In vivo calcium imaging shows that dorsal root ganglion stimulation predominantly activates large-sized sensory neurons in mice

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Abstract

Dorsal root ganglion (DRG) stimulation is an effective treatment for patients with refractory neuropathic pain. However, the exact mechanisms through which DRG stimulation exerts its analgesic effects, especially in relation to the recruitment of different fiber types present within the DRG, remains largely unknown. Here, we examined the effect of DRG stimulation (20Hz) on sensory neuron fiber recruitment and neuronal responses induced by mechanical hind paw stimulation using *in vivo* calcium imaging in mice. GCaMP6s was delivered to sensory neurons via an adeno-associated viral vector of serotype 9 (AAV9) injected subcutaneously at P2-5. In adult mice, a stimulation electrode was placed over the L4 DRG, and *in vivo* calcium imaging was performed. The proportion of responsive neurons and response magnitude was assessed during 30 seconds of DRG stimulation at different stimulation amplitudes (33%, 50%, 66%, 80%, 100% of motor threshold (MT)) for small, medium and large-sized subpopulations. In a second experiment, the effect of 30 minutes of DRG stimulation was assessed at 66% MT on neuronal responses induced by mechanical hind paw stimulation during and following DRG stimulation. We show that 20Hz DRG stimulation at clinically relevant amplitudes mainly activates large-size sensory neurons in mice, both in terms of the proportion of neurons responding as well as response magnitude. There was a small decrease in the proportion of neurons responding to mechanical stimulation during DRG stimulation, which did not recover 30 minutes after stimulation had been switched off. By contrast, DRG stimulation did not alter the response magnitude of responding neurons.

Introduction

Dorsal root ganglia (DRG) play a crucial role in the pathophysiology of neuropathic pain. DRG are present at each segmental level of the spinal cord at the intersection of the peripheral and central nervous system [30]. These DRG house the cell somata of all three neuronal fiber types (Aβ, Aδ, C), and act as a pivotal relay station for electrical impulses containing information on touch and nociception traveling from the periphery to the spinal cord [12; 29]. Following nerve damage, upregulation of neuropeptides, receptors and ion channels can lead to the production of ectopic, aberrant discharges at the level of the DRG [1; 32; 42; 46]. Such discharges can lead to central sensitization, thereby contributing to the development and maintenance of chronic neuropathic pain [18; 22; 24].

Neuromodulation with electrical stimulation has been shown to be an effective treatment for patients with chronic neuropathic pain. Specifically, DRG stimulation has been used as a last resort treatment approach for patients who do not respond adequately to pharmacological intervention [10; 31]. This type of stimulation relies on the transcutaneous implantation of one or multiple leads into the epidural space, after which the lead(s) are advanced laterally over the DRG of interest and provide continuous stimulation [11]. Over the last decade, DRG stimulation has seen consistent use in treating a wide variety of pain disorders [10; 13; 14; 20; 31], and has shown to be effective in treating chronic pain attributed to complex regional pain syndrome (CRPS) [10] in a randomized clinical trial (RCT). Despite frequent use in the clinic, we still do not fully understand the mechanism of how DRG stimulation exerts its analgesic efficacy.

Initially, it was hypothesized that DRG stimulation might engage γ-aminobutyric acid (GABA)ergic gating mechanisms in the spinal cord dorsal horn via activation of large sized myelinated Aβ fibers [17; 19; 25; 43]. In contrast, some studies suggest that DRG stimulation predominantly suppresses the excitability of slow conducting medium sized Aδ or small sized C fibers [5; 23]. The latter effect is often attributed to the unique pseudo-unipolar design of DRG neurons, which might cause the DRG to act as an impedent or low-pass filter for electrical impulses travelling from the periphery to the spinal cord [30]. Given this discrepancy in DRG stimulation literature, more research is needed to investigate which DRG fiber types are modulated by DRG stimulation.

In vivo calcium imaging is a relatively novel method for studying DRG neuronal function. Using this technique, hundreds of cells can be measured simultaneously, where neuronal fiber type (Aβ, Aδ, C) can be characterized based on soma diameter[9; 21]. In this study, we set out to determine the effect of DRG stimulation at different stimulation amplitudes on DRG fiber recruitment in GCaMP6s mice. Furthermore, we also assessed the effects of DRG stimulation on neuronal responses induced by mechanical stimulation of the hind paw.

Methods

Animals

Seven adult C57BL/6J male (n=3) and female (n=4) mice (Charles River, UK) were used in this study. Mice were housed on a 12/12 h light/dark cycle with a maximum of 4 mice per cage,

with food and water available *ad libitum*. All experiments were performed in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act (1986).

Administration of calcium indicator

We utilized the genetically encoded calcium indicator GCaMP6s for imaging sensory neuron activity [8]. GCaMP6s was delivered to sensory neurons via an adeno-associated viral vector of serotype 9 (AAV9), which was administered to mouse pups at P2-P5 as previously described [45]. Briefly, groups of 3-4 mice were separated from their mother. 6 µl of AAV9.CAG.GCaMP6s.WPRE.SV40 (Addgene, USA) was injected subcutaneously in the nape of the neck, using a 10 µL Hamilton syringe with a 30G needle. Mice were separated from their mother after weaning and then used for *in vivo* imaging from 12 weeks after the injection.

***In vivo* calcium imaging**

In vivo imaging was performed as previously described [21]. Briefly, mice were anesthetized using a combination of drugs: 1-1.25 g/kg 12.5% w/v urethane administered intraperitoneally and 0.5-1.5 % isoflurane delivered via a nose cone. Body temperature was maintained close to 37°C using a homeothermic heating mat with a rectal probe (FHC). An incision was made in the skin on the back, and the muscle overlying the L3, L4, and L5 DRG was removed. The bone surrounding the L4 DRG was carefully removed in a caudal-rostral direction. Bleeding was prevented using gelfoam (Spongostan™; Ferrosan, Denmark). The DRG was washed and kept moist using 0.9 % sterile saline. The exposure was then stabilized at the neighboring vertebrae using spinal clamps (Precision Systems and Instrumentation, VA, USA) attached to a custom-made imaging stage. Finally, the DRG was covered with silicone elastomer (World Precision Instruments, Ltd) to maintain a physiological environment. Prior to imaging, we administered a subcutaneous injection of 0.25 ml 0.9 % sterile saline to keep the mouse hydrated. It was then placed under an Eclipse Ni-E FN upright confocal/multiphoton microscope (Nikon), and the microscope stage was diagonally orientated to optimize focus on the L4 DRG. The ambient temperature during imaging was kept at 32°C throughout. All images were acquired using a 10× dry objective. A 488-nm Argon ion laser line was used to excite GCaMP6s, and the signal was collected at 500–550 nm. Time lapse recordings were taken with an in-plane resolution of 512 × 512 pixels and a partially (~2/3rd) open pinhole for confocal image acquisition. All recordings were acquired at 3.65 Hz.

