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Behavioral Survey of Effects of Pulsed Radiofrequency on Neuropathic and Nociceptive Pain in Rats: Treatment Profile and Device Implantation

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Objectives: Pulsed radiofrequency (PRF) stimulation is widely used for intractable pain; however, there is no consensus on treatment protocols and appropriate types of pain. We compared effectiveness of bipolar and unipolar PRF on neuropathic or inflammatory pains, and of targets at the dorsal root ganglion (DRG) and sciatic nerve (SN). We also examined efficacy of repetitive PRF stimulations. This preclinical study could serve as an extensive survey before human trials.

Materials: Spare nerve injury (SNI)-induced neuropathic pain and complete Freund's adjuvant (CFA) injection-induced inflammatory pain were used. Behavioral responses were measured using von Frey test, acetone test, and Hargreave's test at preinjury and postinjury time points. In both models, we evaluated results of DRG stimulation with unipolar PRF (45 V) versus bipolar PRF (5 V), stimulation at DRG vs. SN, and repetitive stimulations.

Results: Both unipolar and bipolar PRFs reduced SNI- or CFA-induced pain for a similar duration. In the SNI model, PRF-DRG had a stronger effect on tactile pain than PRF-SN but lower effect on cold allodynia, whereas in the CFA model PRF-DRG and PRF-SN showed similar effects. Repetitive PRF stimulation, by open technique or implantation method, produced analogous effect by each stimulus, and no evident analgesic tolerance or neurological deficit was shown.

Conclusions: PRF temporarily attenuates neuropathic and inflammatory pain. Bipolar PRF generates significant analgesia with a much lower electrical power than unipolar PRF. Meanwhile, the minor variant effects between PRF-DRG and PRF-SN may indicate distinct mechanisms. The sustained-analgesia by repetitive treatments suggests implantation technique could be a promising choice.

Keywords: Pulsed radiofrequency, neuropathic pain, nociceptive pain, implantation, rats

Conflict of Interest: Dr. Yeong-Ray Wen is the scientific consultant of Gimer Medical Co., which is developing an implantable spinal cord device. Dr. Wen is a stockholder of Gimer Medical Co. The other authors have no conflict of interest in this study.

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INTRODUCTION

Pulsed radiofrequency (PRF) is widely used in the treatment of neuropathic pain. PRF stimulation of the dorsal root ganglion (DRG) reduces cervical and lumbar radicular pain resulting from a herniated disc and spinal stenosis (1–4). In addition, PRF application at peripheral nerves is effective in occipital neuralgia (5,6), postherpetic neuralgia (7,8), carpal tunnel syndrome (9), meralgia paresthetica (10,11), pudendal neuralgia (12) chronic inguinal neuralgia (13,14), and chronic cluster headache (15).

PRF inhibits hyperalgesia in the inflammatory pain models by its actions on sensory nerves and nociceptive transmission (16,17), thus attenuated human surgery-related persistent pain (18), shoulder pain due to rotator cuff injuries or impingement (19–21), discogenic pain (22,23), knee osteoarthritis (24–26), and hip joint pain (27).

However, meta-analyses and Cochrane review have ranked PRF efficacy in pain management from "recommended" or "suggested" (1B to 2C) (2,28) to "poor" or "limited" levels (29–32) due to inadequate methodology or small sample sizes. Consequently, most U.S. insurance companies do not provide reimbursement for PRF treatment of chronic pain (1,33).

The analgesic mechanisms of PRF are not fully understood although knowledge from *in vivo* and *in vitro* studies is growing (34). Direct inhibition on presynaptic neurotransmission releases and postsynaptic neuronal activation, spinal long-term depression, glial inactivation, neuroinflammatory modulation, gene up- or down-regulation and activation of descending inhibitory pathway were proposed in animal studies (16,17,35–38). There are differences in results among human studies as well as between human and animal studies, even though identical PRF devices and parameters were used. For instance, PRF reduced radicular pain by over 50% in humans for a duration varied from weeks to months (31); however, PRF attenuated neuropathic pain in rodents for only weeks, even days (39–41).

The objectives of this study include (1) compare the effectiveness of unipolar and bipolar PRF in reducing pain in neuropathic and inflammatory pain models; (2) compare the effect of PRF treatment at the dorsal root ganglion (DRG) and sciatic nerve (SN), and (3) evaluate the efficacy of repetitive PRF stimulation of the DRG in prolonging pain relief using an implantable PRF electrode.

MATERIALS AND METHODS

Animal Preparation

Adult male Sprague Dawley rats (220–250 g; BioLASCO, Taiwan) were housed in groups of two to three in plastic cages at a constant temperature (22°C), relative humidity (40–60% [vol/vol]), and in a 12-hour light/dark cycle environment for at least five days before experiments and were provided food and water *ad libitum*.

Study protocols were approved by the Institutional Animal Care and Utilization Committee, China Medical University Hospital, Taichung, Taiwan. All experiments followed the ethical guidelines for animal managements from the institute and the International Association for the Study of Pain (42).

