Title

Application of pulsed radiofrequency currents to rat dorsal root ganglia modulates nerve injury-induced tactile allodynia.

Journal

Anesthesia and analgesia, 113(3)

ISSN

1526-7598

Authors

Perret, Danielle M
Kim, Doo-Sik
Li, Kang-Wu
et al.

Publication Date

2011-09-19

Peer reviewed

Published in final edited form as:
Anesth Analg. 2011 September ; 113(3): 610–616. doi:10.1213/ANE.0b013e31821e974f.

Application of Pulsed Radiofrequency Currents to Rat Dorsal Root Ganglia Modulates Nerve Injury-Induced Tactile Allodynia

Danielle M Perret, MD,
Dept. of Anesthesiology and Perioperative Care and Dept. of Physical Medicine & Rehabilitation, University of California, Irvine

Doo-Sik Kim, M.D., Ph.D.,
Dept of Anesthesiology and Perioperative Care, University of California, Irvine, and Kosin University School of Medicine

Kang-Wu Li, M.D., Ph.D.,
Department of Anesthesiology & Perioperative Care, School of Medicine, University of California Irvine, Irvine, CA

Karin Sinavsky, MD, MS,
Dept of Anesthesiology and Perioperative Care, University of California, Irvine

Robert L Newcomb, PhD,
Department of Statistics, University of California Irvine, Irvine, CA

Jason M Miller, M.D., and
Pacific Pain Medicine Oceanside, CA

Corresponding Author: Z. David Luo, Department of Anesthesiology & Perioperative Care University of California, Irvine Medical Center Bldg 53, Room 227 101 The City Drive South Orange, CA 92868, Phone: 714-456-7962, FAX: 714-456-7903, zluo@uci.edu, Reprints will not be available from the authors..

The authors declare no conflicts of interest.

DISCLOSURES:
Name: Danielle M Perret, MD
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Danielle M Perret has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Doo-Sik Kim, MD, PhD
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Doo-Sik Kim has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Kang-Wu Li, MD, PhD
Contribution: This author helped conduct the study and write the manuscript.
Attestation: Kang-Wu Li has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Karin Sinavsky, MD, MS
Contribution: This author helped write the manuscript.
Attestation: Karin Sinavsky has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Robert L Newcomb, PhD
Contribution: This author helped analyze the data and write the manuscript.
Attestation: Robert L Newcomb has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Jason M Miller, MD
Contribution: This author helped design the study, conduct the study, and analyze the data.
Attestation: Jason M Miller has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Name: Z. David Luo, MD, PhD
Contribution: This author helped design the study, analyze the data, and write the manuscript.
Attestation: Z. David Luo has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Z. David Luo, MD, PhD
Dept of Anesthesiology and Perioperative Care, University of California, Irvine

Abstract

Background—Application of pulsed radiofrequency (PRF) currents to the dorsal root ganglia (DRG) has been reported to produce relief from certain pain states without causing thermal ablation. In this study, we examined the direct correlation between PRF application to DRG associated with spinal nerve injury and reversal of injury-induced behavioral hypersensitivity in a rat neuropathic pain model.

Methods—Neuropathic lesioning was performed via left L5 spinal nerve ligation on male adult Sprague-Dawley rats. Once the injured rats had developed tactile allodynia, one group was then assigned to PRF treatment of the L5 DRG and another group was assigned to the sham treatment to the DRG. Behavioral testing was performed on both the control and treated paws using the von Frey filament test before the surgery and at indicated days. The resulting data were analyzed using a linear mixed model to assess the overall difference between the treatment groups and the overall difference among the study days. Cohen's d statistic was computed from paired difference-from-baseline scores for each of the 14 study days after treatment and these measures of effect-size were then used to descriptively compare the recovery patterns over time for each study group.

Results—Spinal nerve injury resulted in the development of behavioral hypersensitivity to von Frey filament stimulation (allodynia) in the hindpaw of the left (injury) side. Mixed Linear modeling showed a significant difference between the treatment groups ($p = 0.0079$) and a significant change of paw withdrawal threshold means over time ($p = 0.0006$) for all 12 animals. Evaluation of Cohen's d (effect size) revealed that the PRF-treated animals exhibited better recovery and recorded larger effect-sizes than the sham-treated animals on 10 of the 14 post-PRF treatment days and exhibited moderate to strong effects posttreatment at days 8–10 and at and beyond day 32.

Conclusions—Findings from this study support that PRF of the DRG causes reversal of nerve injury (spinal nerve ligation)-induced tactile allodynia in rats. This allodynia reversal indicates that nonablative PRF acting via modulation of the DRG can speed recovery in nerve injury-induced pain.

Introduction

Traditional radiofrequency (RF) of the dorsal root ganglion (DRG) was first used for the treatment of low back pain in 1974 using a needle electrode connected to an RF generator (1). Continuous RF ablation has the effect of neurodestruction, similar to the end result of other neurolytic procedures (2). Postprocedure discomfort may result (3).

Pulsed RF (PRF) may be an excellent alternative to traditional RF. A randomized prospective clinical study suggests that low temperature PRF energy is a safe and effective modality when applied adjacent to the DRG for the management of radicular neuropathic pain (4). During PRF administration, the electric current is delivered in pulses. The heat generated dissipates between pulses and neurodestructive temperatures are never reached (5–8). At 42 degrees Centigrade, the temperature at which PRF stimulation occurs, neuroma formation is not likely to occur (9) leading to a decreased risk of a neuritis-like reaction. In addition, PRF has been demonstrated to be less damaging than traditional RF in a protocol examining cell survival in cortical cultures (10).