DRG stimulation

Bipolar electrodes were carefully placed either side (caudal and rostral) of the left L4 DRG using a microscope on the dissection table (See Figure 1a for a diagram of where electrodes were typically positioned). The DRG were stimulated with a constant current stimulator (Digitimer, Welwyn Garden City, United Kingdom), coupled with a voltage stimulator (Pulsemaster A300, World Precision Instruments, Ltd). Following electrode placement, the motor threshold (MT) was accurately determined by gradually increasing the stimulation intensity using a frequency of 2 Hz and a pulse width of 200 µs. The MT was defined as the current inducing observable contractions of the ipsilateral lower trunk or hind limb. The mouse was then placed onto the microscope stage and the MT was re-checked to ensure that the electrodes had not changed position whilst moving the mouse. 20 Hz DRG stimulation with a 200 µs pulse width was applied for 30s at 33%, 50%, 66%, 80%, 100% MT, with 1-minute in between each stimulation for recovery in n=5 experiments. In two experiments, only 33-80% MT was tested. The order of stimulation was randomized for each experiment using randomize.org. For the mechanical stimulation studies, DRG stimulation was delivered for 30 minutes at 20 Hz, using a pulse width of 200 µs and an amplitude of 66% MT.

Mechanical stimulation of sensory afferents

In some mice (n=5), A custom-made mechanical stimulator was used to mechanically stimulate sensory afferents in the hind paw (See [34] for details). The paw was positioned face up and was fixed into position using blue tack. The tip of the mechanical stimulator, which contains 6x4 blunt pins (2mm in height and measuring approximately 6x4mm in total), was positioned 1-2mm above the medial plantar surface of the hind paw. Three forces of 200g, 500g and 800g were applied sequentially for 1s duration, allowing 1 minute in between each stimulation for recovery. Each series of force steps was repeated twice at baseline and at different timepoint during and after stimulation of the DRG (Figure 1C). To optimize neuron detection by the activity algorithm in Suite2p, we applied a pinch stimulus to the hind paw using serrated forceps, maximizing the number of neurons automatically identified.

Imaging data analysis

Timelapse recordings were scaled to 8-bit in Fiji/ImageJ, Version 1.53. The image analysis pipeline Suite2P (v 0.9.2)[35] was utilized for motion correction, automatic region of interest (ROI) detection and signal extraction. ROIs that were not automatically detected in suite2p

were added manually. Further analysis was undertaken with a combination of Microsoft Office Excel 2013, and Python (Version 3.9). A region of background was selected, and its signal subtracted from each ROI. To generate normalized data, the following calculation was used: $\Delta F/F0 = (Ft-F0)/F0$, where Ft is the fluorescence at time t and F0 is the fluorescence average over the first 30 seconds of the recording. $\Delta F/F0$ is expressed as a ratio. To correct for fluctuations in F_o values over time, such as minor drifts in the fluorescent baseline, $\Delta F/F_o$ values for each event were recalculated using F0 values averaged from the 10 frames immediately preceding the event onset. To avoid aberrant amplification due because of small F0 values, cells with very low basal fluorescence after background subtraction (<1.5 F.U.) were excluded.

The area of each neuron in the L4 DRG was estimated by using the radius output from Suite2p. Cells were grouped into small ($<400\mu m^2$) to medium (≥ 400 - $<800\mu m^2$) and large-sized ($\geq 800\mu m^2$) neurons. Cells that could not clearly be identified and had substantial overlap with neighboring cells were excluded from the analysis.

For electrical stimulation, a positive response was taken if the average signal during stimulation was 8*SD ≥ the relative baseline. A positive response to mechanical stimulation was taken if the maximum signal during stimulation was 6*SD ≥ the relative baseline fluorescence. For each of the neurons responding to mechanical stimulation, the average magnitude of the response during the stimulation period was calculated.

There were a small proportion of neurons that responded to mechanical stimulation and 66% MT DRG stimulation in experiments examining the changes to mechanically induced responses. These neurons were excluded from the mechanical response analysis because the responses during DRG stimulation could not be accurately measured I.e The $\Delta F/F0$ for these neurons was reduced because the baseline level of fluorescence was raised by DRG stimulation.

Data loss and exclusion

One experiment was excluded because the GCaMP6s labelling was very poor. The data from this experiment was not analyzed.

Quantification and statistical analysis

Graphing and statistical analysis was undertaken with a combination of Excel 2013, Python (Version 3.9), GraphPad Prism (Version 10) and SPSS (Version 29.0). We used a linear mixed-

effects model (LMM) to analyze the effects of stimulation strength and neuron size on responses and to assess effects of DRG stimulation on mechanically induced responses. Details of sample sizes are recorded in the appropriate figure legend.

