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Abstract

Pulsed-radiofrequency neuromodulation (PRF) is a pain management technique that involves placing a needle electrode near nerves and generating electrical current pulses in order to modulate the transduction of somatosensory information through those nerves. This technique evolved from a similar radiofrequency (RF) procedure in which constant current is distributed to a nerve or neural structure. RF interrupts nerve conduction and prevents somatosensory information from reaching the brain. In the case of continuous radiofrequency, however, the destructive lesion can cause further complications and unwanted side effects. According to research, PRF, unlike RF, is non-destructive yet still induces analgesia and consequently represents a more advantageous technique. Only a handful of previous studies have attempted to determine the neural effects of PRF. The current study seeks to develop an animal model of PRF using the spared nerve injury model (SNI) and, through molecular analysis of neurological tissues harvested from rats, examines mechanisms by which PRF causes analgesia. The study found that there was a significant difference between the SNI lesion model groups and the groups that did not receive the SNI lesion model. However, for the rats with SNI lesions, the analgesic effects of PRF appear to be inconclusive.

Pulsed Radiofrequency Neuromodulation of Peripheral Nerve Injury

Pain is a universal sensation that is unavoidable in the course of a lifetime. Acute pain is a term which applies to the immediate experience of pain. However, if a specific pain is experienced for more than three months, then it is known as chronic pain (Turk and Okifuji, 2001). A type of chronic pain known as neuropathic pain is a debilitating condition which seriously impacts a patient's quality of life. Neuropathic pain is the result of injury to the peripheral or central nervous system, and the resulting barrage of pain messages makes the condition overwhelming and difficult to manage (Bonica, 1992; Millan 1999). As a result of the enhanced transmission of pain signals, noxious stimuli are perceived as even more painful—a condition known as hyperalgesia—and normally harmless stimuli, such as brushing up against clothing, elicit pain—a circumstance known as allodynia (Luongo, 2009). Neuropathic pain has been a subject of study for many years and numerous methods to relieve a patient's suffering have been investigated. One discovery was the surgical technique of radiofrequency (RF) which lessens the experience of pain by damaging the nerve and preventing transmission of stimuli. However, further research discovered that pulsed radiofrequency (PRF) has been found to be an alternative technique that reduces pain with moderately high voltages near the nerve and it does not produce any significant lesion (Wu and Groner, 2007). The purpose of this study is to test whether PRF will significantly reduce chronic pain.

Pain has a purpose: it is fundamental for protecting the body and preventing further tissue damage. Signals sent to the brain and spinal cord indicate when damage is taking place so the person or animal can react appropriately to the situation. Sharp, immediate pain, known as acute pain, encourages the body to avoid the injurious stimulus. Pain travels through C-fibers in the ascending neural pathways through the spinal cord into the brain (Casals-Diaz, Vivo and

Navarro, 2009). Sometimes pain is persistent until the damaged tissue is healed; this continued pain results because of activity-dependent functional plasticity in the spinal dorsal horn that persists beyond the stimulus to remind the person or animal to protect the wounded area (Woolf and Decosterd, 1999). However, there are times when the injured area continues to experience pain even though the damaged tissue has healed. This continuing, chronic pain renders a person dysfunctional and debilitated and can make daily activities nearly impossible for those suffering from it (Sah, Ossipov, and Porreca, 2003).

The study of chronic pain has largely focused on how to treat its effects or, at the very least, lessen its severity. Historically, one of the first techniques used to treat the condition was the use of drugs such as opiates. These drugs are effective in lessening the perception of pain, yet they often leave the patient with many significant negative side effects such as drowsiness and addiction (Rosenblum, Marsch and Joseph, 2008). Subsequently, surgery became a viable solution to improving chronic pain. Physicians attempted to resolve the pain by surgically locating a problem and attending to its dysfunction.   As surgical techniques and knowledge of the functions and interactions of physical structures improved, increasingly sophisticated procedures were developed.

One promising category of procedures involved applying electrical and thermal stimulation to interfere with the transmission of pain signals. The first of these procedures to be developed that met with some success is radiofrequency (RF), which involves inserting a small needle with an electrically active tip into a nerve and activating the tip continuously for short intervals at high temperatures approximately 75°C or higher (Ruiz-Lopez and Erdine, 2002). This procedure was first used as a neuroablative technique and the heat produced by the needle was administered until the cells of the nerve that were causing the chronic pain were destroyed

(Ischia, Luzzani, Ischia and Maffezzoli, 1984; Bogduk, 2006). Slappendel et al. (1997) demonstrated that the use of radiofrequency was shown to reduce pain significantly. Thus, the success of this technique allowed patients to have a new treatment option for their pain. Nevertheless, although this procedure lessened pain, it did have some complications. Radiofrequency uses high temperatures on the tissues which produce thermal lesions on the nerve and result in both neuroablation and deafferenation (Abejon and Reig, 2003). For this reason, radiofrequency has been shown to work, and work well, when larger lesions are desired (Kapural, et al., 2008). Nevertheless, changes were made to the procedure to improve upon its technique. Researchers subsequently discovered that the nerve did not need to be completely destroyed for subjects to get relief from the pain. By changing the procedure so that the temperature was 67°C instead of 80-90°C at the tip of the needle, they were able to prevent total destruction of the nerve (Bogduk, 2006).

In 1997, there was a fundamental shift in the approach to radiofrequency, and new insight into the way radiofrequency was thought to produce its therapeutic effect. Slappendel et al. (1997) demonstrated that patients receiving radiofrequency at 40°C and 67°C had no significant difference between their levels of pain relief. This experimental result indicated that another mechanism was responsible for radiofrequency's previous success and it was not a consequence of the thermal lesion. Thus, it was inferred that the electrical current was the source of the therapeutic effects and the approach to radiofrequency was significantly changed. The new hypothesis was that using bursts of electrical current and allowing time between each burst, so that the heat produced would have time to dissipate through the tissue and not damage the nerve, would still provide the electrical field that would result in pain relief (Bogduk, 2006). This

theoretical supposition gave rise to the method of treatment known as pulsed radiofrequency neuromodulation.

Pulse radiofrequency neuromodulation (PRF) was a modification of the older radiofrequency technique. The method of action of PRF is identical to that of conventional radiofrequency therapy. Both treatments employ an insulated needle that is only activated at the tip, and in both techniques heat and an electric current pass through the needle tip. The resulting electric field is the movement of the electrons within the tissues when they are exposed to the charge produced by the needle's tip. However, one of the crucial differences between the two approaches involves the temperature required to create a reversible lesion: lesions created by temperatures above 45°C produce irreversible injury, whereas lesions produced between 42°C and 44°C are reversible (Abejon and Reig, 2003). It is the "pulsed" component of PRF that prevents the heat of the insulated needle from reaching temperatures that would destroy the nerve. Because the pulses are administered with a pause between each one, the heat can dissipate by thermal conductance into the surrounding area, and therefore only mild lesions are created, making PRF safer and consequently preferable to radiofrequency (Abejon and Reig, 2003; van Boxem et al., 2008; Tun et al., 2009; Liliang et al., 2009). There is a tremendous advantage to PRF because of the remarkable decrease in aversive side effects; there is no indication of significant damage done to the motor and sensory nerves after the application of PRF (Slappendel et al., 1997). The reason for this improvement is that PRF provides treatment to the C-fibers and spares large, myelinated fibers, keeping them intact and preventing deafferentation syndromes (Abejon and Reig, 2003).