Currently, RF and PRF are used for the treatment of facet joint disease (11), radicular pain (11,12), sacroiliac joint disease (11), and trigeminal (11,13) and other neuralgias (14–18). Overall, there is a decreased incidence of side effects and complications from PRF when

compared to RF treatment (11,12). PRF, therefore, is rapidly gaining acceptance as an alternative pain intervention. Although widely used clinically (1,4,11,14,16,19–27), technique evaluation and mechanism(s) of action have not been well identified. There is a paucity of data examining behavioral outcomes to PRF application.

What appears to be the first-ever behavioral study using PRF was published in 2008 and demonstrated improvement in tactile allodynia with percutaneous (not DRG-directed) PRF (28). In this study, neuropathic pain was induced by the spinal nerve ligation (SNL) procedure described by Kim and Chung (29). In this model, allodynia on the ipsilateral hindpaw secondary to unilateral L5/6 SNL peaks within 2 weeks postsurgical SNL and recovers gradually from approximately week 10 postinjury (30–32). On the 14^{th} postoperative day after SNL, percutaneous PRF was applied to the plantar surface of the ipsilateral hindpaw (28). Dynamic plantar aesthesiometer (weight and paw withdrawal time) and Von Frey Filament testing showed that PRF application for 120 seconds significantly improved allodynia on the first through 14^{th} post-PRF day compared to placebo (28). Aksu et al (33) applied PRF to the L5 and L6 dorsal roots in a rabbit neuropathic pain model and found that both mechanical and thermal hyperalgesia decreased 2 and 3 weeks after PRF application, respectively (33). In this behavioral study, we examined the direct effect of PRF adjacent to the DRG associated with spinal nerve injury on reversal of injury-induced tactile allodynia in a neuropathic pain model, which is a technique relevant to current clinical practice.

Materials and Methods

Protocols used in this study were approved by the University of California, Irvine, Institutional Animal Care and Use Committee and conform to the National Institutes of Health Guidelines for Animal Use. Fourteen adult male Sprague-Dawley rats (149–177g, Harlan Industries, Indianapolis, IN, USA) were housed with a 12-hour light-dark cycle and with access to food and water *ad libitum*. Rats were kept in a similar environment for 7 days for acclimation before SNL surgery. The study timeline is summarized in Table 1.

Neuropathic lesioning

Previous studies indicate that tactile allodynia, a form of neuropathic pain as manifested by reduced threshold to Von Frey Filament stimulation (mechanical light touch), develops in hindpaws ipsilateral to the nerve injury approximately four days after nerve ligation (29,32). In addition, the tactile allodynia state recovers after 10 weeks of injury in SNL rats (30,31). This provides an excellent model for studying recovery from nerve injury after an applied treatment, namely RF modulation.

SNL was performed as described by Kim and Chung (29) on all 14 adult rats after baseline testing one day before the surgery. Anesthesia was induced with 5% isoflurane in O_2, and maintained with 2% isoflurane in O_2 during the operation. The back fur was shaved and the animal was placed prone for surgery. The skin was scrubbed with betadine, then 70% EtOH. Under a surgical microscope, skin and percutaneous tissues were incised. The left L4-S1 paraspinal muscles were bluntly dissected from the spinal processes allowing visualization of the left L5 spinal nerve after removal of the L6 transverse process. The L5 spinal nerve was tightly ligated with 6-0 silk suture. Surgery ended with suturing of the muscle, fascia, and subcutaneous layers with 4-0 chromic suture and skin closure with rat staples. Surgery time for each SNL was approximately 15 minutes. The rats were allowed to recover from surgery for 2 days, then tested for SNL-induced allodynia for 10 days as described below. One rat was killed and excluded from the study after SNL due to surgical complications.

PRF application

As for the SNL procedure, anesthesia was induced with 5% isoflurane in O_2 and maintained with 2% isoflurane in O_2 during the operation. The lumbrosacral area of the rat was shaved and the operative field was prepped with betadine, then 70% EtOH. A 5cm skin incision was made in the posterior lumbrosacral junction with a number 11 blade under a surgical microscope. After blunt muscle dissection, the L6 spinous process was identified at the level of the iliac crest. The interspinous muscle was retracted laterally and the plates of the vertebral arch and ligated L5 spinal nerve were exposed. To expose the L5 DRG, L5–L6 articular processes were removed with a small rongeur. The L5 DRG was confirmed by its proximal location to the L5 SNL ligature (Figure 1). Bleeding was controlled with a small electrocautery device (Advanced Meritech International, CAT #CH-H1, 86-38 53rd Ave, Suite 100, Flushing NY 11373, USA) and with the application of direct pressure.