Unipolar Versus Bipolar PRF Electrodes

We used two different PRF stimulation systems: a commercial unipolar PRF (uni-PRF) system and a specially designed bipolar PRF (bi-PRF) system. The uni-PRF system includes a 10-cm 22G RF introducer needle with a 5-mm active tip (Baylis Medical, Montreal, Canada), a 10-cm PRF electrode probe that can be inserted through the introducer needle, a ground pad that can be adhered onto the shaved abdominal skin as a reference electrode, and an RF generator (Cosman G4, Cosman Medical, Burlington, MA, USA) that generates 2-Hz, 20-ms pulse-width RF pulses at 500-kHz and 45-volt pulse amplitudes. The bi-PRF systems were designed in our lab (35,43) and included three types (Fig. 1). The first has two 32G electrodes, with one electrode tip bent at 100° for insertion into the L5 foraminal canal and the other reference electrode maintained straight for contact with the surrounding nonneural tissues (Fig. 1a). The second type has two active, parallel tips at 0.5 mm apart used for SN stimulation (Fig. 1b). The third type is an implantable wire electrode ends which can be fixed at the L5 foramen close to the DRG and the other end percutaneously externalized for the generator connection (Fig. 1c). The PXI-5402 Function Generator (National Instruments, Austin, TX, USA) can generate 2-Hz biphasic pulses with each pulse having 500-kHz electric waves, 25-ms pulse width, and oscillating amplitudes of ±2.5 volts. Treatment duration with either uni- or bi-PRF was 300 sec. These parameters were based on clinical settings (1,44).

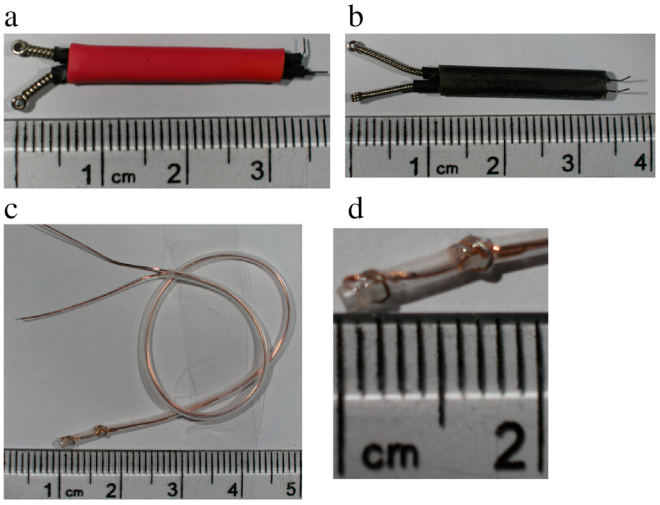

Figure 1. Three different electrodes used in this study. a. A bipolar electrode with two exposed tips at an angle of 90° for DRG stimulation in the SNI and CFA models. b. A bipolar electrode with two parallel exposed tips for sciatic nerve stimulation in the SNI and CFA models. c. A flexible insulated bipolar electrode for implantation in the SNI model. d. The implanted tips of the wired bipolar electrode. [Color figure can be viewed at wileyonlinelibrary.com]

Pain Models

Spare Nerve Injury-Induced Neuropathic Pain Model

To produce a spare nerve injury (SNI), a longitudinal 2-cm incision was made at the posterior mid-thigh of the left hind limb under isoflurane anesthesia to expose the trification of SN. The common peroneal and tibial nerves, two of three SN branches, were ligated with a 5-0 silk suture, then cut, and a 3-mm portion of the distal nerve was removed. We ensured that the third branch, the sural nerve, remained intact and did not stretch during procedures. The control rats received identical skin and muscle incisions but no nerve ligation or cutting. The thigh muscles and skin were sutured and the rat was placed back into the cage for recovery. Nociceptive behaviors were measured two days

postsurgery. We selected this model because it causes diffuse and nondermatomal foot pain in SN territory, which is analog to complete Freud's adjuvant (CFA) injection-induced pain in the paw.

CFA-Induced Inflammatory Pain Model

The left hind paw was intradermally injected with 100 μL of CFA (Sigma-Aldrich, St. Louis, MO, USA), and the control rats were injected with an identical volume of saline.

Nociceptive Behaviors

The mechanical threshold was evaluated using von Frey filaments (Stoelting, Wood Dale, IL, USA) with the up-down method (45) and calculated using a 50% withdrawal threshold. Animals were individually placed in a chamber of a transparent Plexiglas box and tested daily from at least two days before SNI or CFA, and the determined time points after SNI/CFA and PRF/Sham stimulation.

The heat threshold was measured using paw withdrawal latencies (PWLs) to radiant heat stimulation by using a plantar test device (Plantar Test Apparatus, IITC, Woodland Hills, CA, USA). The cutoff latency was set at 20 sec to prevent thermal injury. PWL at each time point was an average of three withdrawal measurements at a 5-min interval.

The cold threshold was measured by detecting paw withdrawal responses to acetone application. Pure acetone (100 μL) was dripped from a syringe with a 20-gauge Teflon needle at a 2 cm height above the dorsal surface of the left foot. Time spent in the complete elevation of the foot within 1 min, including flinching and licking, was recorded.

Study Designs

Our study design contained several different combinations: two nociceptive modalities (SNI and CFA), five types of PRF stimulations (uni-PRF at DRG, bi-PRF at DRG, bi-PRF at SNI, repeated open PRF stimulation, and implanted-PRF stimulation). The Sham controls were always included in separate experiments for objective validation.

We studied the following comparisons in SNI and CFA models (Fig. 2): uni-PRF (45 V) versus bi-PRF (5 V) at DRGs; bi-PRF application at DRG versus at SN; effect of repeated bi-PRF at DRG in SNI-induced pain; and an implantation of a bi-PRF electrode to examine the long-term efficacies.