By definition, neuropathic pain results from a lesion of the nervous system (Campbell and Meyer, 2006). Peripheral neuropathic pain is produced by the interaction of multiple

physiological mechanisms which operate at different times and in diverse sites. PRF has significantly reduced chronic pain for patients in a clinical setting (Wu and Groner, 2007), yet clinical studies cannot always provide the same level of control and continued participation as experimental, empirical studies. In the attempt to create an animal model of such pain, most experimental investigations use a combination of injured and uninjured nerves (Decosterd and Woolf, 2000). Partial sciatic nerve injury (SNI) was designed to produce a sensation in the rat that is similar to the sensation of pain experienced by human beings (Kim and Chung, 1992; Decosterd and Woolf, 2000). In the SNI model, the tibial and common peroneal nerves, which are two of the three terminal branches of the sciatic nerve, are severed (lesioned), but the third branch of the nerve, the sural nerve, is left intact (Chiasson, R.B., 1958). The result of SNI is the production of consistent, enduring hypersensitivity in the area surrounding the spared sural nerve (Bourquin et al., 2005). An experimental SNI model performed on mice suggests that injuring only the tibial nerve results in mechanical hypersensitivity, yet injuring both the peroneal and sural nerves does not result in hypersensitivity to areas surrounding the uninjured tibial nerve (Bourquin et al., 2005). Therefore, the SNI model seems to show the most promise for future experimental investigation.

Several experimental studies have demonstrated that SNI alters the sensitivity to thermal and mechanical stimuli (Casals-Diaz, Vivo and Navarro, 2009; Baliki, Calvo, Chialvo and Apicarian, 2005). A range of mechanical stimuli, such as Von Frey probes and pinpricks, and a variety of thermal applications, both hot (hot plates) and cold (cold plates and the application of acetone) have been tested on rats (Dowdall, Robinson and Meert, 2004; Baliki, Calvo, Chialvo and Apicarian, 2005). These experiments were designed to elicit escape behaviors in the rat, such as lifting the injured paw and moving away from the thermal plate, since such actions are

evidence of a reduced pain threshold (Baliki, Calvo, Chialvo and Apicarian, 2005). Further investigations of mechanical and thermal sensitivity should provide evidence from which a model of peripheral neuropathic pain in rats could be constructed.

One way to observe the neurological effects of chronic pain is to examine and analyze tissues of rats for altered gene expression. Similar to the process in people, when a rat experiences chronic pain, glial activation marker cells alter their gene expression and release tumor necrosis factor-α, interleukin-1β, and interleukin-6 at the injured area; each of these cytokines plays an important role in chronic pain (Cunha, Poole, Lorenzetti, and Ferreira, 1992). There is substantial support for the relationship of tumor necrosis factor-α and neuropathic pain, as evidenced by a correlation between the expression level of tumor necrosis factor-α and the development of allodynia or hyperalgesia in neuropathic pain models (DeLeo, Sorkin and Watkins, 2007). Interleukin-6 is also expressed during chronic pain and elevated serum levels have been identified in patients who have disorders associated with hypersensitivity and tenderness in the tissues, such as neuropathies, burn injuries, and autoimmune and chronic inflammatory conditions (DeLeo, Sorkin and Watkins, 2007). Additionally, interleukin-1β, is a potent proinflammatory cytokine which is apparently involved in neuropathic pain (Sommer et al., 1999; Schafers et al., 2001). Interleukin-1β is secreted in circumstances that are linked to medical conditions that are accompanied by increased pain and hyperalgesia (Watkins et al., 1999). Interestingly, for the purposes of experimental research, studies have shown that gene expression of interleukin-1β is increased in the sciatic nerve 7 days after nerve injury, timing which coincides with peak thermal hyperalgesia (DeLeo, Sorkin and Watkins, 2007). This peak in interleukin-1β provides an ideal time frame for behavioral testing in experiments on rats.

The tissues which are most likely to reveal higher levels of gene expression associated with the experience of chronic pain are located near the injury site. For the purposes of this study, those are the sciatic nerve, left and right dorsal root ganglion as well as the spinal cord. The altered expression of the genes will further indicate that the rat was in a state of chronic pain.

Empirical studies conducted on PRF have found significant results for its analgesic properties. However, there has not been a study that has observed PRF's beneficial effects with a SNI lesion model on rats. Thus, the current study will enhance the knowledge of PRF and contribute to the present literature.

The present study tested PRF on the sciatic nerve of rats using the SNI model (Decosterd and Woolf, 2000) to observe their responses to pressure exerted on the plantar surface of each hind paw using a Specialized Force Transducer. Acetone was also used on the hind paws to test for indications of thermal hypersensitivity. Rats in the experimental group had an initial surgery to create the SNI model and a second surgery with PRF stimulation. After the initial surgery the rats had behavioral testing done on days 1, 3 and 7. On the 7th day after the behavioral testing the rats had their second surgery. The rats had behavioral testing on days 8, 10 and 14 after the initial surgery (see Figure 1). On day 14 the tissues of the rats were harvested to test for interleukin-1β, interleukin-6, and tumor necrosis factor-α as further evidence of pain. A repeated measure ANOVA was used to statistically test for significant results. This study attempted to demonstrate that rats receiving PRF stimulation had a significant increase in pain relief as compared to the group not receiving PRF stimulation.

Method

Subjects

Eighteen white Sprague-Dawley male laboratory rats weighing approximately 250 grams at the beginning of the experiment were tested during the course of the research. Each rat was allowed food and water *ad libitum* and their weights were monitored. Care for the rats followed the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, D.C., 1996) as well as the protocols of Illinois Wesleyan's Institutional Animal Care and Use Committee (IACUC). Each rat was individually housed and the temperature was kept at 70° Fahrenheit with a light:dark cycle of twelve hours.

Procedure

Initial Surgery. Isoflurane gas was initially administered at 0.04 Liters/minute to put the rats to sleep, and the rats were then given 0.03-0.035 L/m isoflurane during the course of the surgery. While unconscious, each rat was placed on an isothermal heating pad to keep its body temperature at its normal baseline. The neuropathic pain model used in the experiment was the Spared Nerve Injury model (Decosterd and Woolf, 2000), which requires a small lesion above the sciatic nerve of the left leg of the rat. In the initial surgery, the skin of each rat was shaved on the left leg over the sciatic nerve and sterilized with a betadine scrub to prevent infection before the opening incision was made. An incision one inch long on the left-hindlimb was made, exposing the biceps femoris and gluteus superficialis muscles which were then gently separated with tissue spreaders to keep the skin and muscle apart and expose the sciatic nerve. Once the sciatic nerve was located, it was either lesioned by use of a scalpel for the experimental group (N = 10) or spared to serve as the control group (N = 8). After lesioning, the skin was stapled shut and the rat was allowed to recover in its cage.