An RF electrode with a built-in thermocouple for temperature monitoring was placed adjacent to the L5 DRG via direct visualization; Figure 1 demonstrates a dorsal view of the surgical field. The RF probe (Radionics, Burlington, MA, USA) used was a standard SMK RF (5cm length) probe (for a needle with a 5mm active tip), which was modified as follows. The RF probe was placed into a plastic tube (pipette tip) allowing 2mm active distal end exposed for PRF treatment. The electrode was then connected to an RFG-3C Plus radiofrequency lesion generator (Radionics, Burlington, MA, USA) to expose the DRG to RF fields. After the electrode was placed adjacent to the L5 DRG and the leads were connected, each animal was placed randomly by the surgeon into two treatment groups, sham versus PRF:

Group 1 (n = 7): PRF treatment

In this group, the DRG was exposed to approximately 25V (peak voltage) 500 KHz RF pulses for 20 milliseconds. The pulses were delivered at a rate of 2 Hz for a period of 120 seconds. Temperature was limited to 42°C. Electrode impedance, DRG tissue temperature and current were recorded at 15-second intervals throughout the period of PRF exposure. At the end of the treatment, muscle/fascial layers and the subcutaneous tissue were sutured with 4-0 chromic suture and skin closure was completed with rat staples. The animal was allowed to recover from anesthesia on a heating pad maintained at 37°C by a temperature controller. During recovery, 5mL of sterile saline was injected intraperitoneally for intravascular volume replacement.

Group 2 (n = 6): Sham treatment

In this group, the electrode was maintained adjacent to the DRG for 120 seconds, without passing current through the electrode. Electrode impedance, DRG tissue temperature and current were recorded at 15-second intervals throughout the period of exposure. At the end of the treatment, muscle/fascial layers and the subcutaneous tissue were sutured with 4-0 chromic suture and skin closure was completed with rat staples. The animal was allowed to recover from anesthesia on a heating pad maintained at 37°C. During recovery, 5mL of sterile saline was injected intraperitoneally for intravascular volume replacement. Surgery time for each DRG PRF and sham procedure was approximately 30 minutes. One rat in the sham group was killed due to study complications, epidural abscess formation as revealed by postmortem evaluation.

Behavioral Testing

Behavioral testing was performed over 50 days as indicated on the control (right) and treatment (left) paws of all animals using the Von Frey Filament Test as described by Chaplan et al (34). All testing was performed by the same physician, who was not the

surgeon, and who was blinded to the animal group assignment. The animals were placed in a clear plastic cage with a wire mesh bottom for at least 15 min acclimation before the 50% paw withdrawal threshold (PWT) to Von Frey filaments was determined. Briefly, a series of filaments of varying buckling weights were applied in consecutive sequence to the plantar surface of the hindpaw in the L5 dermatomal distribution. A pressure was applied causing the filament to buckle. Paw lifting or licking indicated a positive response and was followed by the use of the next weaker filament. Absence of a paw withdrawal after 5 seconds prompted the use of the next higher weight filament. The process was repeated until the completion of evaluation according to the up-down method (34). The 50% response threshold, derived from probability distribution patterns, was calculated using the equation 50% gm threshold = (10^(Xf + kd))/10,000, where Xf is the value (in log units) of the final Von Frey filament used, k is the value of the pattern of positive versus negative responses and d is the mean difference (in log units) between stimuli (34).

Statistical Methods Used

Two-way ANOVA analysis with Bonferroni posttests was used to assess the effects of SNL on behavioral hypersensitivity before PRF treatment. Statistical significance was indicated by p value < 0.05 (Figure 2). For data analysis after PRF treatment, a repeated measures analysis based upon mixed linear modeling with left-hindpaw withdrawal thresholds (LHPWT) as the dependent variable, and with PRF versus Sham treatment as a fixed effect and time (study days) as random effect was performed using SAS Statistical Software (version 9.2, SAS Institute Inc.). Time was considered to be a random effect since measurements were gathered on 14 unequally spaced days during the 51-day recovery period. To appropriately fit the covariance structure, where days were unevenly spaced, we used the spatial power law model ($Cov[LHPWT_{t1},LHPWT_{t2}] = \sigma^2\rho^{|t1-t2|}$) which is widely used for these kinds of data (35). The resulting output provided a test of the difference between the LHPWT means of the sham and PRF treated groups and a test of the overall differences among the LHPWT means for all rats across all post-PRF treatment study days. Finally, a description of the post-PRF treatment recovery patterns registered by each treatment (sham and PRF) across the fourteen post-PRF treatment study days was provided by computing Cohen's d to measure the treatment-by-day effect-size (36). For this purpose, Cohen's d was computed directly from the treatment mean and standard deviation of the group's LHPWT paired-difference scores for each posttreatment day (each animal's LHPWT score at the indicated posttreatment time point minus the animal's baseline LHPWT score). In addition, these effect sizes also provided a description of the difference between treatments on each of the fourteen post-PRF study days (Figure 4).

Results

Neuropathic pain states were induced by tight ligation of the left L5 spinal nerve (SNL) as described by Kim and Chung (29). PWT to Von Frey filament stimulation was tested at designated times after injury as shown in Figure 2. For each SNL rat, the contralateral paw was used as an internal control. The PWT of the right paw for each animal remained at 13–15 gm over the entire 50 days of the study. This indicates that the control paw did not have any significantly increased sensitivity to the mechanical stimulation. However, SNL induced a gradual reduction in PWT at the injury side, which reached a hypersensitive state to the mechanical stimulation (PWT to < 5gm) in about two weeks after injury, indicating induction of tactile allodynia (Figure 2).