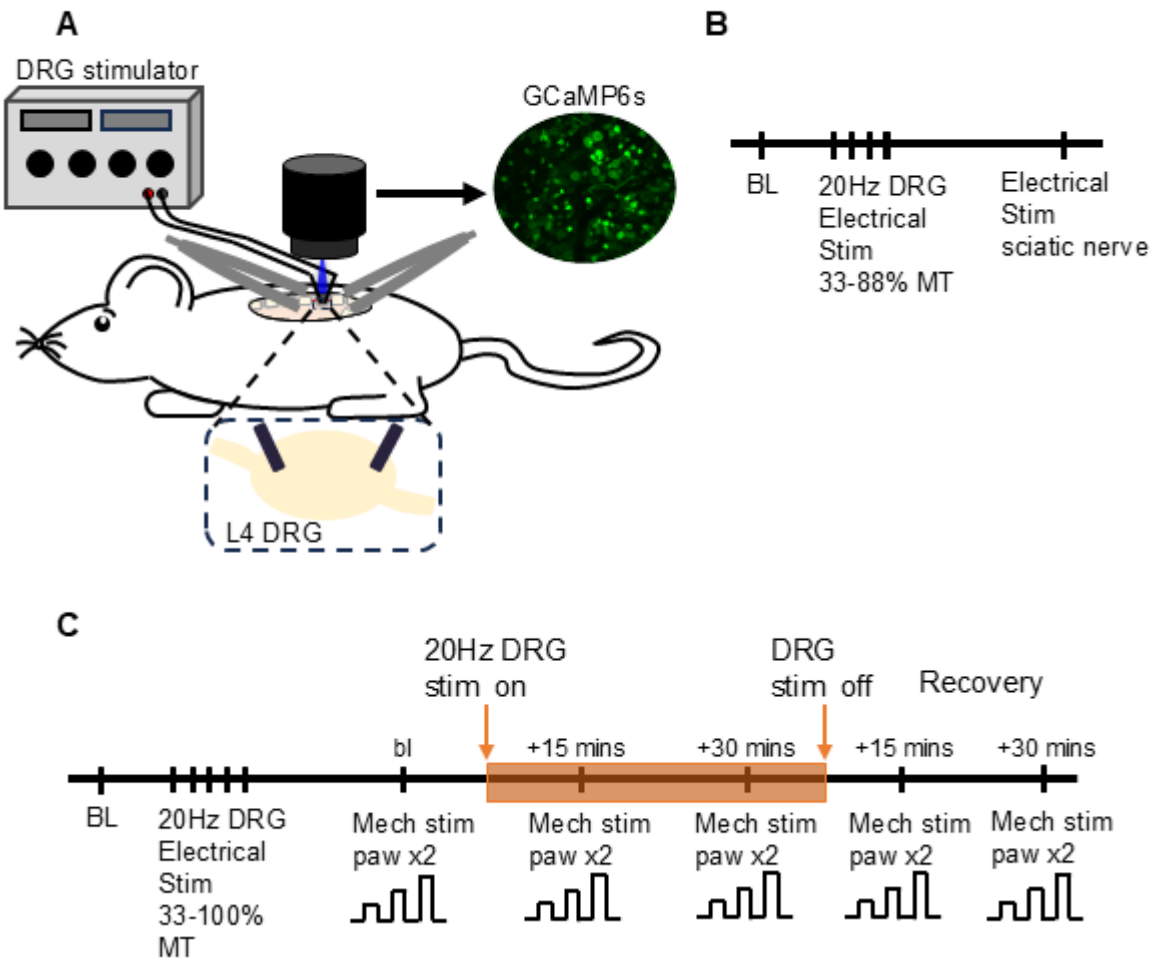

Figure 1. Diagram of experimental set up and stimulation paradigms A) Schematic showing experimental set up. A constant current stimulator was used to deliver pulse of 20Hz stimulation to the L4 Dorsal root ganglion (DRG). Responses were recorded from neurons labelled with GCaMP6s using a confocal microscope. B) Schematic of experimental paradigm 1, n=2 mice. C) Schematic of experimental paradigm 2, n=5 mice.

Results

Electrodes were carefully positioned on the surface of the L4 DRG and then electrical stimulation was applied to determine the MT. The mean motor threshold was 50.6 µV (+/- 9.1, n=7 mice; Figure 2a). Following threshold determination, GCaMP6s-labelled DRG neurons were imaged using a confocal microscope. Electrical stimulation was applied at varying intensities relative to the MT to identify which types of DRG neurons responded. As expected, increasing the stimulation strength caused an increase in the proportion of responding neurons (F (4,75) [Stimulation strength] = 44.4, p<0.0001, linear mixed model; Figure 2 B&C). At every stimulation strength tested, large sized neurons were preferentially activated in comparison to small and medium sized neurons (F (2,75) [Size] = 107, p<0.0001, linear mixed model, n=7). At 66% of the MT, a commonly used stimulation intensity in preclinical pain behavior experiments that resembles clinical settings, 29.4% (± 6.8, n=7) of large-sized neurons were activated (Figure 2 B&C). In contrast, relatively few small- and medium-sized neurons were activated at 66% MT (5.7% +/- 2.9 small neurons; 10.2% +/- 2.5 medium neurons, n=7). Similarly, the response magnitudes during DRG stimulation were greater in large sized neurons compared to small and medium sized neurons (F (2,58) [Size] = 24.4, p<0.0001, linear mixed model, n=7; Supplementary Figure 1). Electrical stimulation at 66% of the MT increased the GCaMP fluorescence of large-sized neurons by 3.5 fold (+/- 0.39, n=7), while small increases occurred in small and medium-sized neurons (1.5 fold +/-0.2 and 2.2 fold +/-0.4, respectively, Supplementary figure 1).

DRG stimulation at 20Hz is reported to induce analgesic responses in both rodents and humans [10; 13; 14; 20; 27; 31; 36; 44] . However, the mechanisms of analgesia are not completely understood. To determine if DRG stimulation reduces neuronal activity within cells bodies of the DRG, mechanically induced responses were measured before, during and after 20Hz DRG stimulation. Neurons in the hind paw ipsilateral to the DRG being imaged were stimulated using a mechanical stimulator (Figure 1C). As expected, increasing force activated a greater number of neurons (F(2,176) = 144.8, p < 0.0001, Linear mixed model, n = 5) and increased their response magnitude (F(2,170) = 44.1, p < 0.0001, Linear mixed model, n = 5; Figure 3A-C). Following application of DRG stimulation, the graded nature of responses to increasing force remained consistent over time F(8,176) [Force*Time] = 1.1, p =0.35, Linear mixed model, n = 5). Similarly, there was no significant change in the response magnitude of