Initial Behavioral Testing. Data was recorded on days 1, 3, and 7 after the first surgery. In order to remove any biases in data collection and to protect the validity of the research, data

collectors were blind as to which rats had their sciatic nerve lesioned and which rats did not. For the data collection, the rats were placed in cages that have elevated wire mesh floors to allow the data collector to go below the cage and have access to the plantar surface of both the ipsilateral and contralateral hind paw. Each rat was allowed 15-30 minutes to acclimate to the new environment before data collection began. Von Frey filaments (IITC Life Science Incorporated, Woodland Hills CA) were applied to the plantar surface of the rat's hind paw; the filaments were connected to an Electric von Frey Anesthesiometer, Model 2390 Series (IITC Life Science Incorporated, Woodland Hills, CA), otherwise known as a specialized force transducer, which measured the amount of force each of the individual rat's hind paws was able to withstand before the rat raised the paw to relieve the pressure. Responses from the right hind leg and left hind leg of each rat were recorded with three Von Frey probes of varying rigidities: Filament 1 with a flex force of 10.4 grams (least rigid), Filament 2 with a flex force of 37.3 grams (semi-rigid) and Filament 3 with a flex force of 131.4 grams (most rigid). Flex force is the amount of pressure the filament can sustain before it will bend. Each of the probes measured the pressure six times on each paw for each probe; thus both the right hind paw and left hind paw were probed 18 times per testing session. One minute of recovery time was allowed between recordings, and the data collection alternated between the left and right paw.

The SNI pain model also creates a potential hypersensitivity to thermal stimulation. To assess the effects of thermal hypersensitivity on the rat's behavior, acetone was applied to each paw a total of six times per day of testing, three tests per hind paw. As the acetone evaporates it creates a cooling sensation that the rats may perceive. The rat was judged to have responded to the acetone if it moved its paw away quickly in a time interval of about one second from the

source of the stimulus or if the rat started to lick its paw, which is a normal behavior rat's exhibit in response to a painful stimulus.

Second Surgery. The second surgery took place seven days after the initial surgery. The same procedures of the initial surgery were implemented to prevent infection and aid in recovery. The staples from the initial surgery were removed, and the skin was gently pulled apart at the site of the incision as dead skin was carefully cut away. Identical procedures were implemented from the initial surgery to locate and expose the sciatic nerve. However, for this second surgery, the pulse radiofrequency probe was placed on the nerve of every rat in both the control and experimental groups. Of the four groups of rats, the two groups exposed to the PRF stimulation are designated "experimental," and the two groups not receiving the stimulation make up the "control" component of the study. All rats were fitted with the PRF probe. However, the pulse radiofrequency stimulation was delivered only to the 6 rats in the SNI-PRF experimental group (rats which were lesioned and received PRF) and to the 4 rats in the Sham-PRF experimental group (rats which were not lesioned and received PRF). The 4 rats in the SNI-Sham control group (rats which were lesioned but received no PRF) and the other 4 rats in the Sham-Sham control group (rats which were not lesioned and received no PRF) received no stimulation. The pulse radiofrequency probe was placed medial to the lesion and lateral to the spinal cord of the damaged nerve. The PRF probe delivered 50,000Hz pulses in 20µs (microseconds), at a frequency of two per second. Thus, the stimulus was delivered for 20µs and then turned off for 480µs and then delivered again for 20µs and turned off for 480µs; this electrical pulsing took place in the course of one second. Forty-five volts were administered, and the temperature of the probe approached 42°C. However, it was not permitted to exceed that temperature because excessive heat would damage neurons. Because the voltage required

depends on the impedance provided by the tissue, in order not to exceed the maximum

temperature, the voltage was delivered below 45V on occasion to make sure that the temperature

of the probe did not exceed $42^{o}C$ (Cahana, Van Zundert, Macrea, van Kleef and Sluijter, 2006).

Stimulation was delivered in three intervals of one minute each and the probe was placed in three

different locations on the sciatic nerve. The first stimulation was 0.5mm from the lesion on the

sciatic nerve and moved about 0.5mm proximal (toward the spinal cord) for the second

stimulation and proximal another 0.5mm for the third stimulation.

Second Behavioral Testing. Data was then collected on the 8, 10 and 14th day after the

initial surgery to measure the effects of the pulse radiofrequency on the rats' behavioral

responses to the pressure exerted on the specialized force transducer. The procedures used for

the Initial Behavioral Testing were the same for the Second Behavioral Testing in order to

provide a consistent basis on which to compare the data.

Data Analysis

Tissue Harvest. The tissue harvest took place after the second behavioral testing data was

completed. Rats received an overdose of Isoflurane gas and then were placed in a CO_2 chamber

for several minutes. In this experiment, the sciatic nerve and sections of the spinal cord located

proximal to the L_5 spinal nerve, as well as the left and right dorsal root ganglion that is

associated with the rats' L_5 spinal nerve, were collected in order to analyze the altered gene

expression that was induced by the PRF and to assess the accuracy of the PRF probe's placement

on the nerve. The glial activation marker cells are responsible for releasing interleukin-1β,

interleukin-6, and tumor necrosis factor-α, and their presence is evidence of the altered gene

expression that accompanies the presence of pain. These specific tissues were collected to study

the local effects of the injury site. Once the tissues were removed they were placed into small

vials filled with trireagent, which is a solution that preserves DNA, RNA and protein, and was sent (Illinois State University, Normal IL) to be analyzed for altered gene expression.

<div align="center">Results</div>

Efficacy of SNI Lesion Model

To determine whether the SNI lesion model successfully induced chronic pain sensitization, a series of independent t-tests were performed, comparing SNI lesioned rats and control rats. First the non-lesioned groups were compared to the SNI lesion model groups to determine the efficacy of the SNI model. Overall, there was a significant difference between the non-lesioned groups and the SNI lesion model groups on all three filaments tested, indicating that the initial SNI surgery was successful for creating a state of chronic hypersensitivity for the rats.

As the above indicates, there was a significant difference between the non-lesioned groups and the SNI lesion model groups for Filament 1 (see Figure 2). A paired sample t-test was first run for the behavioral testing of Days 1, 3, and 7 for Filament 1. The Day 1 Sham groups ($M = 34.1$, $SD = 7.9$), had significantly greater pressure values than the Day 1 SNI groups ($M = 20.0$, $SD = 4.3$), $t(6) = 3.626$, $p = 0.011$. The Day 3 Sham groups ($M = 33.0$, $SD = 7.2$), had significantly greater pressure values than the Day 3 SNI groups ($M = 20.3$, $SD = 5.2$), $t(7) = 3.164$, $p = 0.016$. Also, the Day 7 Sham groups ($M = 32.6$, $SD = 7.1$), had significantly greater pressure values than the Day 7 SNI groups ($M = 16.8$, $SD = 4.5$), $t(7) = 4.326$, $p = 0.003$.

