recordings, 50-Hz Aβ-ES inhibited a larger proportion of pIN neurons (19%) but almost no additional pEX neurons (3%, two clusters; Fig. 8D). On the other hand, 90 Hz inhibited a much larger proportion of pEX neurons (17%) than pIN neurons (9%) after bicuculline. The increase in inhibition of pEX neurons after bicuculline comprised increases to both monotonic inhibition (+13%) and nonmonotonic low amplitude inhibition (+4%) responses. Overall, these results demonstrate that while in vivo responses to stimulation were heterogeneous, there was an interaction between stimulation parameters (frequency and amplitude) and the net inhibition of DH neurons. Additionally, local application of bicuculline produced significant changes to the responses of both pEX and pIN neurons with both 50 and 90 Hz, further implicating a segmental GABAergic mechanism (Fig. 6). Nonetheless, 90-Hz Aβ-ES remained effective at inhibiting DH neurons suggesting mechanisms beyond segmental GABAergic inhibition.

**Discussion**

We hypothesized that recently observed low-rate (<200 Hz) subperception SCS (Metzger et al., 2021) produced rapid-onset pain relief by sparse activation of DC axons, which engaged surround inhibitory mechanisms in the DH. DC axons exhibited irregular patterns of activity during low amplitude SCS both in silico and in vivo. When applied as inputs to a validated DH network model, DC axon activity from low amplitude SCS maximized inhibition of both model WDR and pEX neurons, corroborating the potential role of DC activity in mediating pain relief. Furthermore, the responses of both model neurons and in vivo DH neurons were strongly dependent on the spatial location of stimulation with respect to the “center” painful receptive field, supporting a mechanistic role for surround inhibition. The degree to which SCS inhibited the excitatory neurons was dependent on stimulation parameters, and some paradigms (e.g., low-amplitude, 90-Hz stimulation) produced consistent inhibition across pain states. Blocking local GABAergic inhibition with bicuculline affected neuronal responses, corroborating a role for a segmental GABAergic mechanism, and stimulation frequency had a strong effect on net inhibition, even during low amplitude stimulation. Collectively, these results implicate surround inhibition as a driver of the effects of rapid-onset, low-rate subperception SCS and provide insight into possible strategies for further optimizing SCS.

**Surround inhibition**

The strong net inhibitory effect of low amplitude stimulation depended on the location where DH neurons were recorded. Tibial Aβ-ES that primarily activated the L4 and
L5 nerve roots produced strong inhibition of neurons at L3 compared with mixed responses at L4 and excitation at L5 (Swett et al., 1991). These responses are consistent with previous recordings of DH neurons that demonstrated inhibition from electrical stimulation of a nerve from a different area of the receptive field (Hillman and Wall, 1969; Foreman et al., 1976). Additionally, Aβ-ES of nerve roots adjacent to C-fiber stimulation was much more likely to produce inhibition in both excitatory interneurons and Lamina I projection neurons than Aβ-ES at the same root as C-fiber stimulation (Luz et al., 2014; Fan and Sdrulla, 2020). The striking parallels between the effects of spatial targeting in the computational model (Fig. 5C) and the spatial distribution of responses of pEX neurons to Aβ-ES in vivo (Fig. 7C) demonstrates the importance of considering and exploiting the spatial organization of inputs to the DH (Hillman and Wall, 1969). Additionally, these spatial effects were specific to pEX neurons and pIN neurons did not have the same striking differences in responses based on their spatial distribution (Extended Data Fig. 7-1).

Our results suggest that activation of afferents from surround receptive field areas is important for achieving pain relief, while clinical reports indicate that a high degree of overlap between the pain area and paresthesia area is required for pain relief. However, these are not necessarily contradictory observations because of topographic differences between pain and paresthesia sensations. Crush stimulation preferentially activated pEX neurons in the rostral zones (Fig. 7E), consistent with previous descriptions of a rostral bias in the strength and location of Aδ and C-fiber inputs to Lamina I projection neurons (Pinto et al., 2010; Fernandes et al., 2016). Unlike crush stimulation, brushing the hindpaw facilitated pEX neuron activity regardless of recording position. The difference in spatial responses between Aβ-ES and crush suggest somewhat distinct somatotopic representations of pain and paresthesia sensations within the dorsal horn and may also be explained by the more extensive Aβ fiber collateralization versus Aδ and C fiber collateralization (Kuehn et al., 2019).

Importance of stimulation parameters

In contrast to higher amplitude SCS, SCS applied just above activation threshold generated irregular patterns of DC axon activity (Fig. 4). These activity patterns reduced WDR firing rates in the DH network model more so than DC axon activity synchronized with the stimulation train, predicting that stimulation amplitude plays a role in the mechanisms of action SCS. SCS applied at amplitudes as low as 60% of the predicted sensory threshold (30% of predicted MT) activated model DC axons, and subsequently reduced model WDR firing rates across all tested frequencies. However, changes in WDR firing rate depended nonmonotonically on both stimulation amplitude and frequency. Maximum suppression occurred at 75–85% of sensory threshold (40% of predicted MT) in the computational model, and 50–90 Hz led to greater inhibition of WDR neurons over a broader range of amplitudes than other frequencies (Fig. 5). This matches with clinical observations of pain relief with subperception SCS applied at a frequency of 90 Hz and pulse width of 210 ± 50 μs (Metzger et al., 2021).