The first PRF stimulation was performed at seven days after SNI injury (Fig. 2a) or immediately after CFA injection (Fig. 2b). In repetitive study, we first tried open surgeries for PRF stimulation (Fig. 2c), then we implanted a wire electrode for prolonged stimulations (Fig. 2d). When behavioral tests and PRF stimulation were conducted on the same day, behavioral tests were always performed before PRF. Implantation of the wire electrode was on the

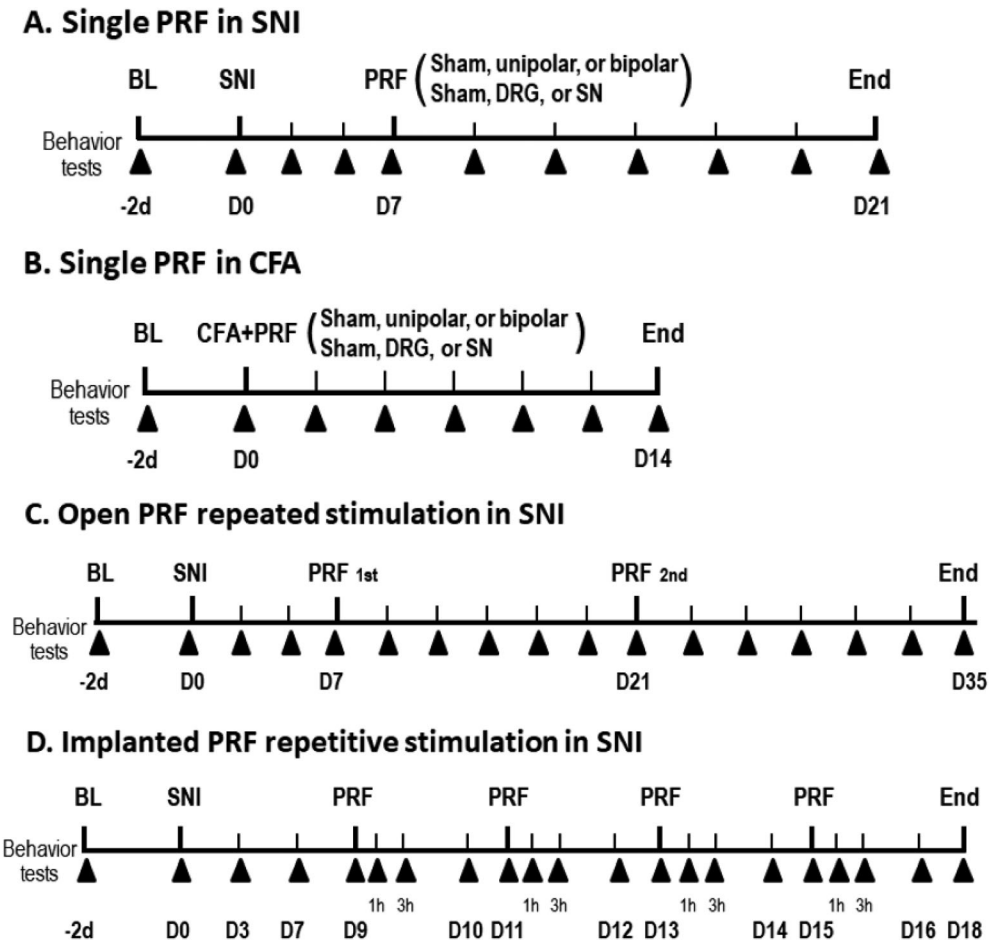

Figure 2. Study protocols. In the figures, time points of pain model (SNI surgery and CFA injection) and PRF treatments were marked. Solid triangles indicate time points for behavioral test.

same day of SNI surgery, and the accuracy of the lead position was confirmed by performing brief motor tests immediately after implantation, every two to three days afterward, and one day before every PRF delivery. The control group received the same surgery and Sham needle or electrode applications but without PRF electric delivery.

The baseline (BL) data were an average of at least two stable measurements from two days efore SNI and CFA injection (D0 in Fig. 2) and post-SNI injury data were obtained on the third, fifth, and seventh post-SNI day (D3, D5, D7 in Fig. 2a,c,d). Post-PRF data were obtained on time points after PRF/Sham stimulation (D1, D3, D5, D7, D12, D14 in Figs. 2b, 4, and 6 for CFA model and D8, D10, D12, D14, D19, D21 in Fig. 2a, 3, 5, and 7 for SNI model). No behavioral data were obtained on the surgical day to avoid various effects of surgery and anesthesia. Particularly, we could easily conducted behavioral measurements in the implantation rats because all of them fast recovered from the short 6–10 min anesthesia for PRF stimulation (Fig. 9).

Statistical Analysis

Data were presented as the mean ± standard error of the mean. All behavioral data were analyzed using two-way RM-ANOVA for group differences, followed by the post hoc Student–Newman–Keuls test (SigmaPlot v.11, Systat Software, Chicago, IL, USA). A p value of <0.05 was considered significant.