There was a significant difference between the non-lesioned groups and the SNI lesion model groups for Filament 2 (see Figure 3). A paired sample t-test was also run for the behavioral testing of Day 1, 3 and 7 for Filament 2. The results for The Day 1 Sham groups ($M = 34.7$, $SD = 6.8$), was not significantly greater in pressure values than the Day 1 SNI groups (M

= 22.9, SD = 11.0), $t(6)$ = 1.873, p = 0.110. The Day 3 Sham groups (M = 33.9, SD = 6.6), had

significantly greater pressure values than the Day 3 SNI groups (M = 20.2, SD = 6.5), $t(7)$ =

3.224, p = 0.015. Also, the Day 7 Sham groups (M = 34.8, SD = 6.4), had significantly greater

pressure values than the Day 7 SNI groups (M = 17.8, SD = 5.3), $t(7)$ = 4.559, p = 0.003.

Additionally, there was a significant difference between the non-lesioned groups and the

SNI lesion model groups for Filament 3 (see Figure 4). A paired sample t-test was run for the

behavioral testing of Day 1, 3 and 7 for Filament 3. The Day 1 Sham groups (M = 34.1, SD =

9.5), was not significantly greater in pressure values than the Day 1 SNI groups (M = 21.6, SD =

6.4), $t(6)$ = 2.198, p = 0.070. The Day 3 Sham groups (M = 37.2, SD = 5.1), had significantly

greater pressure values than the Day 3 SNI groups (M = 20.5, SD = 11.6), $t(7)$ = 3.648, p =

0.008. Also, the Day 7 Sham groups (M = 41.4, SD = 4.7), had significantly greater pressure

values than the Day 7 SNI groups (M = 17.6, SD = 7.0), $t(7)$ = 6.321, p = 0.000.

A paired sample t-test was also conducted to make sure there was a significant difference

between the non-lesioned and SNI groups for the thermal hypersensitivity acetone test. There

was a significant difference between the non-lesioned groups and the SNI lesion model groups

for the thermal hypersensitivity testing, indicating that the Sham groups were less reactive to the

acetone cooling sensation than the SNI lesion model groups (see Figure 5). The Day 1 Sham

groups (M = 0.095, SD = 0.163), did not have a significantly different reaction to acetone than

the Day 1 SNI groups (M = 0.286, SD = 0.230), $t(6)$ = -1.922, p = 0.103. The Day 3 Sham

groups (M = 0.042, SD = 0.118), had a significantly lower reaction to acetone than the Day 3

SNI groups (M = 0.375, SD = 0.214), $t(7)$ = -3.742, p = 0.007. Also, the Day 7 Sham groups (M

= 0.000, SD = 0.000), had a significantly lower reaction to acetone than the Day 7 SNI groups

(M = 0.417, SD = 0.295), $t(7)$ = -3.989, p = 0.005.

Efficacy of PRF Treatment

Once it was established that the SNI model successfully induced chronic pain sensitization, and that the lesioned and non-lesioned groups responded significantly differently to mechanical stimulation, a repeated measures analysis of variance (ANOVA) was used to test whether the PRF application was successful in alleviating the SNI induced chronic hypersensitivity. In order to analyze the data symmetrically, Day 1 (one day after the initial SNI surgery) was compared with Day 8 (one day after PRF treatment). Similarly, Day 3 (three days after the initial SNI surgery) was compared to Day 10 (three days after the PRF treatment). Similarly, Day 7 (seven days after the initial SNI surgery) was compared to Day 14 (seven days after the PRF treatment). See Figure 1 for time frame.

The three sets of daily comparisons of pre- and post-PRF treatment did not reveal significant analgesic effects for Filament 1 (see Figure 6). There was no significant main effect for treatment when comparing Day 1 and Day 8 hypersensitivities, $F(1,13) = 1.486$, $p = 0.245$; nor was there any significant day by treatment interaction effect for Day 1 and Day 8, $F(3,13) = 0.894$, $p = 0.470$. There was no significant main effect for treatment when comparing Day 3 and Day 10 hypersensitivities, $F(1,12) = 2.126$, $p = 0.170$; nor was there any significant day by treatment interaction effect for Day 3 and Day 10, $F(3,12) = 1.454$, $p = 0.276$. There was also no significant main effect for treatment when comparing Day 7 and Day 14 hypersensitivities, $F(1,14) = 0.024$, $p = 0.880$; nor was there any significant day by treatment interaction effect for Day 7 and Day 14, $F(3,14) = 0.829$, $p = 0.500$. Overall, PRF treatment did not significantly reduce the hypersensitivity to Filament 1.

Next, the reaction to Filament 2 was tested for significance. The PRF treatment was not significant in producing the desired analgesic effects while using Filament 2 (see Figure 7).

There was no significant main effect for treatment when comparing Day 1 and Day 8 hypersensitivities, $F(1,13) = 2.461$, $p = 0.141$; nor was there any significant day by treatment interaction effect for Day 1 and Day 8, $F(3,13) = 0.753$, $p = 0.540$. There was no significant main effect for treatment when comparing Day 3 and Day 10 hypersensitivities, $F(1,12) = 0.667$, $p = 0.430$; nor was there any significant day by treatment interaction effect for Day 3 and Day 10, $F(3,12) = 0.447$, $p = 0.724$. There was also no significant main effect for treatment when comparing Day 7 and Day 14 hypersensitivities, $F(1,14) = 0.458$, $p = 0.510$; nor was there any significant day by treatment interaction effect for Day 7 and Day 14, $F(3,14) = 0.252$, $p = 0.859$. Overall, PRF treatment did not significantly reduce the hypersensitivity to Filament 2.

Next, the reaction to Filament 3 was tested for significance. As with the other two probes, application of Filament 3 did not produce any analgesia following PRF treatment (see Figure 8). There was no significant main effect for treatment when comparing Day 1 and Day 8 hypersensitivities, $F(1,13) = 1.061$, $p = 0.322$, however there was a significant day by treatment interaction effect for Day 1 and Day 8, $F(3,13) = 3.722$, $p = 0.039$. There was no significant main effect for treatment when comparing Day 3 and Day 10, $F(1,12) = 0.300$, $p = 0.594$; nor was there any significant day by treatment interaction effect for Day 3 and Day 10, $F(3,12) = 0.922$, $p = 0.460$. There was also no significant main effect for treatment when comparing Day 7 and Day 14 hypersensitivities, $F(1,14) = 1.807$, $p = 0.200$; nor was there any significant day by treatment interaction effect for Day 7 and Day 14, $F(3,14) = 0.252$, $p = 0.859$. Overall, PRF treatment did not significantly reduce the hypersensitivity to Filament 3.

Also, a repeated measures analysis of variance (ANOVA) was used to analyze the PRF treatment for the thermal hypersensitivity acetone test. No significant difference in reactivity to the thermal acetone testing following PRF treatment was observed (see Figure 9). There was no

significant main effect for reactions when comparing Day 1 and Day 8 hypersensitivities, $F_{(1,13)} = 0.005$, $p = 0.946$; nor was there any significant day by reaction interaction effect for Day 1 and Day 8, $F_{(3,13)} = 0.584$, $p = 0.636$. There was no significant main effect for reactions when comparing Day 3 and Day 10 hypersensitivities, $F_{(1,12)} = 0.028$, $p = 0.871$; nor was there any significant day by reaction interaction effect for Day 3 and Day 10, $F_{(3,12)} = 0.286$, $p = 0.835$. There was also no significant main effect for reactions when comparing Day 7 and Day 14 hypersensitivities, $F_{(1,14)} = 2.364$, $p = 0.146$; nor was there any significant day by reaction interaction effect for Day 7 and Day 14, $F_{(3,14)} = 0.017$, $p = 0.997$. Overall, PRF treatment did not significantly reduce the hypersensitivity to the acetone test.

