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Effects of Low Amplitude Pulsed Radiofrequency Stimulation with Different Waveform in Rats for Neuropathic Pain*

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***Abstract*— Pulsed-radiofrequency (PRF) electrical stimulation has been widely used for chronic pain treatment. It has been demonstrated with advantages of low temperature over traditional continuous radiofrequency (CRF) lesions with higher amplitude and mono polar electrode to treat pain in clinics (frequency 500 KHz, Pulse duration 20 msec, Amplitude 45 V, Treatment 2 min). We compare the effects of different pulse waveforms and PRF parameters (Pulse duration 25 ms, Treatment duration 5 min, low amplitude of 2.5/1.25 V) with a miniature bi-polar electrode on Dorsal root ganglion (DRG). The pain relief effect due to PRF is evaluated by using Von Frey method for the pain threshold index based on behavior response to mechanical stimulus of various strengths. Experimental results of Von Frey Score show that the sinusoidal group has higher responses than the square wave one. Both fast and secondary expressed proteins of c-fos and pp38 are measured from spinal cord tissue sectioning slides to characterize the pain associated inflammatory responses and their responses due to PRF stimulation.**

I. Introduction

Neuropathic pain is a common neuronal problem in many patients. It occurs from damage or disease affecting the somatosensory system that found in spinal cord injury or even strokes. In many cases, the effective treatment of neuropathic pain is still a very difficult task and requires novel solution for unmet needs. For example, many pain killing drugs are known to have side effects and possible long-term damage. It has been a common practice in clinics to use Radiofrequency (RF) for the treatment of neuropathic pains and associated diseases in recent years. However, even the state of art, i.e. using Pulsed Radio-Frequency (PRF) on Dorsal root ganglion (DRG), the effect can only last for 3-6 months and thus require repeated procedures for some patients [1].

PRF was developed to replace the conventional radiofrequency ablative lesion [2, 3]. High-frequency radiofrequency waves are pulsed to disseminate the heat to avoid thermal injury and potential squeals, such as de-afferentation pain. Accumulating data proved that single PRF treatment at the dorsal root ganglion (DRG) reduced neck and back pain, especially nerve-related radicular pain [4-7].

Clinicians generally agree that PRF is non-destructive, but not many cellular or molecular researches had been done to explore the changes. In summary, histological studies revealed that morphological alterations in nervous tissues following PRF were reversible, which could only be detectable under electron microscopy [8, 9]. PRF stimulation at DRG induces Fos expression in the superficial dorsal horn at early (3 hours) and delayed (7 days) periods, implicating increased spinal neuron activities by stimulation [10, 11]. We have also studied and reported PRF effect by examining behavioral and biomolecular changes [12]. In this report, we compare the effects of different pulse waveforms and PRF parameters (Pulse duration 25 ms, Treatment duration 5 min, low amplitude of 2.5/1.25 V) with a miniature bi-polar electrode on Dorsal root ganglion (DRG). The pain relief effect due to PRF is evaluated by using Von Frey method for the pain threshold index based on behavior response to mechanical stimulus of various strengths.

II. Procedure And Method

A. Components of Electrical stimulation

We have developed a PRF electrical stimulator that used the LabVIEW and PXI-5402 Function Generator (National Instruments, USA) to provide programmable stimulation parameters. These include frequency (f), amplitude (A), waveform, pulsed duration (pd), and period. Fig 1 shows a typical PRF stimulation waveform with amplitude of 1.25/2.5V and sinusoid/square waveform of 500KHz in the pulsed duration of 25ms.

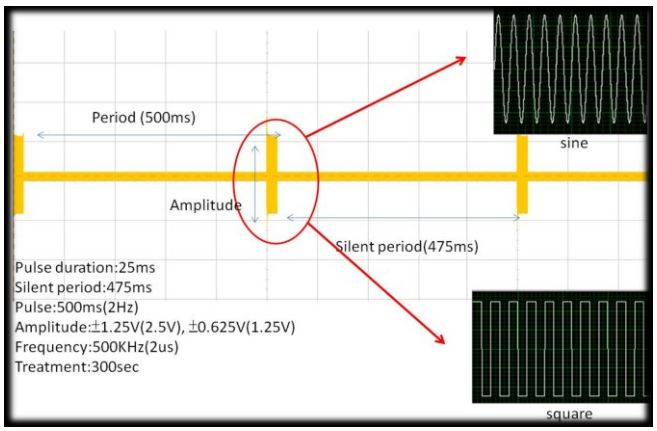

Figure 1. Output of programmable low power pulsed radiofrequency (PRF) waveform.