In the rats that received PRF, the PWT on the injury side increased to > 10g 8–10 days after PRF. The PWT then decreased slightly but began to increase again after day 18 post-PRF towards the pre-SNL baseline (Figure 2). Overall, PWTs in the injury side of the SNL and PRF group (group 1) were higher than that in the injury side of the SNL and sham PRF

group (group 2) (Figure 3). Appropriately, the mixed linear analysis exhibited a statistically significant result for the test of no difference between the sham treatment and PRF treatment means ($p = 0.0079$) and produced a statistically significant result for the test of no difference among the fifteen study day means ($p = 0.0006$). To go beyond these overall findings, by treatment effect-sizes based upon paired-difference scores between baseline and subsequent day PWT scores are presented graphically (Figure 4) for every one of the fourteen post-PRF treatment days. It is noteworthy that the PRF-treated animals recorded larger effect-sizes than the sham-treated animals on ten of the fourteen post-PRF treatment days and exhibited moderate to strong effects after day 32. Taken together, these results show that PRF induced a greater recovery from tactile allodynia than the recovery associated with the sham treatment.

Discussion

This study demonstrates that PRF adjacent to the DRG induces an allodynia reversal in an animal model of neuropathic pain. Even though its maintenance effect is difficult to determine due to the natural recovery of the SNL model, effect size comparison data support that PRF induces a greater allodynia recovery than sham treatment.

These findings are complementary to data published by Ozsoylar et al. (28) or Aksu et al. (33), who found significant reductions in nociception after PRF application to the rear paw of SNL rats, or to the L5/L6 dorsal roots of a rabbit tight sciatic nerve ligation model, respectively. In our experience, tactile allodynia is the best modality for behavioral testing in the SNL model, while the consistency with thermal hyperalgesia testing is less robust. Nevertheless, inclusion of other behavioral testing modalities in pain models is also recommended in future experiments. Since spontaneous (37), enhanced (38) DRG activities, and sustained ectopic DRG neuron firing (39) are the main cause of radicular pain, DRG is likely the most important target to limit ectopic impulse generation in patients with radicular pain. Thus, the allodynia reversal effect of PRF may be applicable to both radicular pain because well as peripheral neuropathic pain as both animal models produce similar allodynic outcomes (40). However, since mechanisms underlying pain states may differ based on etiologies, studying the effects of DRG-adjacent PRF in other pain models will assist in the determination of clinical selection criteria.

We targeted the L5 DRG in this study assuming that injured DRG is the source of injury signals to the central nervous system that leads to central sensitization. Due to L5 tight ligation and fiber degeneration, intact L4 fibers, through the sciatic nerve, may be the major pathway to carry action potentials to the central nervous system. It is possible, however, that non-noxious sensory signal propagated through intact L4 fibers can still trigger behavioral hypersensitivity by activating the sensitized L5 sensory circuit through projection neurons and their collateralization directly or through interneurons indirectly at the dorsal horn level. An experimental PRF design comparing the modulation effects of both L4 DRG and L5 DRG in pain state relief in a larger group of animals may be warranted in future experiments.

While it is not practical to compare the magnitude and duration of antiallodynia effects of PRF in humans and animal models, our data support that PRF-induced antiallodynic effects occur about one week after PRF treatment and last for the duration of SNL-induced allodynia. However, humans may report analgesia after PRF within the first hour of treatment. This discrepancy may reflect the fact that analgesia and tactile allodynia reversal are not synonymous. Early PRF-induced analgesia may reflect that changes in local factors, such as pain-modulators at the PRF site, or central factors, such as the release of endogenous endorphins, may play a role in the perception of pain relief, which was not tested in this

animal model. Later allodynia reversal may reflect long-term changes in gene expression, resulting in more permanent changes in sensory neuron excitability. In fact, many investigators recommend DRG targeting for this reason, although other reasons are also cited (12).

The mechanism of action of PRF is still being investigated. Findings from several studies support that PRF-induced changes seem reversible and do not rely on thermal injury. After exposing rat DRG or sciatic nerve to PRF, RF, and conductive heat, Podhajsky et al (41) reported that PRF caused transient minor structural changes, fibroblast activation and collagen deposition. In contrast, thermal RF lesions cause nerve fiber Wallerian degeneration. Data from similar studies evaluating short-term PRF effects (1 hr) on rat DRG (42) and long-term PRF effects (21 days) on the sciatic nerve (43) showed that unmyelinated nerve fibers were macroscopically normal in both studies. Myelinated axons, however, showed severe nerve degeneration post-RF (43), but only a separation in the sciatic nerve (43), and interrupted myelin coverage in DRG (42) post-PRF.

Erdine et al (44) showed that PRF exposure results in injuries relatively selective to small fibers (C-fiber and A-delta fiber) with changes in the morphology of mitochondrial membranes, disruption and disorganization of microfilaments within the axons; and presumably microscopic changes in the axon membrane, such as changes in ion channels or pumps. It seems that PRF electrical and current fields better penetrate the axonal cell membranes of the C and A-delta fibers, causing greater disruption to inner structures. The authors suggest that damages to mitochondria via their membrane fragility causes an interruption in the essential adenosine triphosphate-mediated cellular functions and in cellular metabolism that may impede the generation of pain signals; the damage to microtubules and microfilaments may similarly impede the transmission of pain impulses.