<p style="text-align:center">Discussion</p>

The primary purpose of the present study was to determine whether PRF produces analgesic effects on rats with SNI lesions. The study hypothesized that SNI-lesioned rats receiving PRF stimulation would have a significant increase in pain relief as compared to the SNI-lesioned group that was not receiving PRF stimulation. Three different Von Frey filaments were applied to test the pressure pain-threshold on each of the 18 rats in the experiment, and thermal hypersensitivity was also analyzed by using acetone to cool the plantar surface of the rats' paws. The data collected for each filament did not attain a level of significance: PRF stimulation on SNI-lesioned rats did not appear to provide substantial relief from pain. Data collected on thermal hypersensitivity produced similar results.

The experiment showed that in both SNI groups, the lesion created a state of chronic hypersensitivity. However, for Filament 2, when Day 1 non-lesioned groups were compared to Day 1 SNI groups, the data indicated that these groups were not significantly different from each other in creating the desired chronic hypersensitivity. This was also the case for Filament 3 and

the acetone test as well. This effect was expected, because it usually takes more than twenty-four hours for the SNI pain model to develop (Woodroofe et al, 1991). Overall, however, for Filament 1 there was a significant difference between the non-lesioned groups and the SNI groups for each day. This consistent difference was observed for Filament 2 and 3 as well; there was a significant difference between the SNI model and the rats that did not receive the SNI lesion. However, for Filaments 1, 2 and 3, as well as for the thermal hypersensitivity data, the experiment found that PRF did not produce the desired analgesic effects with the induced SNI lesion model. The results of the PRF stimulation are inconclusive with the SNI lesion model in rats.

The study's hypothesis was based on the results of previous research: both clinical and empirical studies conducted on PRF have found significant results for its analgesic properties (Liliang et al., 2009; Kapural et al., 2008; Wu and Groner, 2007; Bogduk, 2006; Li, Yang, Meyerson, and Linderoth, 2006). Since there was some evidence that the result of SNI is the production of consistent, enduring hypersensitivity in the area surrounding the spared sural nerve (Bourquin et al., 2005), and since clinical studies cannot always provide the same level of participation as experimental, empirical studies, the next logical step seemed to be a study such as this one that observed PRF's beneficial effects with a SNI lesion model on rats. In spite of the inconclusive results for PRF, this study does support previous findings that the SNI lesion model produces observable escape behaviors in the rat, such as lifting the injured paw and moving away from the cold stimulus, thus providing further evidence of a reduced pain threshold. This research therefore suggests that the SNI model holds promise for the investigation of chronic pain, and contributes to the present literature. Further investigations of mechanical and thermal

sensitivity should provide evidence from which a model of peripheral neuropathic pain in rats could be constructed.

The literature indicates that the SNI lesion model should produce chronic hypersensitivity in the rats, and the results of the current experiment are consistent with the literature and further support the theory that this pain model accurately creates a state of chronic hypersensitivity to both mechanical and thermal stimuli. However, the results of the PRF stimulation did not match the expectations created by previous research. According to the literature, PRF stimulation should be able to relieve peripheral neuropathic pain, including that induced by the SNI lesion model, because the SNI model is a specific type of peripheral nerve injury and PRF has lessened the severity of chronic peripheral nerve injury pain according to research conducted in the past. Perhaps the results of the PRF stimulation of the SNI model were inconclusive in this study because there was an insufficient amount of current that was allowed to pass through the tissue of the sciatic nerve. The voltage is restricted for PRF because it depends on the impedance of the tissue and the temperature cannot rise above 42° C or the neural tissue of the sciatic nerve will develop irreversible lesions. However, it would be possible to increase the length of time the current is delivered to the sciatic nerve. It is possible that this change in duration could have altered the results of the study to bring it more in line with previous findings; only further research will be able to answer this question.

After the data had been analyzed, some specific trends appeared which are worth noting. Based on the data, the SNI-PRF group had the lowest pain tolerance to all the filaments and the greatest reactivity to the acetone test as compared to the Sham-Sham, Sham-PRF and SNI-Sham groups. It would appear, therefore, that the PRF treatment is counterproductive with the SNI lesion model. However, there is another trend in the data that is quite interesting. It appears that

the Sham-PRF group had the highest pain tolerance to all the filaments and had the least reaction to the acetone test as compared to the Sham-Sham, SNI-Sham and SNI-PRF groups. Based on this trend, it appears that PRF did indeed generate measurable analgesic effects. If this trend is taken into account, then, as the literature indicates, PRF did indeed produce analgesic effects; perhaps not enough is known about the sciatic nerve to understand why PRF did not create measurable pain relief on the rats with a SNI lesion. These trends and the results of the data collected in this study suggest that PRF should not be used on a lesion similar to a SNI lesion if pain relief is the desired outcome.

Limitations

It is possible that the results were not significant because of limitations of the study. For example, since Woodroofe et al (1991) determined that peak concentrations of the cytokines occur seven days after the onset of injury, it is possible that the 14-day span of data collection could have interfered with the analgesic effects of the PRF stimulation. If the data collection took place in the course of one week for each rat and PRF was delivered in the middle of the week, perhaps the results would be different.

Additionally, there was the limitation of a relatively small sample size; a total of 18 rats may not have been enough to represent the entire population accurately. This limitation is unlikely to have affected the results, however, because the data was collected by two different people, and each set of collections had data that was consistent. Each data collector was also blind to which rats had received the SNI model and PRF stimulation. On the other hand, there was a significant difference in responses between the rats that had the SNI lesion and those with no lesion which indicates that enough rats were used to be able to observe differences between these two groups.

Other limitations that could have affected the results of the study are the placement of the electrodes on the sciatic nerve and the impedance of the tissue (the resistance of the tissues the electrical current passes through). The electrodes may have been placed on the nerve in a way that would limit the amount of voltage that could pass through the tissue; placement is important because the higher the impedance is, the lower the amount of that current passes through the nerve tissue. Therefore, a high impedance in the tissue at the site where the electrodes were applied to the sciatic nerve could potentially decrease the analgesic affect of PRF. Since the mechanism for PRF is unclear, it is unknown if the electrical current is what causes analgesia or if it is some other property of the electromagnetic field that affects the chemical messengers in the sciatic nervous tissue.

Future Research

The information gathered in this study suggests possibilities for further research. One promising option might be to shorten the window of the data collection days. In the current study, the rats had seven days to develop their chronic pain sensation and at the end of the seventh day the PRF was administered. Then seven more days were allowed to study the effects of the PRF stimulation; therefore the entire data collection took place over the course of 14 days for one rat. However, the next research project could shorten the data collection to a total time frame of seven days and the PRF stimulation could be administered in the middle of the week. This altered time frame may produce significant results because the cytokines would be at their peak levels by the end of the seven days after the lesion was induced. If this theory proved accurate, the information would indicate that PRF would need to be utilized before the peak concentrations of the cytokines in order to produce its analgesic effects.