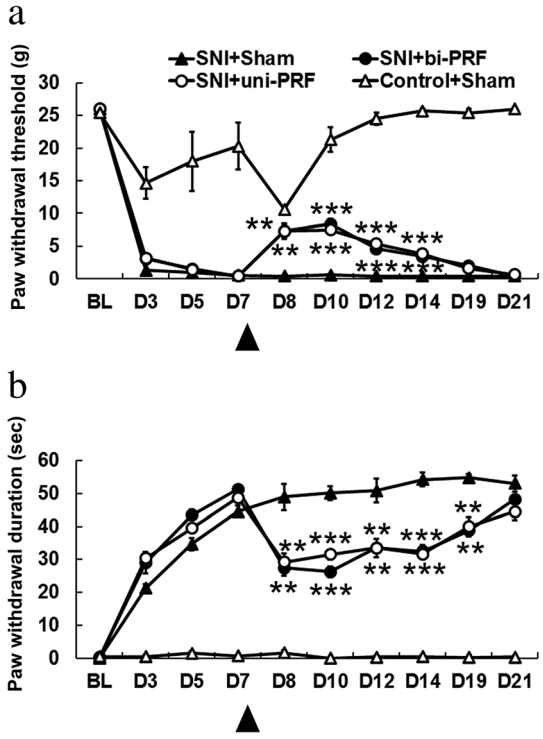

Figure 3. Comparison of unipolar and bipolar PRF stimulation in SNI-induced neuropathic pain. a. Mechanical withdrawal threshold by von Frey test. b. Cold withdrawal threshold by acetone test. BL: baseline data; black triangle: PRF stimulation. $^{**}p < 0.01$, $^{***}p < 0.001$ for SNI + bi-PRF and SNI + uni-PRF vs. the SNI + Sham by two-way ANOVA with Student–Newman–Keuls post hoc test. The significant differences between the Control + Sham and SNI + Sham are not marked. N = 6, 7, 7, and 6 for SNI + Sham, SNI + bi-PRF, SNI + uni-PRF, and Control + Sham groups, respectively.

RESULTS

Effects of Bi-PRF Versus Uni-PRF on SNI- and CFA-Induced Pain

The SNI and CFA studies had four groups respectively, which were SNI/CFA plus Sham PRF, SNI/CFA plus bi-PRF, SNI/CFA plus uni-PRF, and Sham SNI/saline plus Sham PRF.

In the SNI model, surgery caused marked drop of mechanical and cold threshold in the Control + Sham group from 26.00 to 1.30 ± 0.09 in von Frey test and from 0.00 to 21.25 ± 1.33 in acetone test, at baseline and Day 3, respectively (Fig. 3a,b). The hypersensitive state maintained until the end of study at 21 days. Application of uni-PRF or bi-PRF at the SN proximal to the SNI lesion on Day 7 attenuated mechanical and cold hypersensitivity to a similar extent ($p < 0.05$). Reduction in mechanical hypersensitivity maintained for seven days ($p < 0.001$ for SNI + bi-PRF/SNI + uni-PRF vs. SNI + Sham) and reduction in cold hypersensitivity kept for 12 days ($p < 0.01$ for SNI + bi-PRF/SNI + uni-PRF vs SNI + Sham). Maximal reversal occurred one to three days after PRF and the effect declined over time. No significant difference was observed between bi-PRF and uni-PRF. Notably in the control group (Control + Sham), mechanical thresholds dropped twice due to two surgeries (D3 for Sham SNI and D8 for Sham PRF Fig. 3a); however, mechanical and cold thresholds returned to their baseline levels soon.

CFA paw injection reduced mechanical thresholds to <3 g up to 14 days and heat thresholds to <4 sec for five days (CFA plus Sham group, Fig. 4a,b). Both uni-PRF and bi-PRF partly alleviated mechanical hypersensitivity for seven days and largely reversed heat hypersensitivity to basal thresholds for 5 (bi-PRF vs. Sham; 7.79 ± 0.63 vs. 3.57 ± 0.50, $p < 0.01$) and seven days (uni-PRF vs. Sham; 10.87 ± 0.51 vs. 7.24 ± 0.84, $p < 0.001$). No statistical difference was noted between the two types of PRFs. The Control + Sham group rapidly returned to baseline thresholds after Sham PRF surgery.

Comparisons of bi-PRF Application at DRG Versus at SN in SNI and CFA Models

We compared effects of bi-PRF at the DRG or at the SN in two pain models. Each study had four groups: SNI/CFA plus Sham PRF, SNI/CFA plus PRF-DRG, SNI/CFA plus PRF-SN, and Sham SNI/saline plus Sham PRF (control). Both PRF-DRG and PRF-SN similarly reversed mechanical hypersensitivity from D8 (144.71 ± 2.48 and 12.74 ± 1.64 vs. 3.45 ± 0.21, all $p < 0.01$) to D14 (9.71 ± 1.66 and 5.37 ± 1.08 vs. 2.22 ± 0.244, $p < 0.05$; Fig. 5a). Notably, PRF-DRG exerted a longer effect than PRF-SN to D19. Both PRFs similarly depressed cold pain through D8–D19 (Fig. 5b).

No differences were observed between PRF-DRG and PRF-SN in the CFA model. Both PRF-DRG and PRF-SN reduced mechanical and heat hypersensitivity for seven and five days, respectively (Fig. 6a,b).

Effect of Repeated PRF Stimulations on SNI-Induced Pain

Because effects of PRF in both pain models were short, we tested if duration could be prolonged by repeated stimulations. We only examined SNI model because CFA-induced pain was less than one week. Repeated PRF stimulations at D7 and D21 produced significant depression on nociceptive hypersensitivities as single stimulation (Fig. 7a,b). The maximal reversal effect of the second stimulation in von Frey and acetone tests was lower than the first stimulation, but there were no statistical differences