the responding neurons following 20Hz DRG stimulation ($F(8,170)$ [Time] = 0.08, $p=1.0$, linear mixed model; $n=5$; Supplementary figure 1). By contrast, the proportion of neurons responding to mechanical stimulation significantly decreased following DRG stimulation ($F(4,176)$ [Time] = 2.4, $p = 0.048$, linear mixed model, $n = 5$; Figure 3B). The proportion of neurons responding to mechanical stimulation declined after DRG stimulation (Mean difference: Baseline vs. +30 min = 4.2, SE = 1.9, 95% CI: -1.2 to 9.6), but responsivity did not recover after stimulation ceased (Mean difference: +30 min vs. Recovery +30 min = 0.4, SE = 1.9, 95% CI: -4.9 to 5.8). Pairwise comparisons with Bonferroni correction did not reveal significant differences between individual time points (all $p>0.05$). The reduction in the proportion of responders was consistent across all neuron size groups and forces, as there were no significant interactions between time and neuron size ($F(8,176) = 0.40$, $p = 0.92$) or time and force ($F(8,176) = 1.1$, $p = 0.35$; linear mixed model).

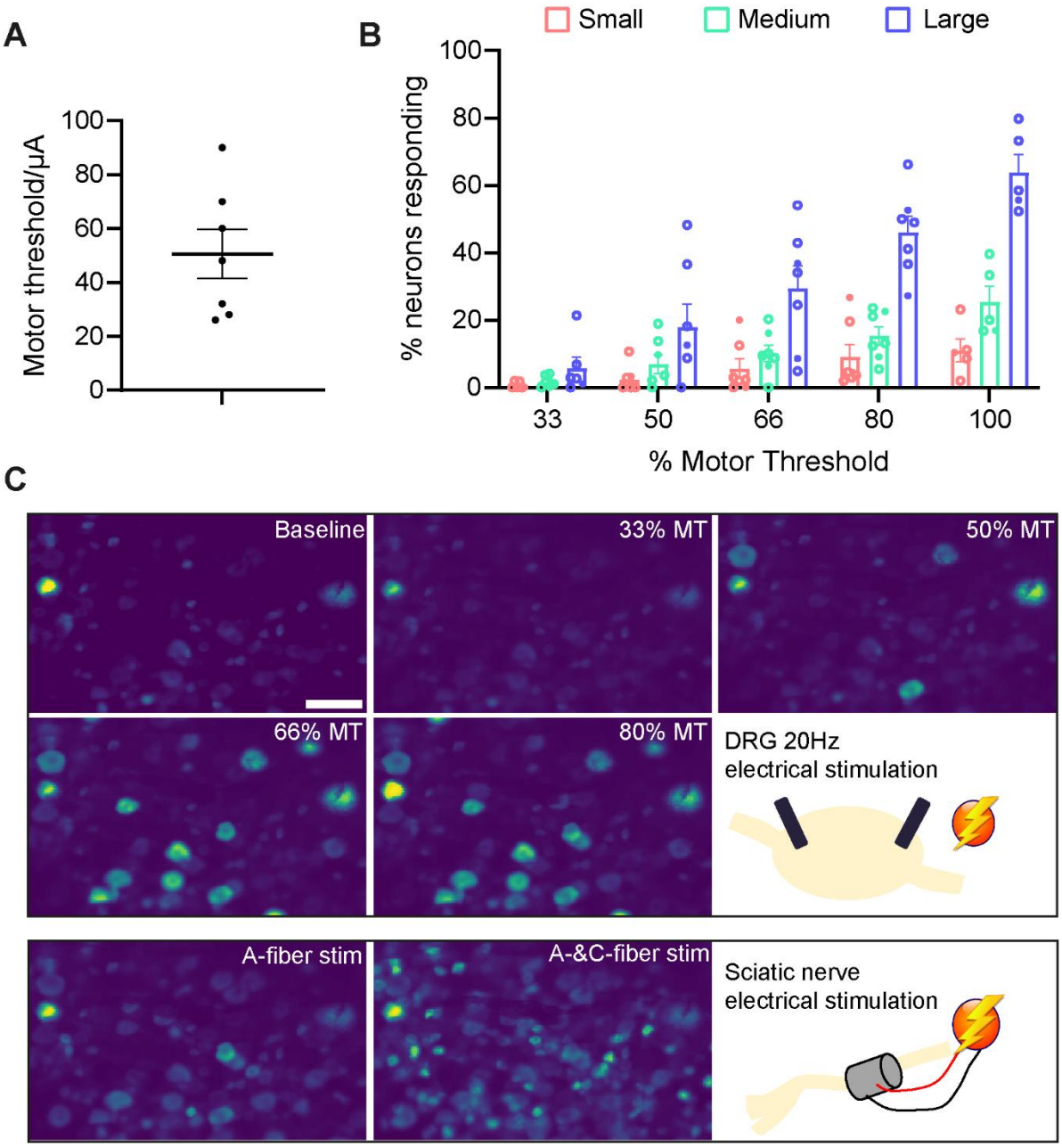

Figure 2. 20Hz DRG stimulation mostly activates large sized neurons when stimulating between 33%-100% of the motor threshold. A) Graph showing the motor threshold for each experiment. Each dot represents an experiment. B) The proportion of neurons in each size subcategory responding to 33-100% of motor threshold (MT) 20hz DRG stimulation. n=5 mice for 33-100% MT and n=7 mice for 33-80% MT stimulation. N=2 males (closed circles) and n=5 females (open circles). n=123–274 neurons/mouse. C) Example average intensity projections taken from 30s clips during different experimental periods. Top panels show average fluorescence intensities at baseline and at different DRG stimulation strengths. Bottom panels show average fluorescence intensities in response to stimulation of the sciatic nerve at low threshold (left) and suprathreshold (right) stimulation strengths. Scale bar = 100um