Further research could be conducted on the procedure of PRF delivery. One potentially advantageous change to the procedure of PRF administration would be to deliver the electrical current for a longer duration. The results of the PRF stimulation in the SNI model were inconclusive; this could be because there was an insufficient amount of electrical current that passed through the sciatic nerve tissue. Although in some previous experiments 45 volts were not used in order to prevent further lesions from occurring in the neural tissue, it is possible that in the present experiment occasionally the temperature and impedance of the sciatic tissue prevented the ideal 45 volts from being administered. In this study PRF stimulation was delivered in three intervals of one minute each and the probe was placed in three different locations on the sciatic nerve; however, a different outcome might result from as simple a change as increasing the time that the current is administered to the nerve. Another study will be able to show if this procedural change could potentially lead to significant results.

Further research is also needed to determine whether it is the chemical components in the neurons or the electrical activity that produces the analgesic effects of PRF stimulation. One approach might be to use lidocaine at the site of injury. Lidocaine is a common local anesthetic, and it blocks the chemical activity between neurons (Catteral, W., 2002). Consequently, the study could be restricted so that only the electrical activity from the PRF would have any effect on the neurons. Thus, if lidocaine is applied and PRF produces analgesia, it might be determined that it is the electrical activity that produces a reduction in pain. On the other hand, if lidocaine is used and PRF provides no analgesia, then a reasonable conclusion might be that it is the chemical communication between neurons that is responsible for the pain reduction that has been observed after PRF stimulation.

One planned extension of the present study involves the analysis of the nerve tissue near the lesion of the rats' sciatic nerve to determine whether those tissues reveal higher levels of tumor necrosis factor-α, interleukin-1β, and interleukin-6. The production of these cytokines would indicate that the rats were indeed experiencing the neurological effects of chronic pain, as evidenced by the changes in the altered gene expression. Such a result would further confirm the success of the SNI model as an experimental method for inducing hypersensitivity similar to that caused by chronic pain.

Conclusion

The current study examined the effects of PRF stimulation on rats with surgical SNI lesions. No other empirical study has analyzed these factors in a four-group animal model. While pulsed radiofrequency neuromodulation is a surgical technique that has been used with some degree of success to produce analgesic effects in individuals suffering from chronic neuropathic pain, the current study's experimental design allowed for far more precision than a clinical study would have been able to provide because the sciatic nerve could be lesioned exactly to the required specifications of the experiment. The knowledge gained from the collected data provides confirming evidence that the surgical technique of SNI does induce a state of chronic hypersensitivity, and it suggests that this technique has potential for use in further research. The current study also adds to the literature of PRF stimulation by suggesting that, while PRF may have some mild analgesic effect, it may not be reliable as a treatment for pain resulting from SNI lesions in rats.

The information acquired from this study can now be applied to future research in PRF stimulation. Further research will be necessary to understand under what conditions and through what mechanisms this procedure can produce analgesia; the more that is learned about PRF

stimulation the better the care will be for individuals suffering from chronic pain. This procedure potentially could be used to reduce a large variety of chronic, peripheral neuropathic pains in patients. Chronic pain can be a debilitating condition, and as people live longer their chances of suffering from this pain increases. Because of this age-related component, it is important to learn about the complexities of chronic pain in order to better ensure that it does not consume the lives of patients and other individuals.

References

Abejón, D., & Reig, E. (2003). Is pulsed radiofrequency a neuromodulation

technique? *Neuromodulation, 6*(1), 1-3.

Baliki, M.,Calvo, O. Chialvo, D., & Apkarian, A. (2005). Spared nerve injury rats exhibit

thermal hyperalgesia on an automated operant dynamic escape task. *Molecular Pain, 18* (1),

1-18.

Bogduk, N. (2006). Pulsed radiofrequency. *Pain Medicine, 7*(5), 396-407.

Bonica, J. (1992). Clinical importance of hyperalgesia. In: Willis, W.D. Jr. (Ed.) *Hyperalgesia*

and Alloynia, Vol 1. New York: Raven Press17-43.

Bourquin, A., Suveges, M., Pertin, M., Gilliard, N., Sardy, S., Davison, A., Spahn, D., &

Decosterd, I. (2006). Assessment and analysis of mechanical allodynia-like behavior

induced by spared nerve injury (SNI) in the mouse. *Pain, 122*, 14.e1-14.e14.

Cahana, A., Van Zundert, J., Macrea, L., van Kleef, M., & Sluijter, M. (2006). Pulsed

radiofrequency: Current clinical and biological literature available. *Pain Medicine, 7*(5),

411-423.

Campbell, J., & Meyer, R. (2006). Mechanisms of neuropathic pain. *Neuron*, *52*, 77-92.

Casals-Díaz, L., Vivó, M., & Navarro, X. (2009). Nociceptive responses and spinal plastic

changes of afferent C-fibers in three neuropathic pain models induced by sciatic nerve injury

in the rat. *Experimental Neurology, 217*(1), 84-95.

Catterall, W.A. (2002). Molecular mechanisms of gating and drug block of sodium channels.

Novartis Foundation Symposium, 241, 206-232.

Chiasson, R.B. (1958) *Laboratory anatomy of the white rat.* Dubuque: Wm. C. Brown.

Cunha, F., Poole, S, Lorenzetti, B., & Ferreira (1992).The pivotal role of tumor necrosis factor α

    in the development of inflammatory hyperalgesia. *Br. J. Pharmacology, 107*, 660-664.

Decosterd, I, & Woolf, C.J (2000. Spared nerve injury: An animal model of persistent peripheral

    neuropathic pain. *Pain, 87*, 149-158.

DeLeo, J.A., Sorkin, L.S. & Watkins, L.R. (Eds.). (2007) *Immune and glial regulation of pain.*

    Seattle: IASP Press.

Dowdall, T., Robinson, I., & Meert, T. (2005). Comparison of five different rat models of

    peripheral nerve injury. *Pharmacology, Biochemistry and Behavior, 80*, 93-108.

Ischia, S., Luzzani, A. Ischia, A., & Maffezzoli,G. (1984). Bilateral percutaneous cervical

    cordotomy: Immediate and long-term results in 36 patients with neoplastic disease. *Journal*

    of Neurology, Neurosurgery, and Psychiatry, 47, 141-147.

Kapural, L., Nageeb, F., Kapural, M., Cata, J. P., Narouze, S., & Mekhail, N. (2008). Cooled

    radiofrequency system for the treatment of chronic pain from sacroiliitis: The first case-

    series. *Pain Practice, 8*(5), 348-354.

Kim, S.H., & Chung, J.M. (1992). An experimental model for peripheral neuropathy produced

    by segmental spinal nerve ligation in the rat. *Pain, 50*, 355-363.