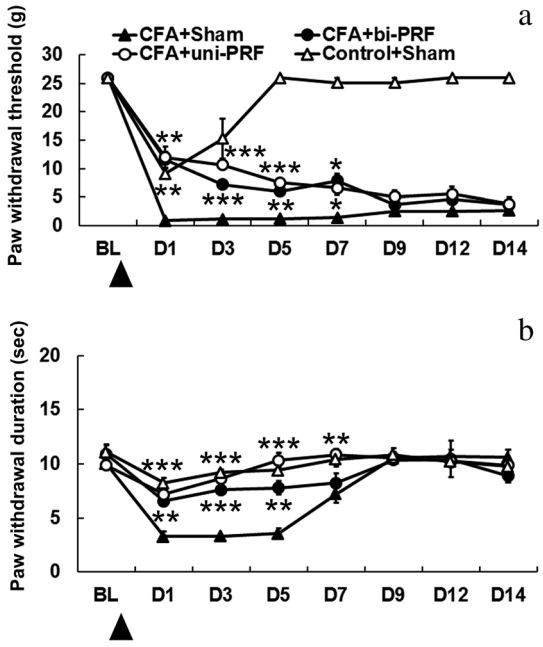

Figure 4. Comparison of unipolar and bipolar PRF stimulation in CFA-induced inflammatory pain. a. Mechanical withdrawal threshold by von Frey test. b. Heat withdrawal threshold by plantar heat test. BL, baseline data; black triangle, PRF stimulation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for CFA + bi-PRF and CFA + uni-PRF vs. the CFA + Sham by two-way ANOVA with Student–Newman–Keuls post hoc test. The significant differences between the Control + Sham and CFA + Sham are not marked. $N = 6, 6, 6$, and 5 for CFA + Sham, CFA + bi-PRF, CFA + uni-PRF, and Control + Sham groups, respectively.

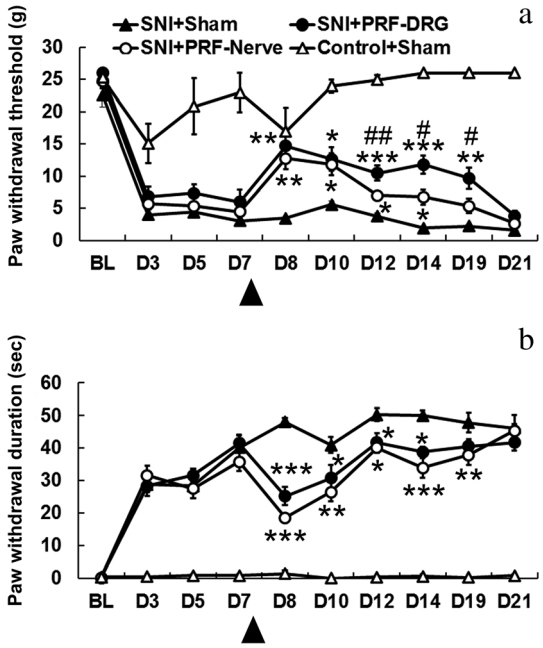

Figure 5. Comparison of effects of bipolar PRF application at dorsal root ganglion (DRG) or at sciatic nerve (SN) in SNI-induced neuropathic pain. a. Mechanical withdrawal threshold. b. Cold withdrawal threshold. BL, baseline data; black triangle, PRF stimulation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for SNI + PRF-DRG and SNI + PRF-Nerve vs. the SNI + Sham; and #$p < 0.05$, ##$p < 0.01$ SNI + PRF-DRG vs. SNI + PRF-Nerve by two-way ANOVA with Student–Newman–Keuls post hoc test. N = 5, 7, 6, and 6 for SNI + Sham, SNI + PRF-DRG, SNI + PRF-Nerve, and Control + Sham groups, respectively.

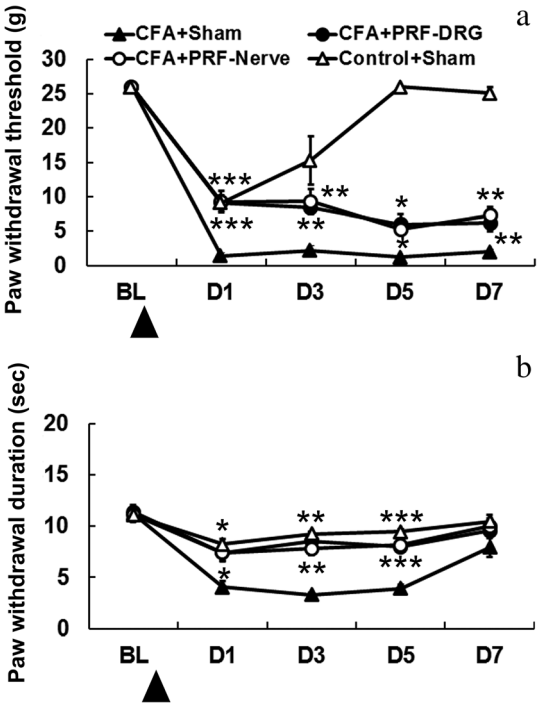

Figure 6. Comparison of effects of PRF stimulation at dorsal root ganglion or at sciatic nerve in CFA-induced inflammatory pain. a. Mechanical withdrawal threshold. b. Heat withdrawal threshold. BL, baseline data; black triangle, PRF stimulation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for CFA + PRF-DRG and CFA + PRF-Nerve vs. the CFA + Sham by two-way ANOVA with Student–Newman–Keuls post hoc test. $N = 7, 6, 6$, and 6 for CFA + Sham, CFA + PRF-DRG, CFA + PRF-Nerve, and Control+Sham groups, respectively.

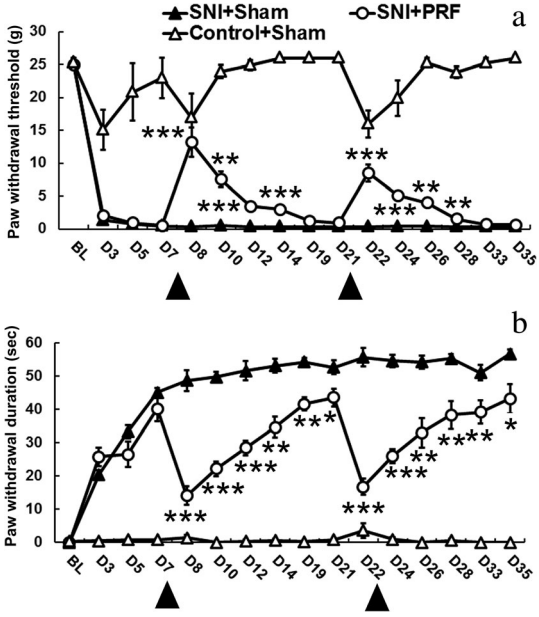

Figure 7. Effect of repeated PRF stimulation by open surgery on SNI-induced neuropathic pain. a. Mechanical withdrawal threshold. b. Cold withdrawal threshold. BL, baseline data; two black triangles, repeated PRF stimulation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for SNI + PRF vs. the SNI + Sham by two-way ANOVA with Student–Newman–Keuls post hoc test. $N = 6, 7$, and 6 for SNI + Sham, SNI + PRF, and Control + Sham groups, respectively.