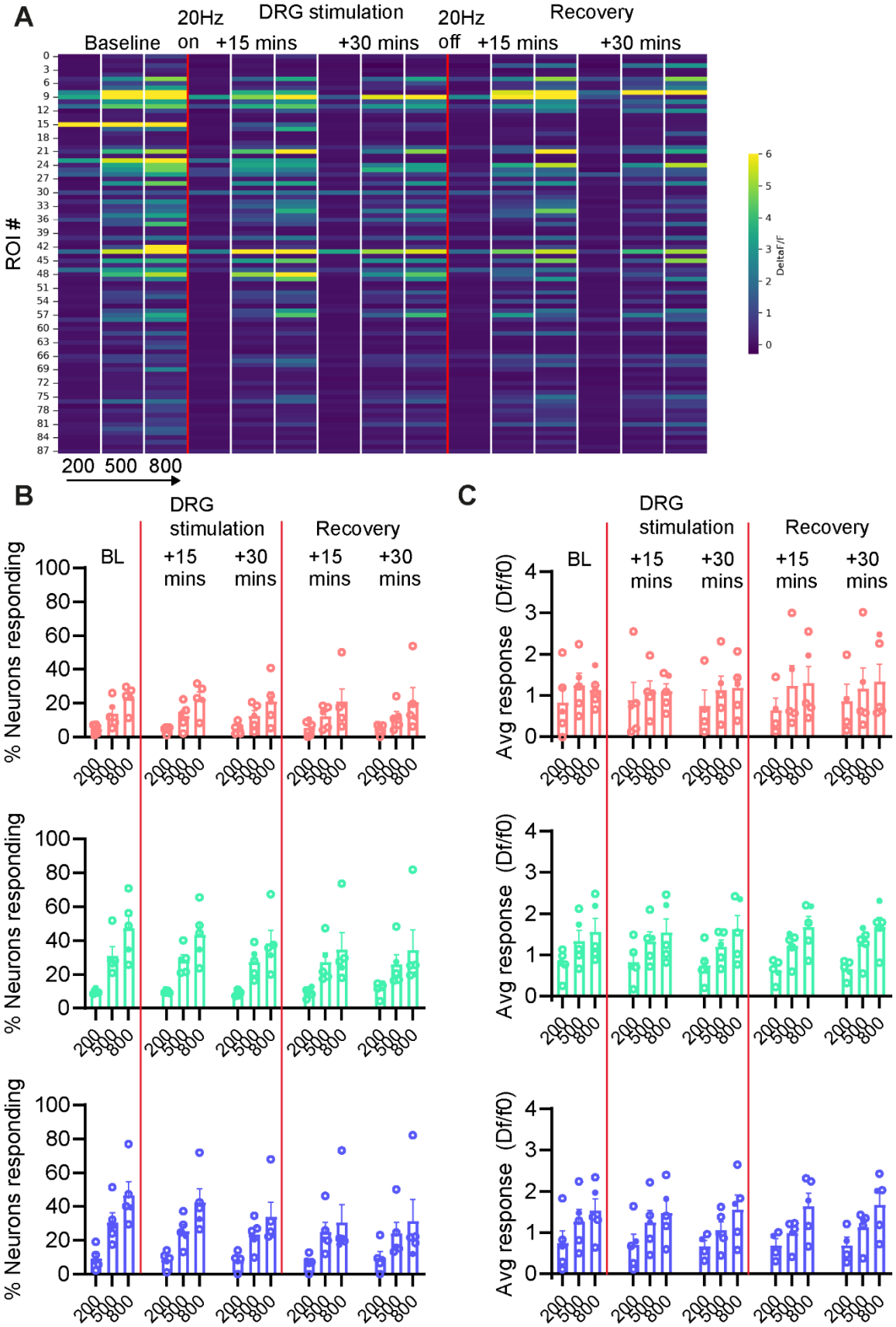

Figure 3. 20hZ DRG stimulation did not attenuate mechanically induced responses generated in hind paw

Figure 3 legend. Heat map showing the DeltaF/F0 normalized data for an example experiment. Each row shows data from a neuron that responded to mechanical stimulation of the paw during the baseline period. Each column represents the average DeltaF/F0 during mechanical stimulation of the hind paw. B) and C) Graphs show the proportion of neurons responding (B) and average responses (C) to mechanical stimulation of the hind paw pre, during and post 20Hz DRG stimulation. Top panel = small sized neurons, middle panel = medium sized neurons, Bottom panels = large sized neurons. Mean +/- SEM

Discussion

In this study, we set out to determine the effect of DRG stimulation at different stimulation amplitudes on DRG fiber recruitment. We found that DRG stimulation at clinically relevant amplitudes mainly recruits large sized A-fibers, and to a lesser extent medium sized A-fibers. In contrast, only very few small-sized C-fiber neurons were recruited. The proportion of neurons responding to mechanical stimulation decreased following DRG stimulation, but this responsivity did not recover after stimulation ceased.

Using *in vivo* calcium imaging we found that DRG stimulation mainly recruits large sized A-fibers across all stimulation amplitudes. At clinically relevant amplitudes of 66% MT, 29.4% of large-sized neurons were activated, whereas relatively few small- (5.7%) and medium-sized (10.2%) neurons were activated. Consistent with this finding, Chao *et al.* reported a negative correlation between cell size and the intensity of DRG stimulation needed to activate neuronal cell bodies in excised rat DRGs[5]. However, the same study found no relationship between DRG stimulation response magnitude and cell diameter when examining the effect of DRG stimulation *in vivo*, contrasting our findings: at 66% MT, large-sized neurons showed a 3.5 fold increase in response magnitude, compared to 2.2-fold in medium and 1.5-fold in small neurons. This discrepancy might be due to the difference in recording set ups: we employed a 'dry' set up where stimulating electrodes are making contact with the DRG and silicone elastomer around it, whereas the DRG was perfused in imaging experiments conducted by Chao *et al,* meaning that the current might have dissipated into the surrounding solution. Our findings are consistent with a computational study that showed that DRG stimulation drives the activity of large myelinated Aβ-fibers, but not C-fibers(17). The importance of large-diameter fiber activation during DRG stimulation was further underscored by Ting *et al.*, who demonstrated that DRG stimulation with clinical stimulation parameters evokes activity in Aα/β-fibers of the sciatic nerve[43]. Collectively, these data suggest that DRG stimulation preferentially activates large-diameter fibers, implicating their importance in DRG stimulation mediated analgesia.