Various studies have noted changes in dorsal horn neuronal activity, and increased cellular stress in small and medium caliber neurons in response to PRF at or near the DRG (42,45–47). Application of PRF, but not conventional RF, to the DRG results in an increase in *c-fos* immunoreactive neurons in the superficial laminae of the dorsal horn 3 hours later, suggesting a heat-independent activation of dorsal horn neurons (47). Van Zundert et al (45) reported an increase in *c-fos* immunoreactive cells in the dorsal horn 7 days after application of both RF and PRF to the cervical dorsal root, representing a late neuronal activation. In addition, Hamann et al (46) revealed an upregulation of ATF3 in DRG neurons, but not in the sciatic nerve, both of which were subjected to PRF, suggesting that the biological response to PRF is tissue or cell type-specific and independent from thermal damages (46). Since both *c-fos* and ATF3 are transcription factors, these findings support that PRF pain relief may derive from long-term modulation of cell functions by altering gene expression, which may include positive PRF effects on synaptic strength and long-term enhancement (48), which is related to central sensitization, a major player in chronic pain development (49).

In this study, PRF was applied similar to clinical variables. The voltage was limited to 25V to avoid a thermal ablation (temperature limited to 42°C) as the other variables (500 KHz pulses, 20 milliseconds in duration, rate of 2 Hz, period of 120 seconds) were fixed for smaller rat DRG. Even though 25 V output is not the mode in clinical practice, it is occasionally cited (6). However, animal studies that vary technical considerations, such as ideal voltage, number of cycles, pulse duration and optimal electrode distance, are also encouraged and will refine the application of PRF in pain treatment. Furthermore, we used an open procedure to ensure reliable DRG-adjacent PRF application since the anesthetized animal cannot report vibration, buzzing, pressure or tingling sensation for probe placement as humans do. However, this invasive approach will not be necessary in clinical practice

because PRF adjacent to the DRG can be performed percutaneously via fluoroscopic guidance. Finally, although it did not affect allodynia recovery in the sham rats, the application of electrocautery, in combination with direct pressure, for bleeding control may cause unnecessary electrical energy at the PRF site, which should be avoided in future studies if possible.

In conclusion, PRF is effective for the treatment of experimental neuropathic pain via DRG modulation. Further elucidation of exact mechanisms underlying PRF-induced DRG modulation in pain state relief, however, is needed.

Acknowledgments

The authors would like to thank Mr. Joshua Lee for his technical assistance during the preparation of the manuscript.

Funding: This study was supported in part by pilot grants from the Committee on Research and Graduate Academic Programs and the Department of Anesthesiology & Perioperative Care; University of California, Irvine Medical Center.

References

1. Uematsu S, Udvarhelyi GB, Benson DW, Siebens AA. Percutaneous radiofrequency rhizotomy. Surg Neurol. 1974; 2:319–325. [PubMed: 4853577]
2. Myers, R. Neuropathology of neurolytic agents with analysis of the causes of failed neurolysis. In: Cousins, MSBP., editor. Neural Blockade and Pain Management in Clinical Anesthesia and Management of Pain. 3rd ed.. Philadelphia: Lippincott; 1998. p. 985-1006.
3. Sluijter ME. The role of radiofrequency in failed back surgery patients. Curr Rev Pain. 2000; 4:49–53. [PubMed: 10998715]
4. Van Zundert J, Patijn J, Kessels A, Lame I, van Suijlekom H, van Kleef M. Pulsed radiofrequency adjacent to the cervical dorsal root ganglion in chronic cervical radicular pain: a double blind sham controlled randomized clinical trial. Pain. 2007; 127:173–182. [PubMed: 17055165]
5. Van Zundert J, Brabant S, Van de Kelft E, Vercruyssen A, Van Buyten JP. Pulsed radiofrequency treatment of the Gasserian ganglion in patients with idiopathic trigeminal neuralgia. Pain. 2003; 104:449–452. [PubMed: 12927617]
6. Munglani R. The longer term effect of pulsed radiofrequency for neuropathic pain. Pain. 1999; 80:437–439. [PubMed: 10204759]
7. Rozen DAJ. Pulsed radiofrequency for the treatment of ilioinguinal neuralgia after inguinal herniorrhaphy. Mount Sinai Journal of Medicine. 2006; 73:716–718. [PubMed: 16878278]
8. Sluijter MCER, Rittman WB, Van Kleef M. The effects of pulsed radiofrequency fields applied to the dorsal root ganglion. A preliminary report. Pain Clin. 1998:109–117.
9. Cohen SP, Foster A. Pulsed radiofrequency as a treatment for groin pain and orchialgia. Urology. 2003; 61:645. [PubMed: 12639676]
10. Cahana A, Vutskits L, Muller D. Acute differential modulation of synaptic transmission and cell survival during exposure to pulsed and continuous radiofrequency energy. J Pain. 2003; 4:197–202. [PubMed: 14622704]
11. von Boxem, KvEM.; Brinkuize, T.; Patijn, J.; van Kleef, M.; van Zundert, J. Radiofrequency and pulsed radiofrequency radiofrequency treatment of chronic pain syndromes: the available evidence. Pain Practice. 2008; 8:385–393. [PubMed: 18721175]
12. Malik K, Benzon HT. Radiofrequency applications to dorsal root ganglia: a literature review. Anesthesiology. 2008; 109:527–542. [PubMed: 18719452]
13. Orlandini G. Pulsed percutaneous radiofrequency treatment of the Gasserian ganglion for therapy of trigeminal neuralgia: technical notes, validity of the method and selection of the patients. Pain. 2004; 108:297–298. author reply 8–9. [PubMed: 15030950]
14. Navani A, Mahajan G, Kreis P, Fishman SM. A case of pulsed radiofrequency lesioning for occipital neuralgia. Pain Med. 2006; 7:453–456. [PubMed: 17014606]