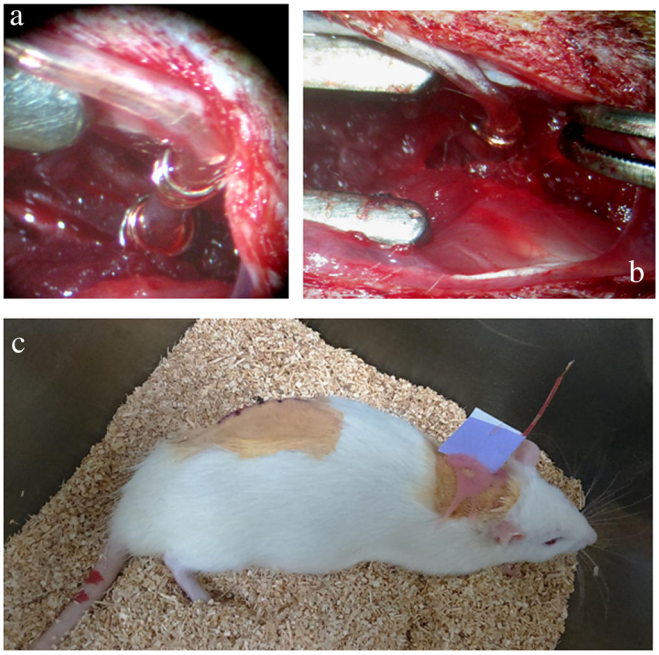

Figure 8. Illustration of an implantable PRF electrode and the implantation technique. a. Implantable electrode before placement. L4, L5 nerve roots were marked. b. Implantable electrode after placement. c. Rat with implantable electrode extended out from nuchal skin. [Color figure can be viewed at wileyonlinelibrary.com]

(11.07 ± 1.81 on D8 vs. 9.25 ± 0.85, $p > 0.05$ in Fig. 7a; 18.79 ± 2.82 vs. 19.20 ± 2.44, $p > 0.05$ in Fig. 7b). In the control group (Control + Sham, Fig. 7a), surgical wound pain (Sham SNI) and two Sham PRF applications caused three corresponding drops in mechanical thresholds.

Prolonged Effect of the Implanted PRF Electrode on SNI-Induced Pain

All rats were implanted with an electrode adjacent to L5 DRG during the SNI/Sham surgery (Fig. 8). There were three groups: SNI plus Sham PRF, SNI plus implantation PRF, and Sham SNI plus Sham PRF. Behavioral tests showed that electrode implantation (the Control + Sham) did not change baseline thresholds. Repetitive PRF stimulation significantly suppressed SNI-induced mechanical and cold hypersensitivities in bilateral hind paws (Fig. 9). PRF every two days depressed mechanical hypersensitivity in between-intervals and maintained pain reduction with a fluctuating pattern. The maximal effect appeared three hours after each PRF stimulation (Fig. 9a). PRF also reversed mechanical allodynia at the contralateral hind paw to the baseline immediately post-treatment (Fig. 9c). For cold hypersensitivity, PRF produced persistent and stable suppression over time at the two paws (Fig. 9b,d). Notably, repetitive PRF stimulation did not result in analgesic tolerance or efficacious attenuation during our study period (D9–D18).