The lack of paresthesia evoked by low-amplitude, low-frequency SCS may be because of desynchronized activity, as only few axons faithfully follow stimulation. A previous study found that constant stimulation produced paresthesia that felt unnatural, but modulation of stimulation pulse width during stimulation produced changes in firing rate across the target population that did not evoke paresthesia (Tan et al., 2014). In our computational model, almost no axons fired at the stimulation frequency ≤110% of their AT, and this increased to only ~50% at 120% of the AT (Fig. 4A). This desynchronized activity was consistent with our in vivo recordings of DC axons (Fig. 4B) and may underlie inhibition without paresthesia. Additionally, a human scale patient-specific model of SCS found remarkable similarity between absolute values of clinical sensory thresholds and model predicted thresholds using the assumption that the sensory threshold occurred when >10% of the model DC axons were activated (Lempka et al., 2020). Supporting DC activation during low-amplitude SCS, prior studies recorded evoked compound action potentials (ECAPs) from the DCs below 50% of MT (or 100% of PT) in a preclinical model (Yang et al., 2015), and clinical reports with closed-loop SCS showed that ECAPs occur below sensory threshold in some patients (Pilitsis et al., 2021). A study of continuous 50-Hz subperception SCS found decreased mechanical hypersensitivity and altered theta rhythms in awake freely moving rats (Koyama et al., 2018). Finally, electroencephalography (EEG) recordings during electrical stimulation below PT indicated that stimulation can have significant direct effects on somatosensory responses without evoking perception (Iliopoulos et al., 2020).

DH neuron responses following disinhibition

Neuropathic pain leads to disinhibition in DH networks through a variety of mechanisms (Sandkuhler, 2009). Applying different modes of disinhibition to the network model increased baseline firing rates but did not change the overall trend of responses to SCS (Fig. 5). Responses to SCS were more variable across different neuropathic pain states, and higher stimulation amplitudes led to greater relative inhibition of WDR neurons than in the healthy model conditions. Additionally, optimal stimulation parameters depended strongly on stimulation conditions and showed greater heterogeneity compared with naïve conditions (Fig. 5H), indicating that additional patient-specific mechanisms, or computational models personalized to individual patients, may be necessary to optimize stimulation (Lempka et al., 2020).

Following application of bicuculline in vivo, there was a significant increase in pEX neuron spontaneous activity and responses to brush (Fig. 8). Receptive fields of pEX neurons expand following disinhibition, thus unmasking additional excitation and reducing some of the effects of surround inhibition (Lee et al., 2019). Similarly, we observed a substantial shift of neurons to the low threshold
excitatory cluster following bicuculline and decreased inhibitory effect from lower amplitudes of Aβ-ES on pEX neuron activity, suggesting a GABAergic contribution to the segmental effects of surround inhibition. Prior studies also demonstrated reduced inhibition of projection neurons from SCS following application of bicuculline (Duggan and Foong, 1985; Zhang et al., 2015), and chronic pain models demonstrated reduced GABAergic inhibition compared with glycineergic inhibition (Moore et al., 2002). The partial rescue of inhibitory effects by increasing stimulation amplitude supports recruitment of additional inhibitory mechanisms, e.g., glycineergic neurons (Braz et al., 2014), but inhibition remained impaired.

Limitations
We used a previously published classification system to segregate neural types in our recordings (Lee et al., 2019), but classification based on waveform shape is a relatively new method and may miss some of the heterogeneity within DH populations. In our study, pIN neurons were generally suppressed by stimulation, which goes against what we would expect to find with gate-control SCS. However, pIN neurons exhibited a wide array of responses, and pIN neurons that were inhibited by stimulation may be a part of other neural microcircuits that are quite complex (Prescott and Ratte, 2012; Prescott, 2015). Second, to enable receptive field targeted stimulation, we delivered Aβ-ES to a peripheral nerve, and future experiments should explore effects of spatial targeting with DC stimulation. Third, the amplitudes for effective subperception SCS (70–100% PT) were higher than those reported in a clinical study of subperception low-rate SCS (20–70% PT; Metzger et al., 2021). However, this could be because of differences between rat and human anatomy, or because motor thresholds are an inexact method for determining stimulation amplitude (Koyama et al., 2018). Also, we correlated model WDR projection neuron activity with in vivo responses but did not test whether any neurons were projection neurons; however, we did identify neurons based on their responses to mechanical stimulation. Nonetheless, interneurons play vital roles in gating, integrating, and relaying sensory and nociceptive information through the dorsal horn (Prescott et al., 2014). Finally, our in vivo experiments were only performed on male rats, but previous studies have demonstrated conflicting effects of sex on responses to chronic pain (Coyle et al., 1995; Tall et al., 2001; LaCroix-Fralish et al., 2005), and other preclinical studies (Song et al., 2009; Barchini et al., 2012) and clinical studies (Kumar and Toth, 1998; Mekhail et al., 2022) have found comparable outcomes to SCS between males and females. Therefore, we did not include sex-based differences in our study design, but this may be addressed with future studies.

We made several important assumptions with the computational models. First, specific connections within the network model were based on descriptions of DH recordings and prior models, but specific synaptic conductances are difficult to match. Also, the model did not include the effects of supraspinal inputs, and although a role for supraspinal inputs for conventional SCS that produces paresthesia has been established (Saade et al., 2015; Linderoth and Foreman, 2017), the role of descending modulation during subperception SCS remains unclear. Prior studies of DH circuits indicate that there is significant variability within DH neural populations, but we only accounted for a single neuron for each type in the model. We also assumed that the DCs were the only elements activated by stimulation and ignored potential direct field effects on individual neurons, although previous computational modeling studies have found that the activation thresholds of DH neurons are always higher than DC axons (Rogers et al., 2022).

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