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Due to rat's DRG anatomical constraint, we have designed and fabricated an electrode probe by bundling two 32G stainless steel needles with heat shrinkable tube, except the distal 3-mm tips. Needle tips were blunted; one of the exposed tips was bended upward, and the other exposed tip was left straight. The bended probe tip can be easily inserted into the foramen cannel and coming to contact with nerves trunk of DRG as a stimulating probe. The straight probe contacts with the surrounding non-neural tissues as a grounding electrode (Fig 2).

Figure 2. An assembled bipolar electrode for DRG stimulation in rat.

B. Animal preparation

Sprague–Dawley rats (BioLASCO, Taiwan co., Ltd.; Taiwan) weight around 225g-250g were used in the animal study. Animals were housed in groups of two in plastic cages with soft bedding and acclimatized to the animal facilities.

C. Animal Operation

All surgical protocols were standardized. Under 2% isoflurane anesthesia, a 2-3 cm skin incision was made between lumbar spine and pelvic bone on the left side. The muscles within the space and L4/L5 spinal nerves were exposed when the L5 transverse process was removed. Ligation of the L5 spinal nerve induced the neuropathic pain (Chung Model) with 6-0 silk [13] (Fig 3).

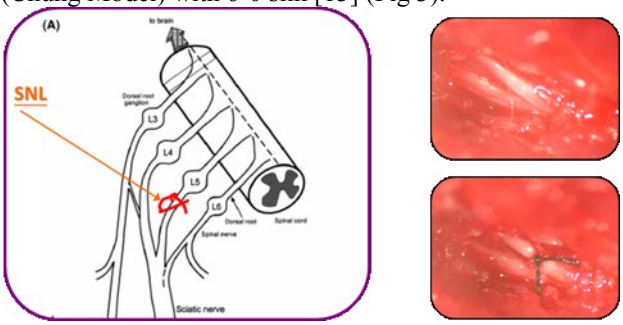

Figure 3. Spinal nerve ligation on the exposed lumbar 5 (L5) nerve trunk.

Electrode crossed L5 nerve to the foramen delivered the designated waveform to the DRG via a pair of bipolar electrodes. We test the proper position of electrode with low frequency stimulation parameter, 2Hz square-wave at a minimal intensity above 0.6-1.2 V, and observe at paw twitch. Started PRF electrical stimulation after confirmed with previously stated procedure after 5 minutes and withdraw the electrode and sutured all tissues with 4-0 chromic.

D. Mechanical allodynia on rat paw

Baseline paw withdrawal threshold (PWT) was measured on left paw using von Frey filaments. After recovery from surgery the rats were returned to their home cage with food and water available ad libitum. To quantify mechanical sensitivity of the foot, foot withdrawal in response to mechanical stimuli was measured as described previously. Mechanical stimuli were applied with von Frey filament. The rat was placed in a transparent cage with metal mesh floor and the von Frey filament was applied 10 times (once every 3–4 s) to the middle plantar surface of the hind paw. The frequency of foot withdrawal was expressed as follows: number of trials accompanied by foot withdrawal/number of total (%) trials × 100%. Mechanical sensitivity was tested on the day prior to and 3 and 6 days after neuropathic surgery. We setup the check point on the baseline (normal rats without surgery), pre-op (before surgery), D1 (operate after one days), D2 (operate after 2 days), D3, D5, D7.

E. Bio-marker Expression in Dorsal Horn

In immunostaining analysis, an additional group of sham SNL surgery was added, which included skin incision, muscle dissection, cut of transverse process, but no nerve ligation nor electrode insertion, to stand for negative control. Rats with or without PRF were killed at various time points after SNL. We will used c-fos and p-p38 in this study. The c-fos and its protein product Fos are expressed in the spinal cord of rats subjected to peripheral noxious stimulation that expression as a valuable tool in pain research. Moreover, recent studies have provided evidence identifying the role of c-fos expression in spinal nociceptive processes [14].