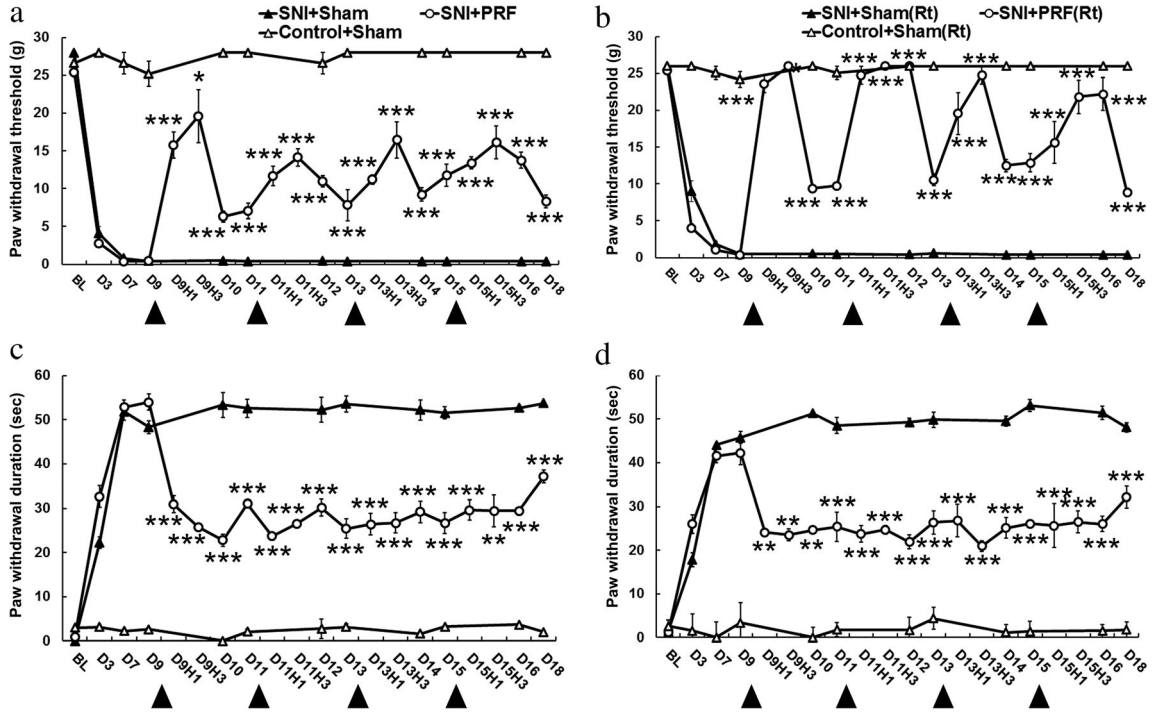

Figure 9. Effect of implantable PRF stimulation on SNI-induced neuropathic pain. a,c. Mechanical withdrawal threshold in the left and right paw, respectively. b,d. Cold withdrawal threshold in the left and right paw, respectively. BL, baseline data; black triangles, repetitive PRF stimulations. At x-axis, D9: post-SNI D9, before PRF stimulation, D9H1: one hour after PRF stimulation on D9, D9H3: three hours after PRF stimulation on D9, and so on. *$p < 0.05$, **$p < 0.01$ for SNI + PRF vs. the SNI + Sham by two-way ANOVA with Student–Newman–Keuls post hoc test. N = 6, 6, and 6 for SNI + Sham, SNI + PRF, and Control + Sham groups, respectively.

Complications, Adverse Effects, and General Concept of Efficacy

Throughout all experiments, including single and implantation stimulations, no animals showed neurological adverse effects or deficits such as constant muscle twitches, clamping, or claudication. One rat had wound infection in the implantation study, so the electrode was removed and the data was excluded from calculation. There was no vocalization, unusual or irritable behaviors, or death during or after the PRF stimulation. To present a clearer overview of therapeutic efficacy between models and between pain modalities, we summarized the outcome data in Supplement Table 1.

DISCUSSION

Our results demonstrated that (1) unipolar and bipolar PRF were equally effective in reducing pain in neuropathic and inflammatory pain models, (2) bipolar PRF treatment at the DRG exerted a greater reduction in mechanical allodynia than PRF treatment of the SN, and (3) repetitive PRF stimulations at DRG using an implantable electrode resulted in prolonged pain reduction. The relative effectiveness of PRF in different conditions was shown in Table 1.

PRF can reduce radicular pain (>50% reduction) in humans for weeks to months (31); while an animal study reported that PRF at the DRG in a partial sciatic nerve ligation model in rabbits reduced heat hyperalgesia for one week and mechanical withdrawal for two weeks (39). PRF at the DRG to treat L5 spinal nerve ligation (SNL) in rats showed moderate-to-strong effects at post-treatment Days 8–10 and beyond 32 (40), whereas another SNL study demonstrated a constant 12-day reduction in mechanical allodynia but not in cold allodynia (41). Our PRF study which included SNI rats showed PRF inhibited mechanical and heat hypersensitivities for 10 and 14 days, respectively, however, another study showed 28-day allodynidc suppression when PRF was applied close to the SN injury site (46). In a CFA-induced inflammatory pain, PRF application at L5 DRG inhibited mechanical allodynia for 14 days, but at SN, it had no effect (47). Taken together, these results imply that application targets and other factors, such as stimulation parameters, may all determine analgesic efficacy and duration. Although animal results may varied from human trials to some extent, preclinical behavioral data can help clarifying some confusions and provide mechanism-based logics before a large-scale human randomized controlled trial studies.

Bipolar vs. Unipolar PRF Stimulation

Our study demonstrated that a bipolar PRF system was able to produce a closed electrical circuit at a low voltage (5 V) and a similar effect to that of an open circuit by a high voltage (45 V) in the unipolar PRF system. We believe that a closed circuit indicated a dense E-field, a critical function determining PRF analgesic effect (48). The SNI model differs from the SNL model in that SNI leads to an injury comprising multiple spinal nerves whereas SNL is usually a mononeuropathy at L5. Therefore, PRF treating at the SN in this study could have a better spinal nerve coverage than applied at the DRG. In previous studies, we successfully applied bipolar PRF in rats with SNL to effectively reduce nociceptive behaviors, selectively blocking C-fiber mediated excitatory postsynaptic potentials, inhibiting activation of mitogen-activated protein kinase (MAPK, such as p-Erk and p-p38) and the release of proinflammatory cytokines (tumor necrosis factor) in the spinal dorsal horns (35,36). In this study, the effects exerted by bipolar and unipolar PRF on SNI-induced nociceptive hypersensitivity were similar, suggesting that a low-voltage bipolar system could provide equivalent analgesic efficacy at a lower power. We speculated that a small, dense E-field circuit between and around two closing electrode tips may avoid large tissue resistance in the unipolar system and can generate high efficacy. A recent human study successfully using bipolar PRF treatment to reduce lumbosacral radicular pain in patients who had poorly responded to monopolar PRF reflected that this hypothesis and demonstrated the superiority (49).