15. Cohen SP, Sireci A, Wu CL, Larkin TM, Williams KA, Hurley RW. Pulsed radiofrequency of the dorsal root ganglia is superior to pharmacotherapy or pulsed radiofrequency of the intercostal nerves in the treatment of chronic postsurgical thoracic pain. Pain Physician. 2006; 9:227–235. [PubMed: 16886031]
16. Rhame EE, Levey KA, Gharibo CG. Successful treatment of refractory pudendal neuralgia with pulsed radiofrequency. Pain Physician. 2009; 12:633–638. [PubMed: 19461829]
17. Rozen D, Ahn J. Pulsed radiofrequency for the treatment of ilioinguinal neuralgia after inguinal herniorrhaphy. Mt Sinai J Med. 2006; 73:716–718. [PubMed: 16878278]
18. Shah RV, Racz GB. Pulsed mode radiofrequency lesioning to treat chronic post-tonsillectomy pain (secondary glossopharyngeal neuralgia). Pain Pract. 2003; 3:232–237. [PubMed: 17147673]
19. Tekin I, Mirzai H, Ok G, Erbuyun K, Vatansever D. A comparison of conventional and pulsed radiofrequency denervation in the treatment of chronic facet joint pain. Clin J Pain. 2007; 23:524–529. [PubMed: 17575493]
20. Balogh S. Transcutaneous application of pulsed radiofrequency: four case reports. Pain Pract. 2004; 4:310–313. [PubMed: 17173614]
21. Misra S, Ward S, Coker C. Pulsed radiofrequency for chronic testicular pain-a preliminary report. Pain Med. 2009; 10:673–678. [PubMed: 19302438]
22. Keskinbora K, Aydinli I. Long-term results of suprascapular pulsed radiofrequency in chronic shoulder pain. Agri. 2009; 21:16–21. [PubMed: 19357996]
23. Wilkes D, Ganceres N, Solanki D, Hayes M. Pulsed radiofrequency treatment of lower extremity phantom limb pain. Clin J Pain. 2008; 24:736–739. [PubMed: 18806541]
24. Liliang PC, Lu K, Hsieh CH, Kao CY, Wang KW, Chen HJ. Pulsed radiofrequency of cervical medial branches for treatment of whiplash-related cervical zygapophysial joint pain. Surg Neurol. 2008; 70 Suppl 1:S1, 50–55. discussion S1:5. [PubMed: 18786711]
25. Chao SC, Lee HT, Kao TH, Yang MY, Tsuei YS, Shen CC, Tsou HK. Percutaneous pulsed radiofrequency in the treatment of cervical and lumbar radicular pain. Surg Neurol. 2008; 70:59–65. discussion. [PubMed: 18207554]
26. Simopoulos TT, Kraemer J, Nagda JV, Aner M, Bajwa ZH. Response to pulsed and continuous radiofrequency lesioning of the dorsal root ganglion and segmental nerves in patients with chronic lumbar radicular pain. Pain Physician. 2008; 11:137–144. [PubMed: 18354708]
27. Kroll HR, Kim D, Danic MJ, Sankey SS, Gariwala M, Brown M. A randomized, double-blind, prospective study comparing the efficacy of continuous versus pulsed radiofrequency in the treatment of lumbar facet syndrome. J Clin Anesth. 2008; 20:534–537. [PubMed: 19041042]
28. Ozsoylar O, Akcali D, Cizmeci P, Babacan A, Cahana A, Bolay H. Percutaneous pulsed radiofrequency reduces mechanical allodynia in a neuropathic pain model. Anesth Analg. 2008; 107:1406–1411. [PubMed: 18806060]
29. Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain. 1992; 50:355–363. [PubMed: 1333581]
30. Kim D-S, Figueroa KW, Li K-W, Boroujerdi A, Yolo T, Luo ZD. Profiling of dynamically changed gene expression in dorsal root ganglia post peripheral nerve injury and a critical role of injury-induced glial fibrillary acidic protein in maintenance of pain behaviors. Pain. 2009; 143:114–122. [PubMed: 19307059]
31. Valder CR, Liu JJ, Song YH, Luo ZD. Coupling gene chip analyses and rat genetic variances in identifying potential target genes that may contribute to neuropathic allodynia development. J Neurochem. 2003; 87:560–573. [PubMed: 14535940]
32. Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. J Neurosci. 2001; 21:1868–1875. [PubMed: 11245671]
33. Aksu R, Ugur F, Bicer C, Menku A, Guler G, Madenoglu H, Canpolat DG, Boyaci A. The efficiency of pulsed radiofrequency application on L5 and l6 dorsal roots in rabbits developing neuropathic pain. Reg Anesth Pain Med. 35:11–15. [PubMed: 20048653]
34. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994; 53:55–63. [PubMed: 7990513]