The possible involvement of p38 mitogen-activated protein kinase (MAPK) activation in spinal cord and dorsal root ganglion (DRG) cells in the development of peripheral neuropathic pain has been explored. Ligation of the L5 spinal nerve (SNL) on one side in adult rats produces an early onset and long-lasting mechanical allodynia

E. Finite Element Analysis

ANSYS is a general purpose simulation based on the finite element analysis. In this topic, we use this method to simulate the electrical and temperature fields generated by PRF. Based on Maxwell's equations, the electric field vector E around an electrode placed in a conductive in tissue is derived from the current density vector field J and the electrical conductivity σ within the tissue, given by equation (1):

$$J = \sigma E \tag{1}$$

At RF frequencies of 500 KHz, the average power deposition density comes from equation (2):

$$P = \frac{0.5J^2}{\sigma} = 0.5\sigma E^2 \tag{2}$$

E is the amplitude, which is assumed to be RF frequency. The distribution of temperature T in the tissue can be calculated using the bio-heat equation (3):

$$\rho C_p \frac{\partial T}{\partial t} = 0.5\sigma E^2 + \nabla(k\nabla T) + W_b C_b (T_b - T) \tag{3}$$

The simulations performed with ANSYS software show that the electrodes relationship between temperature and electric field, we tried to analysis electrode in low voltage environment on the bi-polar electrode. Cosman et.al. showed analysis about the radiofrequency and pulsed radiofrequency experiment, it used the clinical device to simulate the temperature and electric field [15]. We used the following material parameters in our analysis conditions (TABLE I.)

TABLE I. Parameters of materials in finite element analysis

Material	Parameters				
	K(W/m)	*P(kg/m)*	*Cρ(J/Kg)*	*Γ(Ωmm)*	*ε*
muscle	1.2	1000	3400	1.41	2000
stainless	15	7900	500		
PDMS	0.15	965	1460	4×10^{13}	2.5

F. Statistical analysis

Data are reported as mean and standard error of the means (SEM). Behavioral data and cell count data from immunostaining at different time points were analyzed by Student t-test between two groups or one-way ANOVA followed by post hoc Tukey's Method for tests of more groups (SigmaPlot v.12, Systat Software, Chicago, IL). Sequential behavior data along time were compared by two-way repeated measure ANOVA with post hoc test.

III. RESULTS

From experimental results shown in Fig 4, we found that both square and sinusoidal waveforms of the specific PRF parameters (RF 500 KHz, Pulse frequency 2 Hz, Pulse duration 25 msec, and amplitude 1.25 V) can have significant effects on the VF scores up to 5 days comparing to the control group. The responses are higher in the sinusoidal group than the square ones. In the sinusoidal group, we also observed dose-dependent responses under different stimulating amplitude (2.5 V vs. 1.25 V). Moreover, we found that sinusoidal stimulation significantly reduced both c-fos and pp38 after one day and three days, respectively.(Fig 6) However, at 1.25 V of square stimulation, both proteins over-expressed significantly at day 3 after procedure. According to the above mentioned behavior and molecular studies, it clearly demonstrates that both square and sinusoidal stimulation can increase the pain threshold. And yet square wave might induce inflammatory responses even at low amplitude of 1.25 V, while sinusoidal wave can effectively inhibit the expression of inflammatory proteins.

The data showed that the expression of p-p38 after PRF-Sine wave stimulation on post-operative day 1 and day 7 are significantly lower than the control group (Fig 7). C-Fos expression on post-operative day 1 is lower than the control group (Fig 8).

The electrode and tissue model used in the finite-element calculations is shown in Figure 9. The calculated temperature and electric field in Finite Element Analysis showed insignificant raise of tissue temperature between electrodes to about 37.4℃, which is much lower than 42℃ for hyperthermia threshold (Fig 10). The electric field exhibits similar distribution as the temperature field with the fan-shape near the tip.

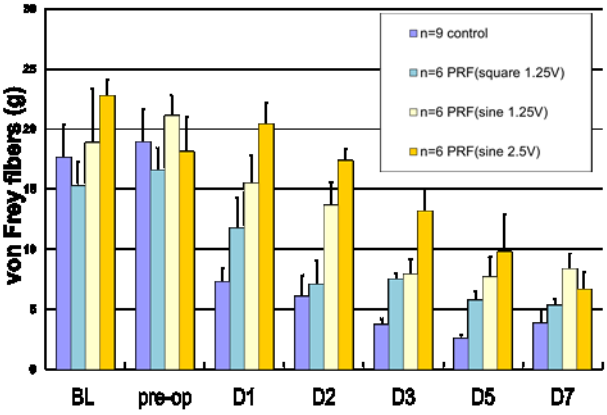

Figure 4. Mechanical allodynia after spinal nerve ligation.

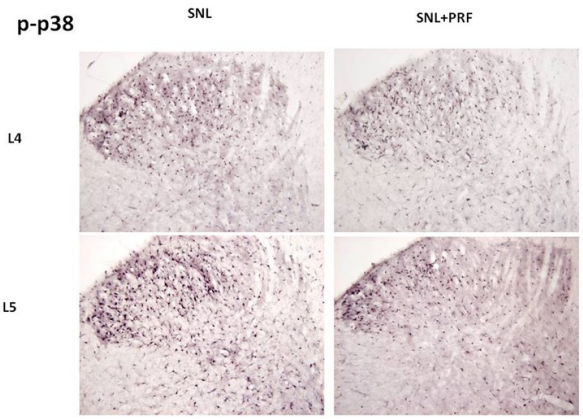

Figure 5. Reduced p-p38 expression in experiment group (SNL+PRF) of both L4 and L5 microsections compare to the sham group (SNL).