Effects of PRF Stimulation on Neuropathic and Inflammatory Pain

This study is the first to simultaneously explore effects of PRF on neuropathic and inflammatory pain. Our results revealed that unipolar and bipolar PRF stimulation generated distinct analgesic patterns between these two models. Both bipolar and unipolar systems partially reduced mechanical and cold allodynia in the SNI model and mechanical allodynia in the CFA model, but almost completely reversed heat hyperalgesia in the CFA model. In CFA-induced pain, unipolar PRF appeared to have a more favorable effect than bipolar PRF (Fig. 4). The behavioral differences could be attributed to pathophysiological differences between SNI and CFA, and their distinct mechanisms underlying mechanical, cold, and heat signaling. SNI causes severe Wallerian degeneration of the injured nerve, and axonotomy elicits direct molecule activations in the nerves and post-synaptic spinal dorsal horns. CFA inflammation induces innate immunity and increases pro-inflammatory cytokines/chemokines in the paw, and an indirect involvement of nervous system (50,51). It is possible that unipolar PRF yields a wider E-field, whereas bipolar PRF has a smaller E-field confined to an area between two electrodes (49). The differences of PRF physics could lead to variations in their function and biological effectiveness (49).

DRG vs. SN Stimulation

In patients with chronic postsurgical thoracic pain, PRF stimulation at the DRG was demonstrated to be superior to PRF at intercostal nerves or pharmacotherapy in terms of pain relief (>50% reduction) and analgesic duration (>3 months) (18). The anti-allodynic effect was greater in PRF-DRG than in PRF-SN in rats with CFA-induced pain in an animal study (47). Our current study was a head-to-head comparison between DRG and SN stimulation in neuropathic and inflammatory pain. In neuropathic pain, PRF-DRG exerted a longer inhibitory effect on mechanical allodynia than PRF-SN (Fig. 5a), suggesting that PRF stimulation at the neuronal soma or neurites varies in blocking nociceptive transmissions. However, their effects on cold allodynia did not differ significantly (Fig. 5b). In inflammatory pain, the effects of PRF-DRG and PRF-SN on both mechanical and cold pain did not differ (Fig. 6). *In vivo* recordings demonstrated that nerve transection caused spontaneous ectopic firings in the affected DRG, generated by A-type neurons from 16 h to 23 days after injury, paralleling the appearance of tactile allodynia at the paw (52). It is possible that PRF-DRG differentially depresses ganglionic neurons in comparison with that of PRF-SN only on the neurites in the nerve.

Because of lack of supporting evidence, further discussion is beyond the scope of this study.

Repetitive PRF Stimulation of DRG

In this study, repeated stimulation by an open surgical technique or an implanted electrode produced similar suppressions on mechanical and cold allodynia without demonstrating analgesic tolerance at four repeats. The second DRG stimulation cycle was shown to extend the duration of analgesia in PRF treatment for chronic inguinal neuralgia (13). In patients with lumbosacral radicular pain, repeated PRF of five or more treatment courses provided long-term effects with constant averaged relief duration (53). However, repetitive PRF treatments via percutaneous technique are potentially risky, and patients might have low satisfaction due to repeated punctures and short analgesic durations. From our results, implantation of a DRG stimulator and repetitive stimulations with low-voltage RF currents may be more favorable.

The technique of electrode implantation has not been reported in either animal or human studies. Our previous study demonstrated the safety of bipolar PRF stimulation at DRG evidenced by no neuronal damage and no increases of ATF3 in the DRG (36). A new DRG implantation device in human is available commercially (54); however, the implantable pulse generator of this device delivers low-frequency electrical current rather than a radiofrequency-high electrostimulation. Thus, repetitive DRG stimulations by an on-demand PRF-mode power generator could be a potential alternative for chronic pain.

Limitations

Our results of PRF stimulation in rats cannot be extrapolated to clinical practice without human studies. For example, the evoked responses in behavioral tests in animal models are neurologically different from the subjectively description of pain in humans (55). Moreover, the implantation in this study lasted for 18 days, we need a longer duration to observe its tissue safety and analgesic effectiveness in animals before we could advocate its clinical applicability as a chronic pain therapy.

CONCLUSIONS

Our study demonstrated that a low-voltage bipolar PRF was equivalent in analgesic efficacy to a high-voltage PRF at the DRG and SN in both neuropathic and inflammatory pain models. PRF stimulation at the DRG appeared to have a greater effect on neuropathic pain than PRF stimulation at the SN. Importantly, we demonstrated that implantation of a PRF electrode for repeated stimulation the DRG may prolong analgesia and can be an innovative PRF technology for improving intractable pain control.

Authorship Statements

Dr. Wen, Dr. Tan, and Student Huang designed the study, interpreted data, conducted statistical analysis, and prepared the manuscript draft. Student Huang, Drs. Tsai, and Dr. Liu conducted the behavioral tests and data collection. Dr. Ho and Dr. Poree helped preparation and revision of the manuscript and had important intellectual input to the final submission. All authors approved the final manuscript and all took public responsibility for appropriate portions of the content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the supporting information tab for this article.

COMMENTS

In an animal model of inflammatory and neuropathic pain pulsed radiofrequency ablation provided acutely a similar reduction in allodynia/hyperalgesia when bipolar or unipolar modes of pulsed radiofrequency were applied to DRG or peripheral nerve. This may have an important future clinical implication when various designs of electrodes are used to deliver pulsed radiofrequency.

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The authors in this article make a very interesting study on the effectiveness of a widely used technique in Europe, pulsed radiofrequency, controversial in recent times in some countries, but widely used in others. The comparison of various modalities and various pathologies, neuropathic pain, and nociceptive pain makes the article richer and the conclusions more useful for readers. Although it is controversial that pulsed radiofrequency is a neuromodulation technique, due to the microlesions and microporation in the membranes that are produced, it does seems to be an interesting topic due to the number of patients who can benefit before proceeding to the implantation of a neuromodulation system.

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