Our mechanical stimulation experiments showed that DRG stimulation does not affect the response magnitude – an indicator of the level of neuronal excitability - in responding neurons. These results appear to contrast a previous study reporting that DRG stimulation reduces cell soma excitability, as assessed by examining conduction velocity [28]. However, it is entirely possible that we would also have observed reduced conduction velocity, but this could not be assessed using GCaMP6s due to its relatively slow kinetics[8]. By contrast, the proportion of neurons responding to mechanical stimulation decreased following DRG stimulation. However, it is important to note that this responsivity did not recover after stimulation ceased, although wash-out effects lasting for at least 15-30 minutes have been described in previous preclinical DRGS studies[15; 16; 36]. As the reduction in the proportion of responders was consistent across all neuron size groups and forces, it may reflect repeated mechanical stimulation inducing inflammation[2], which has been reported to reduce neuronal responsivity[41]. Although we did not observe a substantial reduction of the number of neurons responding to mechanical stimulation at the level of the cell soma, other studies, which examine transmission at the level of the dorsal root, have reported conduction block[4; 5; 37] Specifically, transmission was blocked by DRG stimulation in C-fibers, and to a lesser extent Aδ and Aβ-fibers[5]. This data is further supported by a computational modelling analysis by Kent *et al.*, who showed that DRG stimulation can amplify the natural filtering effect of the DRG, thereby blocking afferent signaling and ectopic activity[23].

DRG stimulation was originally developed to overcome some of the drawbacks related to spinal cord stimulation (SCS) of the dorsal columns, such as difficulty in capturing specific dermatomes (e.g. extremities, groin), high battery usage, and insufficient pain relief. SCS is known to activate non-nociceptive Aβ fibers in the dorsal columns of the spinal cord and engage gate control mechanisms in the spinal cord dorsal horn, thereby inhibiting incoming transmission from nociceptive C-fibers[33]. In theory, DRG stimulation might engage similar gate control mechanisms, through direct activation of Aβ fibers in the DRG. Indeed, our study shows that large-sized fibers, which encompass Aβ fibers, are predominantly activated by DRG stimulation, suggesting the role of gate control mechanisms in DRG stimulation mediated analgesia. However, the gate control mechanism is just one of the theories that has been proposed for analgesia mediated by DRG stimulation. Another theory is that local T-junction filtering occurs at the level of the DRG. DRG somata have a unique pseudo-unipolar design, with one common axon (stem) that branches into two separate axons, connected by T-junction

that connects the branching point to the cell body[30]. DRG stimulation is proposed to depolarize the somatic membrane, thereby creating a reduction in membrane excitability at the T-junction[5; 28]. This loss in membrane excitability at the site of the T-junction is thought to be Ca^{2+} dependent and activate Ca^{2+}-sensitive K^+ channels, producing hyperpolarization in the axon stem and T-junction[5; 23]. Regardless of the precise fiber type that is modulated by DRG stimulation, it is reasonable to expect that ultimately, this leads to a reduction in output of second order projection neurons and pain transmission to supraspinal centers. Indeed, a previous study by Chao *et al.* showed that DRG stimulation can reduce spinal nerve ligation induced spontaneous activity of wide-dynamic-range neurons[3]. Furthermore, Pawela *et al.* showed that DRG stimulation can attenuate blood oxygen-level dependent (BOLD) responses in areas related to sensory and pain-related responses[38].

Our study has limitations. Our study included only 5 mice for the mechanical stimulation experiments, which means that we were only able to detect very large effect sizes. In contrast, previous behavioral studies typically used 8-12 rats to detect an analgesic effect[15-17; 26; 27; 36; 44; 47]. We did not include a sham DRG stimulation control group and therefore we were not able to determine if the progressive decline in mechanical responsivity was an effect mediated by DRG stimulation or repeated mechanical testing. It is important to note that we were only measuring excitability at the level of the soma and so it is entirely possible that the T-junction filtering mechanisms described above, may have caused inhibition of neurons along the dorsal root, which we would have missed. We delivered GCaMP6s to sensory neurons via a viral approach and used this as a proxy of neuronal excitability. Viral delivery via AAV9 labels most neuronal subtypes, including Aα and Aβ fibers [39], but it is important to note that relatively few tyrosine hydroxylase (TH) positive neurons, which are markers of low threshold mechanoreceptor C-fibers (C-LTMR), are labelled using this approach [40]. Therefore, it is likely that the C-fibers activated in our preparations were likely nociceptive and it is not known whether C-LTMRs would be activated. Of note, activation of this subclass of C-fibers and subsequent engagement of the endogenous opioid system was recently suggested to underly DRG stimulation induced analgesia, especially at low frequencies (<20Hz)[6; 7].

In conclusion, our study showed that DRG stimulation at clinically relevant amplitudes mainly recruits large sized A-fibers, and to a lesser extent medium sized A-fibers and C-fibers. The proportion of neurons responding to mechanical stimulation significantly decreased following

DRG stimulation, but this responsivity did not recover after stimulation ceased. These results imply that both DRG stimulation induced spinal gating mechanisms as well as local filtering mechanisms should be considered. Future studies should further investigate the effect of DRG stimulation on specific subtypes of DRG soma, including LTMR C-fibers and a range of DRG stimulation parameters.

Declarations

Ethics approval

All experiments were performed in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act (1986).

Availability of data and materials

Raw data and code for calcium imaging analysis will be shared in the OSF under accession number: DOI 10.17605/OSF.IO/D7XVQ

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Author contributions: G.G. conceived, designed, and performed experiments, analyzed data, and wrote the manuscript. G.F conceived, designed, and performed experiments, analyzed data, and wrote the manuscript.

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Authors' information

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References

[1] Abe M, Kurihara T, Han W, Shinomiya K, Tanabe T. Changes in expression of voltage-dependent ion channel subunits in dorsal root ganglia of rats with radicular injury and pain. Spine (Phila Pa 1976) 2002;27(14):1517-1524; discussion 1525.

[2] Bove GM, Dilley A. The conundrum of sensitization when recording from nociceptors. J Neurosci Methods 2010;188(2):213-218.