Li, D., Yang, H., Meyerson, B. A., & Linderoth, B. (2006). Response to spinal cord stimulation

    in variants of the spared nerve injury pain model. *Neuroscience Letters, 400*(1-2), 115-120.

Liliang, P., Lu, K., Liang, C., Tsai, Y., Hsieh, C., & Chen, H. (2009). Pulsed radiofrequency

    lesioning of the suprascapular nerve for chronic shoulder pain: A preliminary report. *Pain*

    Medicine, 10(1), 70-75.

Luongo, L., Palazzo, E., Tambaro, S., Giordano, C., Gatta, L., Scafuro, M. A., Rossi, F. s.,

    Lazzari, P., Pani, L., de Novellis, V., Malcangio, M., & Maione, S. (2010). 1-(2′,4′-

dichlorophenyl)-6-methyl-N-cyclohexylamine-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide, a novel CB2 agonist, alleviates neuropathic pain through functional microglial changes in mice. *Neurobiology of Disease, 37*(1), 177-185.

Millan, M.J. (1999). The induction of pain: An integrative review. *Progress in Heurobiology 57*(1), 1-164.

Rosenblum, A., Marsch, L. A., Joseph, H., & Portenoy, R. K. (2008). Opioids and the treatment of chronic pain: Controversies, current status, and future directions. *Experimental and Clinical Psychopharmacology, 16*(5), 405-416.

Ruiz-López, R., & - Erdine, S. (2002). *Treatment of cranio-facial pain with radiofrequency procedures* Blackwell Science Inc.

Sah, D. Ossipov, M., & Porreca, F. (2003). Neurotrophic factors as novel therapeutics for neuropathic pain. *Nature Publishing Group, 2,* 460-472.

Schäfers, M., Brinkhoff, J., Neukirchen, S., Marziniak, M., & Sommer, C. (2001). Combined epineurial therapy with neutralizing antibodies to tumor necrosis factor-alpha and interleukin-1 receptor has an additive effect in reducing neuropathic pain in mice. *Neuroscience Letters, 310*(2-3), 113-116.

Slappendel, R., Crul, B. J. P., Braak, G. J. J., Geurts, J. W. M., Booij, L. H. D. J., Voerman, V. F., & de Boo, T. (1997). The efficacy of radiofrequency lesioning of the cervical spinal dorsal root ganglion in a double blinded randomized study: No difference between 40°C and 67°C treatments. *Pain, 73*(2), 159-163.

Sommer, C., Petrausch, S., Lindenlaub, T., & Toyka, K. V. (1999). Neutralizing antibodies to interleukin 1-receptor reduce pain associated behavior in mice with experimental neuropathy. *Neuroscience Letters, 270*(1), 25-28.

Tun, K., Cemil, B., Gurcay, A. G., Kaptanoglu, E., Sargon, M. F., Tekdemir, I., Comert, A., & Kanpolat, Y. (2009). Ultrastructural evaluation of pulsed radiofrequency and conventional radiofrequency lesions in rat sciatic nerve. *Surgical Neurology, 72*(5), 496-500.

Turk, D.C.; Okifuji, A. (2001). Pain terms and taxonomies. In Loeser, D.; Butler, S. H.; Chapman, J.J. et al.. *Bonica's management of pain* (3 ed.). Lippincott Williams & Wilkins. 18–25.

van Boxem, K., van Eerd, M., Brinkhuize, T., Patijn, J., van Kleef, M., & van Zundert, J. (2008). Radiofrequency and pulsed radiofrequency treatment of chronic pain syndromes: The available evidence.*Pain Practice, 8*(5), 385-393.

Watkins, L. R., Hansen, M. K., Nguyen, K. T., Lee, J. E., & Maier, S. F. (1999). Dynamic regulation of the proinflammatory cytokine, interleukin-1β: Molecular biology for non-molecular biologists. *Life Sciences, 65*(5), 449-481.

Woolf, C. J., & Decosterd, I. (1999). Implications of recent advances in the understanding of pain pathophysiology for the assessment of pain in patients. *Pain, 82*(Supplement 1), S141-S147.

Woodroofe, M.N, Sarna, G., Wadhwa, M., Hayes, G. Loughlin, A., Tinker, A., & Cuzner, M. (1991). Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by in vivo microdialysis: evidence of a role for microglia in cytokine production. *Journal of Heuroimmunology, 33*(3) 227-236.

Wu, H., & Groner, J. (2007). Pulsed radiofrequency treatment of articular branches of the obturator and femoral nerves for management of hip joint pain. *Pain Practice, 7*(4), 341-344.

Figure 1. The time frame for testing that each individual rat underwent during the course of the study.
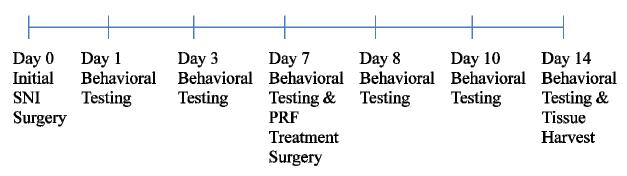

Day 0
Initial
SNI
Surgery

Day 1
Behavioral
Testing

Day 3
Behavioral
Testing

Day 7
Behavioral
Testing &
PRF
Treatment
Surgery

Day 8
Behavioral
Testing
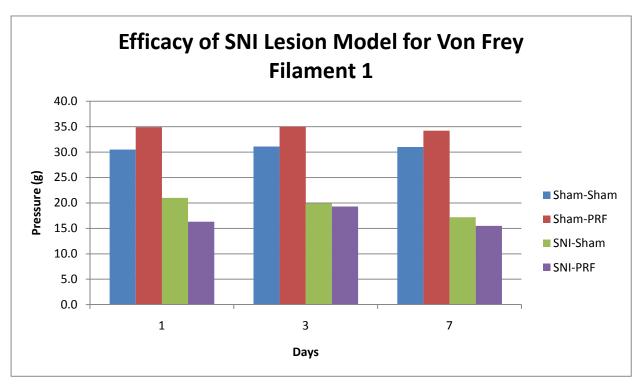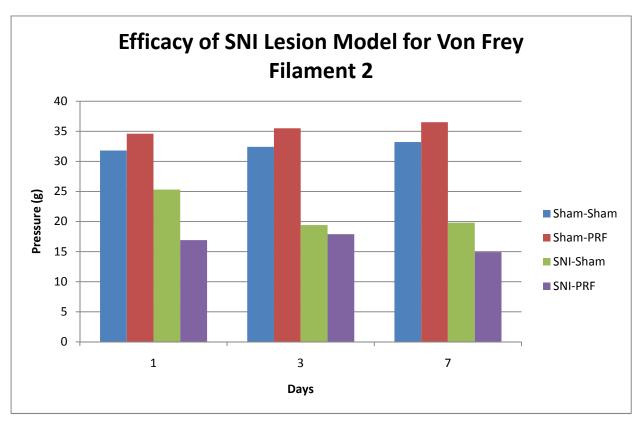
Day 10
Behavioral
Testing