35. Littell, R.; Milliken, G.; Stroup, W.; Wolfinger, R.; Schabenberger, O. SAS for Mixed Models. Second Edition ed.. Cary, NC: SAS Institute Inc; 2010.
36. Ferguson C. An Effect Size Primer: A Guide for Clinicians and Researchers. Professional Psychology: Research and Practice. 2009:1–7.
37. Wall PD, Devor M. Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. Pain. 1983; 17:321–339. [PubMed: 6664680]
38. Howe JF, Loeser JD, Calvin WH. Mechanosensitivity of dorsal root ganglia and chronically injured axons: a physiological basis for the radicular pain of nerve root compression. Pain. 1977; 3:25–41. [PubMed: 195255]
39. Van Zundert J, Harney D, Joosten EA, Durieux ME, Patijn J, Prins MH, Van Kleef M. The role of the dorsal root ganglion in cervical radicular pain: diagnosis, pathophysiology, and rationale for treatment. Reg Anesth Pain Med. 2006; 31:152–167. [PubMed: 16543102]
40. Lacroix-Fralish ML, Tawfik VL, Tanga FY, Spratt KF, DeLeo JA. Differential spinal cord gene expression in rodent models of radicular and neuropathic pain. Anesthesiology. 2006; 104:1283–1292. [PubMed: 16732101]
41. Podhajsky RJ, Sekiguchi Y, Kikuchi S, Myers RR. The histologic effects of pulsed and continuous radiofrequency lesions at 42 degrees C to rat dorsal root ganglion and sciatic nerve. Spine (Phila Pa 1976). 2005; 30:1008–1013. [PubMed: 15864151]
42. Protasoni M, Reguzzoni M, Sangiorgi S, Reverberi C, Borsani E, Rodella LF, Dario A, Tomei G, Dell'Orbo C. Pulsed radiofrequency effects on the lumbar ganglion of the rat dorsal root: a morphological light and transmission electron microscopy study at acute stage. Eur Spine J. 2009; 18:473–478. [PubMed: 19172311]
43. Tun K, Cemil B, Gurcay AG, Kaptanoglu E, Sargon MF, Tekdemir I, Comert A, Kanpolat Y. Ultrastructural evaluation of pulsed radiofrequency and conventional radiofrequency lesions in rat sciatic nerve. Surg Neurol. 2009
44. Erdine S, Bilir A, Cosman ER, Cosman ER Jr. Ultrastructural changes in axons following exposure to pulsed radiofrequency fields. Pain Pract. 2009; 9:407–417. [PubMed: 19761513]
45. Van Zundert J, de Louw AJ, Joosten EA, Kessels AG, Honig W, Dederen PJ, Veening JG, Vles JS, van Kleef M. Pulsed and continuous radiofrequency current adjacent to the cervical dorsal root ganglion of the rat induces late cellular activity in the dorsal horn. Anesthesiology. 2005; 102:125–131. [PubMed: 15618796]
46. Hamann W, Abou-Sherif S, Thompson S, Hall S. Pulsed radiofrequency applied to dorsal root ganglia causes a selective increase in ATF3 in small neurons. Eur J Pain. 2006; 10:171–176. [PubMed: 16310722]
47. Higuchi Y, Nashold BS Jr, Sluijter M, Cosman E, Pearlstein RD. Exposure of the dorsal root ganglion in rats to pulsed radiofrequency currents activates dorsal horn lamina I and II neurons. Neurosurgery. 2002; 50:850–855. discussion 6. [PubMed: 11904038]
48. Pakhomov AG, Doyle J, Stuck BE, Murphy MR. Effects of high power microwave pulses on synaptic transmission and long term potentiation in hippocampus. Bioelectromagnetics. 2003; 24:174–181. [PubMed: 12669300]
49. Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003; 26:696–705. [PubMed: 14624855]

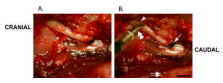

Figure 1. Placement of a radiofrequency electrode adjacent to the left L5 dorsal root ganglion in a rat

Images showing the relative locations of L5 dorsal root ganglia (DRG), L5 spinal nerve ligation site (A) and placement of a radiofrequency electrode adjacent to the L5 DRG (B). The images do not represent the actual surgery performed, but were designed to show the size and location relationships among the pulsed radiofrequency (PRF) probe, L5 DRG and L5 spinal nerve ligation site in a wider surgical view from a postmortem mock-up. Since both images were similar, the labels and scale bar were presented on Panel B only. Arrow – L5 spinal nerve ligature. Arrow head – L5 DRG. Double arrow heads – PRF probe tip. Double arrows – tip of the Iliac crest. Scale bar = 2 mm

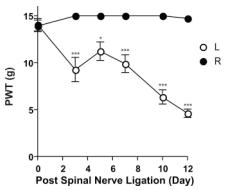

Figure 2. Allodynia development after left L5 spinal nerve ligation (SNL)

Paw withdrawal thresholds (PWT) to von Frey filament stimulation was tested at designated times before and after injury as shown. Two-way ANOVA analysis with Bonferroni post-tests was used to assess the effects of SNL on behavioral hypersensitivity. Statistical significance was indicated by p value < 0.05.

*p < 0.05, ***p < 0.001. L – left (ligation) side; R – right (contralateral) side.

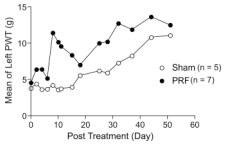

Figure 3. Allodynia recovery after pulsed radiofrequency (PRF) dorsal root ganglia (DRG) neuromodulation

Left hindpaw withdrawal thresholds (PWT) to von Frey filament stimulation were tested after unilateral PRF to the L5 dorsal root ganglia (DRG) of spinal nerve-ligated rats as described in Table 1. Data shown represent the means in the injury (left) side from the number of animals in each group as indicated. Statistical analysis of the data is shown in Figure 4. Behavioral testing data after Day 51 are not shown due to complete recovery of allodynia in both groups.