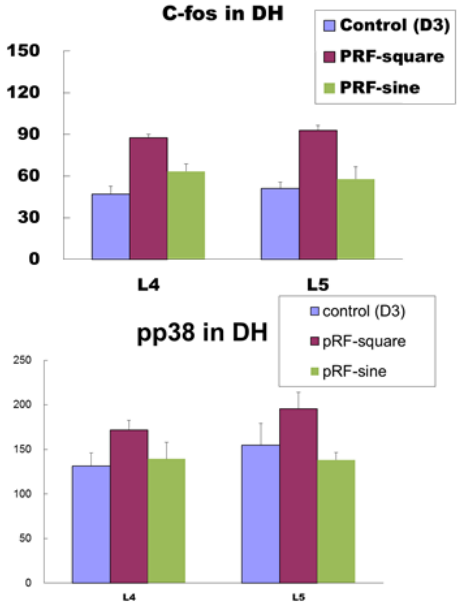

Figure 6. C-fos and p-p38 expression on dorsal horn at Day3 of control, PRF-square and PRF-sinusoidal group. Significant differences in L5 protein expression due to PRF-Sine comparing to PRF-square treatment are noted.

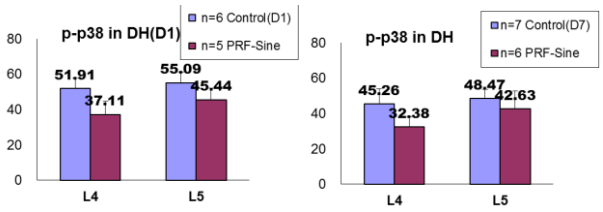

Figure 7. pp38 expression on different days with PRF-sine wave

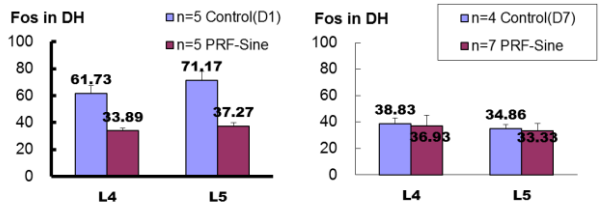

Figure 8.C-fos expression on different days with PRF-square wave

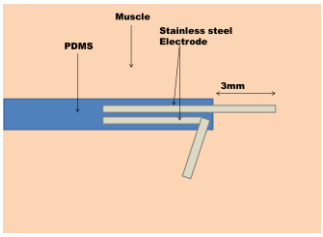

Figure 9.A diagram of the electrode and tissue model used in the finite-element calculations

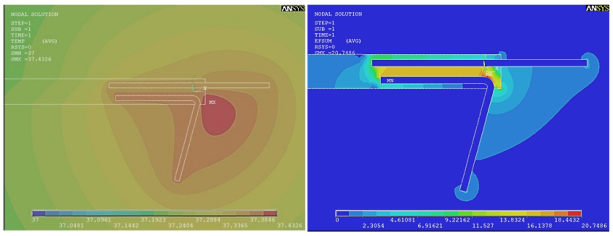

Figure 10. A. temperature B. electrical field stimulated distribution map of bipolar electrodes under designated PRF and tissue parameters in Table I.

IV. Conclusion

It was found that both square and sinusoidal waveforms of PRF treatment produced significant effects on the mechanical hypersensitivity for up to 5 days comparing to those of the control group. In particular, we found that sinusoidal stimulation exhibited dose-dependent responses. At the same amplitude of 1.25 V, square wave significantly activates both c-fos and pp38 but sine wave suppresses the expression.

It also exhibits dose-dependent responses under different stimulating amplitude of sinusoidal waveform. According to the above mentioned behavior and molecular studies, it clearly demonstrates that both square and sinusoidal stimulation can increase the paw threshold of rat.

Low power PRF suppressed early Fos expression and p38 activations at time points agreeing with their highest induction periods, and p38 activation were predominantly co-localized with microglia. We found pp38 in cells were largely increased in both the L4 and L5 dorsal horns at the ligation-ipsilateral side and mildly increased at the contralateral side. Interestingly, there was no segmental difference in number between L4 and L5 in the dorsal horns.

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