[3] Chao D, Mecca CM, Yu G, Segel I, Gold MS, Hogan QH, Pan B. Dorsal root ganglion stimulation of injured sensory neurons in rats rapidly eliminates their spontaneous activity and relieves spontaneous pain. Pain 2021;162(12):2917-2932.

[4] Chao D, Tran H, Hogan QH, Pan B. Analgesic dorsal root ganglion field stimulation blocks both afferent and efferent spontaneous activity in sensory neurons of rats with monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage 2022;30(11):1468-1481.

[5] Chao D, Zhang Z, Mecca CM, Hogan QH, Pan B. Analgesic dorsal root ganglionic field stimulation blocks conduction of afferent impulse trains selectively in nociceptive sensory afferents. Pain 2020;161(12):2872-2886.

[6] Chapman KB, Groenen PS, Vissers KC, van Helmond N, Stanton-Hicks MD. The Pathways and Processes Underlying Spinal Transmission of Low Back Pain: Observations From Dorsal Root Ganglion Stimulation Treatment. Neuromodulation 2021;24(4):610-621.

[7] Chapman KB, Yousef TA, Foster A, M DS-H, van Helmond N. Mechanisms for the Clinical Utility of Low-Frequency Stimulation in Neuromodulation of the Dorsal Root Ganglion. Neuromodulation 2021;24(4):738-745.

[8] Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 2013;499(7458):295-300.

[9] Chisholm KI, Khovanov N, Lopes DM, La Russa F, McMahon SB. Large Scale In Vivo Recording of Sensory Neuron Activity with GCaMP6. eNeuro 2018;5(1).

[10] Deer TR, Levy RM, Kramer J, Poree L, Amirdelfan K, Grigsby E, Staats P, Burton AW, Burgher AH, Obray J, Scowcroft J, Golovac S, Kapural L, Paicius R, Kim C, Pope J, Yearwood T, Samuel S, McRoberts WP, Cassim H, Netherton M, Miller N, Schaufele M, Tavel E, Davis T, Davis K, Johnson L, Mekhail N. Dorsal root ganglion stimulation yielded higher treatment success rate for complex regional pain syndrome and causalgia at 3 and 12 months: a randomized comparative trial. Pain 2017;158(4):669-681.

[11] Deer TR, Pope JE, Lamer TJ, Grider JS, Provenzano D, Lubenow TR, FitzGerald JJ, Hunter C, Falowski S, Sayed D, Baranidharan G, Patel NK, Davis T, Green A, Pajuelo A, Epstein LJ, Harned M, Liem L, Christo PJ, Chakravarthy K, Gilmore C, Huygen F, Lee E, Metha P, Nijhuis H, Patterson DG, Petersen E, Pilitsis JG, Rowe JJ, Rupert MP, Skaribas I, Sweet J, Verrills P, Wilson D, Levy RM, Mekhail N. The Neuromodulation Appropriateness Consensus Committee on Best Practices for Dorsal Root Ganglion Stimulation. Neuromodulation 2019;22(1):1-35.

[12] Devor M. Unexplained peculiarities of the dorsal root ganglion. Pain 1999;Suppl 6:S27-S35.

[13] Eldabe S, Burger K, Moser H, Klase D, Schu S, Wahlstedt A, Vanderick B, Francois E, Kramer J, Subbaroyan J. Dorsal Root Ganglion (DRG) Stimulation in the Treatment of Phantom Limb Pain (PLP). Neuromodulation 2015;18(7):610-616; discussion 616-617.

[14] Eldabe S, Espinet A, Wahlstedt A, Kang P, Liem L, Patel NK, Vesper J, Kimber A, Cusack W, Kramer J. Retrospective Case Series on the Treatment of Painful Diabetic Peripheral Neuropathy With Dorsal Root Ganglion Stimulation. Neuromodulation 2018;21(8):787-792.

[15] Franken G, Debets J, Joosten EAJ. Dorsal Root Ganglion Stimulation in Experimental Painful Diabetic Peripheral Neuropathy: Burst vs. Conventional Stimulation Paradigm. Neuromodulation 2019;22(8):943-950.