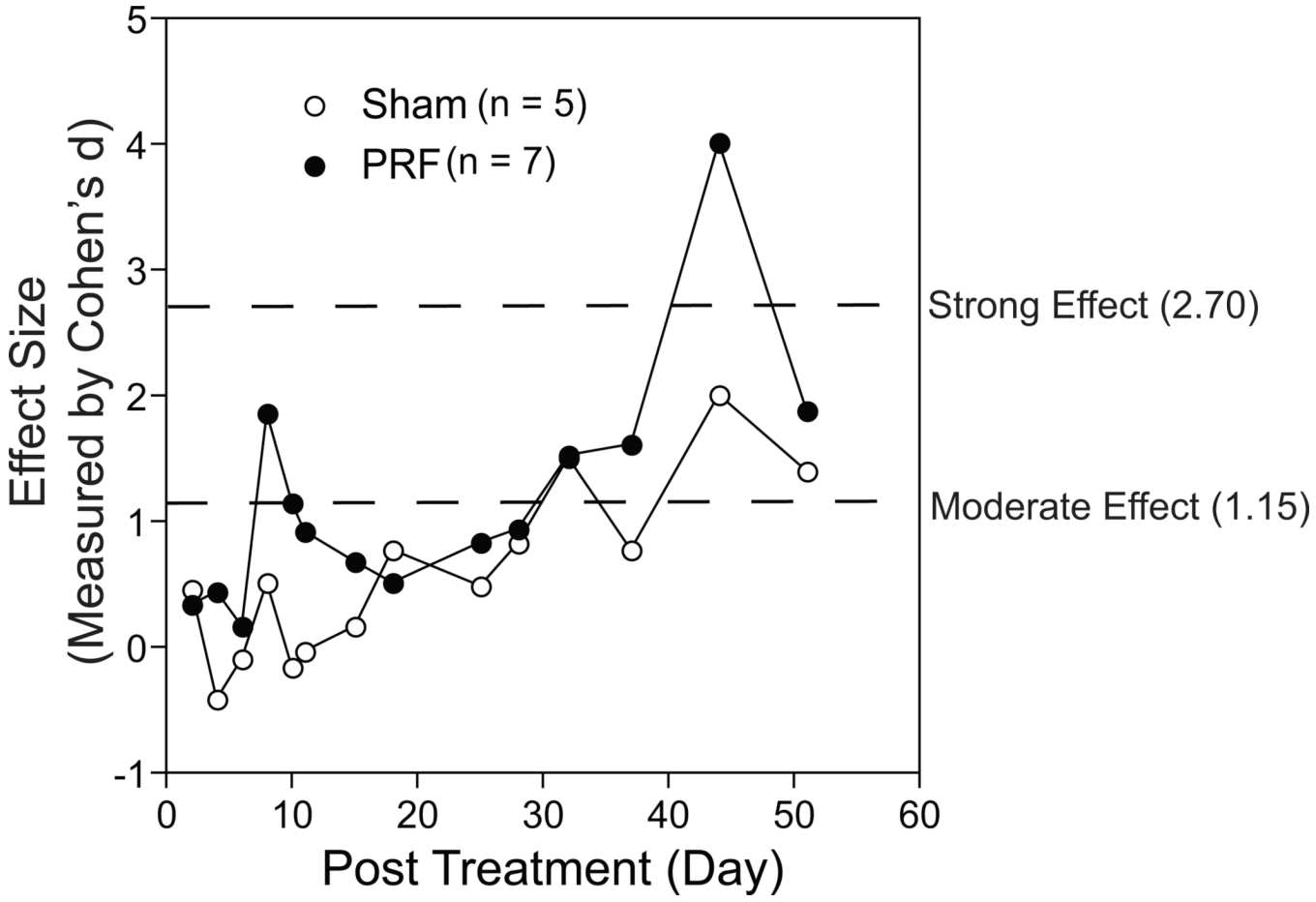

Figure 4. Effect-size measured by Cohen's d

Left hindpaw withdrawal thresholds (PWT) to von Frey filament stimulation were tested after unilateral pulsed radiofrequency (PRF) to the L5 dorsal root ganglia (DRG) of spinal nerve-ligated rats as described in Table 1. Data shown represent the effect-size of the PWT analyzed with Cohen's d method as described. The number of animals in each group is indicated. Dashed lines represent the levels of a moderate (effect-size more than 1.15) or strong (effect-size more than 2.70) effect, respectively.

Table 1

Study Timeline for Experimental Design.

	Day 0–6	**Day 7**	**Day 8**	**Day 9–10**	**Day 11–20**	**Day 21/22**	**Day 24–75**
Group 1 (n=7)	Acclimation	Baseline Test	Left L5 SNL	Recovery	Allodynia Testing *As shown in* Figure 2	Pulsed RF Left L5 DRG	Allodynia Testing *As shown in* Figure 3
Group 2 (n=5)	Acclimation	Baseline Test	Left L5 SNL	Recovery	Allodynia Testing *As shown in Figure 2*	Sham RF Left L5 DRG	Allodynia Testing *As shown in Figure 3*

dorsal root ganglia (DRG)
radiofrequency (RF)
spinal nerve ligation (SNL)