Day 14
Behavioral
Testing &
Tissue
Harvest

Figure 2. There was a significant difference between the SNI lesion model groups and the groups that did not receive the SNI lesion for Filament 1. This indicates that the SNI lesion model effectively put the rats into a state of chronic hypersensitivity.
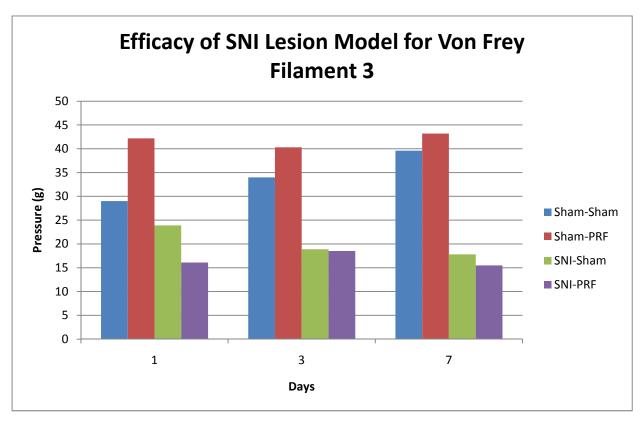
Figure 3. There was a significant difference between the SNI lesion model groups and the groups that did not receive the SNI lesion for Filament 2. This indicates that the SNI lesion model effectively put the rats into a state of chronic hypersensitivity.

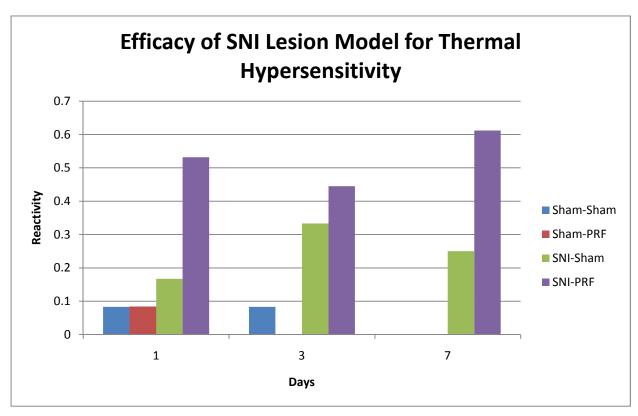Figure 4. There was a significant difference between the SNI lesion model groups and the groups that did not receive the SNI lesion for Filament 3. This indicates that the SNI lesion model effectively put the rats into a state of chronic hypersensitivity.

Figure 5. There was a significant difference between the SNI lesion model groups and the groups that did not receive the SNI lesion for the thermal hypersensitivity Acetone test. This indicates that the SNI lesion model effectively put the rats into a state of chronic hypersensitivity.

Figure 6. For Filament 1 there was no significant main effect for the PRF treatment when comparing Day 1 to Day 8, Day 3 to Day 10, or Day 7 to Day 14 hypersensitivities; nor was there any significant interaction effect for the PRF treatment when the four groups were compared against each other for Day 1 to Day 8, Day 3 to Day 10, and Day 7 to Day 14. Thus, the analgesic effects of PRF appear to be ineffective with the SNI lesion model.
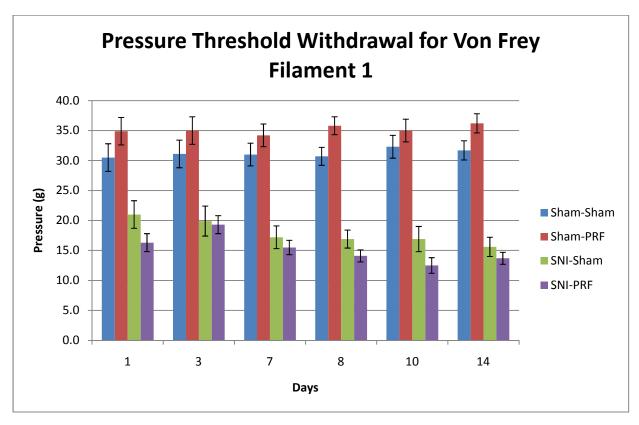
Figure 7. For Filament 2 there was no significant main effect for the PRF treatment when comparing Day 1 to Day 8, Day 3 to Day 10, or Day 7 to Day 14 hypersensitivities; nor was there any significant interaction effect for the PRF treatment when the four groups were compared against each other for Day 1 to Day 8, Day 3 to Day 10, and Day 7 to Day 14. Thus, the analgesic effects of PRF appear to be ineffective with the SNI lesion model.
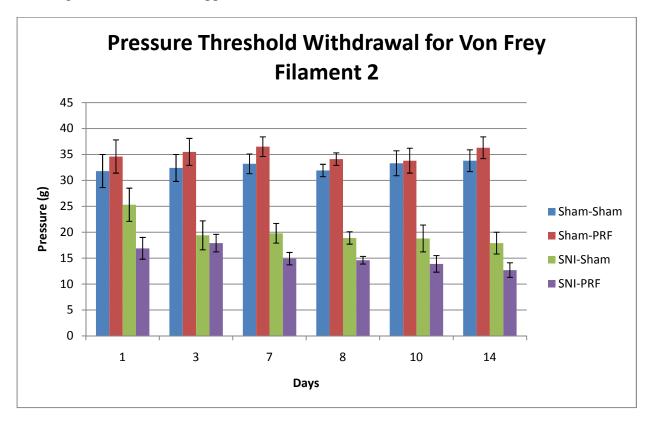
Figure 8. For Filament 3 there was no significant main effect for the PRF treatment when comparing Day 1 to Day 8, Day 3 to Day 10, or Day 7 to Day 14 hypersensitivities; nor was there any significant interaction effect for the PRF treatment when the four groups were compared against each other for Day 1 to Day 8, Day 3 to Day 10, and Day 7 to Day 14. Thus, the analgesic effects of PRF appear to be ineffective with the SNI lesion model.
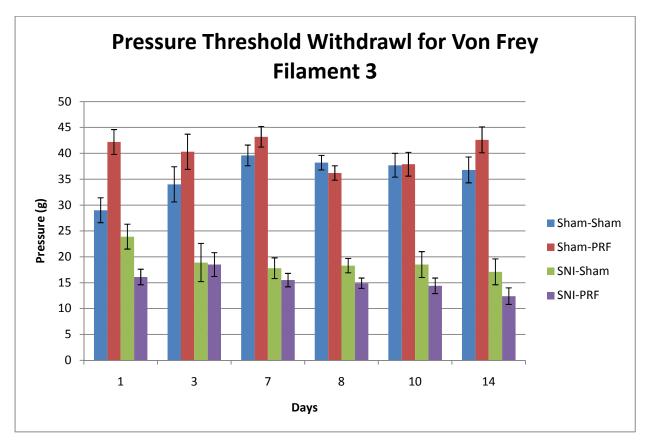
Figure 9. For the thermal hypersensitivity Acetone test, there was no significant main effect for the PRF treatment when comparing Day 1 to Day 8, Day 3 to Day 10, and Day 7 to Day 14 hypersensitivities; nor was there any significant interaction effect for the PRF treatment when the four groups were compared against each other for Day 1 to Day 8, Day 3 to Day 10, and Day 7 to Day 14. Thus, the analgesic effects of PRF appear to be ineffective with the SNI lesion model.