[16] Franken G, Debets J, Joosten EAJ. Nonlinear Relation Between Burst Dorsal Root Ganglion Stimulation Amplitude and Behavioral Outcome in an Experimental Model of Painful Diabetic Peripheral Neuropathy. Neuromodulation 2020;23(2):158-166.
[17] Franken G, Douven P, Debets J, Joosten EAJ. Conventional Dorsal Root Ganglion Stimulation in an Experimental Model of Painful Diabetic Peripheral Neuropathy: A Quantitative Immunocytochemical Analysis of Intracellular gamma-Aminobutyric Acid in Dorsal Root Ganglion Neurons. Neuromodulation 2021;24(4):639-645.
[18] Geng SJ, Liao FF, Dang WH, Ding X, Liu XD, Cai J, Han JS, Wan Y, Xing GG. Contribution of the spinal cord BDNF to the development of neuropathic pain by activation of the NR2B-containing NMDA receptors in rats with spinal nerve ligation. Exp Neurol 2010;222(2):256-266.
[19] Graham RD, Bruns TM, Duan B, Lempka SF. Dorsal root ganglion stimulation for chronic pain modulates Abeta-fiber activity but not C-fiber activity: A computational modeling study. Clin Neurophysiol 2019;130(6):941-951.
[20] Huygen F, Liem L, Cusack W, Kramer J. Stimulation of the L2-L3 Dorsal Root Ganglia Induces Effective Pain Relief in the Low Back. Pain Pract 2018;18(2):205-213.
[21] Ingram S, Chisholm KI, Wang F, De Koninck Y, Denk F, Goodwin GL. Assessing spontaneous sensory neuron activity using in vivo calcium imaging. Pain 2024;165(5):1131-1141.
[22] Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003;26(12):696-705.
[23] Kent AR, Min X, Hogan QH, Kramer JM. Mechanisms of Dorsal Root Ganglion Stimulation in Pain Suppression: A Computational Modeling Analysis. Neuromodulation 2018;21(3):234-246.
[24] Kerr BJ, Bradbury EJ, Bennett DL, Trivedi PM, Dassan P, French J, Shelton DB, McMahon SB, Thompson SW. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. J Neurosci 1999;19(12):5138-5148.
[25] Koetsier E, Franken G, Debets J, Heijmans L, van Kuijk SMJ, Linderoth B, Joosten EA, Maino P. Mechanism of dorsal root ganglion stimulation for pain relief in painful diabetic polyneuropathy is not dependent on GABA release in the dorsal horn of the spinal cord. CNS Neurosci Ther 2020;26(1):136-143.
[26] Koetsier E, Franken G, Debets J, van Kuijk SMJ, Linderoth B, Joosten EA, Maino P. Dorsal Root Ganglion Stimulation in Experimental Painful Diabetic Polyneuropathy: Delayed Wash-Out of Pain Relief After Low-Frequency (1Hz) Stimulation. Neuromodulation 2020;23(2):177-184.
[27] Koetsier E, Franken G, Debets J, van Kuijk SMJ, Perez R, Linderoth B, Joosten EAJ, Maino P. Effectiveness of dorsal root ganglion stimulation and dorsal column spinal cord stimulation in a model of experimental painful diabetic polyneuropathy. CNS Neurosci Ther 2019;25(3):367-374.
[28] Koopmeiners AS, Mueller S, Kramer J, Hogan QH. Effect of electrical field stimulation on dorsal root ganglion neuronal function. Neuromodulation 2013;16(4):304-311; discussion 310-301.
[29] Krames ES. The role of the dorsal root ganglion in the development of neuropathic pain. Pain Med 2014;15(10):1669-1685.
[30] Krames ES. The dorsal root ganglion in chronic pain and as a target for neuromodulation: a review. Neuromodulation 2015;18(1):24-32; discussion 32.
[31] Liem L, Russo M, Huygen FJ, Van Buyten JP, Smet I, Verrills P, Cousins M, Brooker C, Levy R, Deer T, Kramer J. A multicenter, prospective trial to assess the safety and performance of the spinal modulation dorsal root ganglion neurostimulator system in the treatment of chronic pain. Neuromodulation 2013;16(5):471-482; discussion 482.
[32] Liu CN, Wall PD, Ben-Dor E, Michaelis M, Amir R, Devor M. Tactile allodynia in the absence of C-fiber activation: altered firing properties of DRG neurons following spinal nerve injury. Pain 2000;85(3):503-521.
[33] Melzack R, Wall PD. Pain mechanisms: a new theory. Science 1965;150(3699):971-979.

[34] Oehler B, Périer C, Martin V, Fisher A, Lezmi S, Kalinichev M, McMahon SB. Evaluation of Recombinant Botulinum Neurotoxin Type A1 Efficacy in Peripheral Inflammatory Pain in Mice. Front Mol Neurosci 2022;15:909835.
[35] Pachitariu M, Stringer C, Dipoppa M, Schröder S, Rossi LF, Dalgleish H, Carandini M, Harris KD. Suite2p: beyond 10,000 neurons with standard two-photon microscopy. bioRxiv 2017:061507.
[36] Pan B, Yu H, Fischer GJ, Kramer JM, Hogan QH. Dorsal Root Ganglionic Field Stimulation Relieves Spontaneous and Induced Neuropathic Pain in Rats. J Pain 2016;17(12):1349-1358.
[37] Pan B, Zhang Z, Chao D, Hogan QH. Dorsal Root Ganglion Field Stimulation Prevents Inflammation and Joint Damage in a Rat Model of Rheumatoid Arthritis. Neuromodulation 2018;21(3):247-253.
[38] Pawela CP, Kramer JM, Hogan QH. Dorsal root ganglion stimulation attenuates the BOLD signal response to noxious sensory input in specific brain regions: Insights into a possible mechanism for analgesia. Neuroimage 2017;147:10-18.
[39] Perez-Sanchez J, Middleton SJ, Pattison LA, Hilton H, Ali Awadelkareem M, Zuberi SR, Renke MB, Hu H, Yang X, Clark AJ, St John Smith E, Bennett DL. A humanized chemogenetic system inhibits murine pain-related behavior and hyperactivity in human sensory neurons. Sci Transl Med 2023;15(716):eadh3839.
[40] Semizoglou E, Lo Re L, Middleton SJ, Perez-Sanchez J, Tufarelli T, Bennett DL, Chisholm KI. In vivo calcium imaging reveals directional sensitivity of C-low threshold mechanoreceptors. J Physiol 2025;603(4):895-908.
[41] Smith-Edwards KM, DeBerry JJ, Saloman JL, Davis BM, Woodbury CJ. Profound alteration in cutaneous primary afferent activity produced by inflammatory mediators. Elife 2016;5.
[42] Song XJ, Hu SJ, Greenquist KW, Zhang JM, LaMotte RH. Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. J Neurophysiol 1999;82(6):3347-3358.
[43] Ting JE, Hooper CA, Dalrymple AN, Weber DJ. Tonic Stimulation of Dorsal Root Ganglion Results in Progressive Decline in Recruitment of Aalpha/beta-Fibers in Rats. Neuromodulation 2024;27(8):1347-1359.
[44] Vuka I, Marcius T, Kovacic D, Sarolic A, Puljak L, Sapunar D. Implantable, Programmable, and Wireless Device for Electrical Stimulation of the Dorsal Root Ganglion in Freely-Moving Rats: A Proof of Concept Study. J Pain Res 2021;14:3759-3772.
[45] Wang F, Belanger E, Cote SL, Desrosiers P, Prescott SA, Cote DC, De Koninck Y. Sensory Afferents Use Different Coding Strategies for Heat and Cold. Cell Rep 2018;23(7):2001-2013.
[46] Xiao HS, Huang QH, Zhang FX, Bao L, Lu YJ, Guo C, Yang L, Huang WJ, Fu G, Xu SH, Cheng XP, Yan Q, Zhu ZD, Zhang X, Chen Z, Han ZG, Zhang X. Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. Proc Natl Acad Sci U S A 2002;99(12):8360-8365.
[47] Yu G, Segel I, Tran H, Park HJ, Ross E, Hogan QH, Pan B. Analgesic Effects of Tonic and Burst Dorsal Root Ganglion Stimulation in Rats With Painful Tibial Nerve Injury. Neuromodulation 2022;25(7):970-979.