ISSN (Print) 1226-1750
ISSN (Online) 2233-6656
Journal of Magnetics 24(4), 668-673 (2019) https://doi.org/10.4283/JMAG.2019.24.4.668

Effects of Static Magnetic Field on Compound Action Potential of Isolated Frog Sciatic Nerve

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(Received 25 June 2019, Received in final form 29 October 2019, Accepted 29 October 2019)

The use of Static Magnetic Field (SMF) in medicine is currently under consideration. In this experimental study, the aim is to investigate the possible effects of SMF on nerve excitation and conduction characteristics. Our objectives are to take Compound Action Potential (CAP) measurements from an isolated frog sciatic nerve under SMF, and to calculate the amplitude and latency parameters of the CAP in order to assess possible effects. The experimental study was carried on twelve Rana Ridibundas. The sciatic nerve of the frogs were isolated from their locations and transferred to the nerve chamber. The nerve was stimulated with an electrical impulse of 0.2 ms duration and 1.4 V amplitude at the proximal end and its response was recorded at the distal end. The possible effects of SMF on the frog sciatic nerve were examined. A sequence of CAP measurements were taken with and without SMF exposure. Changes in four variables were observed. Two of the measurement variables were peak-to-peak amplitudes. The other two variables were the durations of stimulus artifact from the onset to the appearance of the first negative and first positive peaks respectively. After the first, second and third SMF exposure periods, there was a significant increase in the height of PP-1 and PP-2 which are peak-to-peak variables of CAP in both during and after exposure. Similarly, after the first, second and third SMF exposure periods, there was a significant increase in the length of Latency-1 and Latency-2 which are linked with the duration of CAP. In this study, it was observed that SMF exposure increases both the amplitude and duration of nerve CAP. Our study gave a different perspective on the effects of SMF on neuronal excitation mechanism of sciatic nerves. Besides, it provided a better understanding of the pain perception phenomenon based on transmembrane Na^+ channel dynamics and nerve conduction velocity.

Keywords : static magnetic field, sciatic nerve, compound action potential

1. Introduction

Magnetic Field Therapy means the treatment of some diseases via magnets and magnetism [1]. Although the effects of the magnetic field on the human body are not clear, some clinical studies reveal anti-inflammatory and analgesic effects of the static magnetic field [2]. It was reported that magnetic field therapy was effective on neuralgia-like painful and inflammatory peripheral nervous diseases, reflex regional pain syndrome, brachial plexus syndrome, and myopathy. Also, it speeded up fracture and wound recovery.

When SMF is applied to the inflammatory area, magnetic field penetrates skin, deep tissue and blood flow. Damaged cells interact with the magnetic field, unbalanced ionic equilibrium is present again and accumulated fluid starts to flow out of the cell. Then, cell damage ceases and the recovery period begins. Magnetic field reduces pain and threats the disease via these effects [3].

Magnetic field establishes its analgesic effect in two different ways, one is direct and the other is indirect. The magnetic field can affect nerve tissue, cell membranes, neurorotransmitters, and hormones directly. Some of these direct effects are speeding up neuron firing and recovery, calcium ion movement, membrane potentials, rising endorphin levels in tissues, changes in nitric oxide and dopamine levels, acupuncture effects, and nerve regeneration [4]. The indirect effects of magnetic fields that are bene-

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ficial to physiologic functions are on circulation, muscles, tissue oxygenation, inflammation, recovery, cell metabolism, and cell energy levels.

It is known that the mechanism of action of Electromagnetic Field Therapy hinges on biological tissue and low-energy signal interaction [5]. In our study, we aimed to investigate the effects of Static Magnetic Field on nerve electrical characteristics. Objectives are to take Compound Action Potential (CAP) measurements from isolated frog sciatic nerve under SMF and to calculate amplitude and latency parameters of the CAP to address possible effects.

2. Materials and Methods

2.1. Setup of Materials

This study was performed in Boğaziçi University Biomedical Laboratory with the approval of the Animal Experiments Local Ethical Committee. In this study, the effect of static magnetic fields on isolated frog sciatic nerve CAP was addressed. For that purpose, some set of compound action potential measurements were performed with and without SMF exposure. To assess possible changes in the nerve physiology, the parameters related to CAP waveform were measured (Fig. 1).

2.2. Physiological and Environmental conditions for Subjects

In this study, twelve healthy Rana Ridibundas were used. The average weights of the frogs were 80 gr (40-120 gr) and their ages were 8-10 months. The frogs were kept at 21 ± 1 °C temperature and under a 40-60 % humidity condition and in 12-hour night/day standards under the supervision of a veterinarian. The frogs were fed with nutrition consisting of water and 21 % protein pellet. Each frog was numbered.

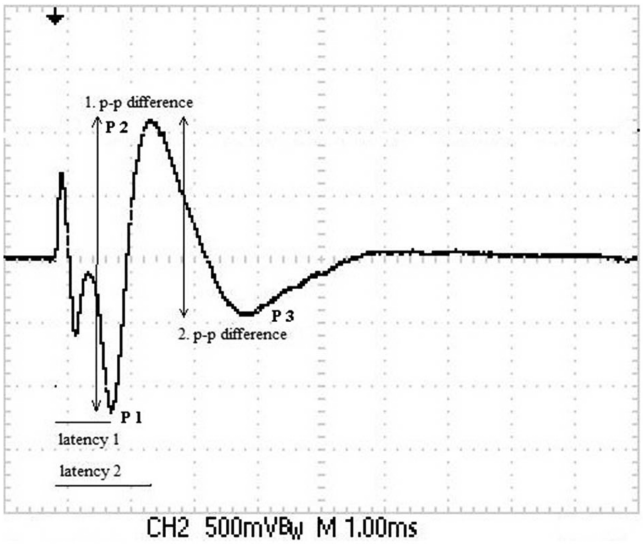

Fig. 1. Compound action potential of hibernated frog.

2.3. Double pithing and subsequent nerve isolation procedure

Before performing the sciatic nerve experiment, the frogs were desensitized to pain by "pithing". Double Pithing is relatively painless to the frog. It is a very common procedure and destroys both the brain and spinal cord. The frog was held in such a posture that the head was directed away and lower extremities were extended. The frog was grasped by two fingers so that the thumb was on the nose and the forefinger was under the jaw. The head was flexed forward (away from the experimenter's body). The pithing needle was moved down the midline over a bump (as a reference point) that is the occipital process until the soft spot of the foramen magnum was reached. The needle quickly was inserted into the cranial vault so that the brain and spinal cord was severed. Whether or not the destruction of sensory perception was successful while keeping the spine intact was tested by Corneal Reflex.

After a successful pithing process, the next stage would be isolating the sciatic nerve. The frog was laid in a dissecting tray. The skin was completely cut around the body at a point just posterior to the forelegs. The cut skin was pulled posteriorly in such a manner that it was everted and removed from the torso and hind legs. The nerve was easily observed when the frog was laid dorsal-side up. The dorsal muscles of the thigh were separated with probes and forceps to reveal the white sciatic nerve and the accompanying blood vessels. Care was taken to avoid stretching the nerve. Any branches that were occured were cut with scissors without touching the main trunk. By making a longitudinal cut in the lateral body wall the internal organs were revealed. The sciatic nerve was located as it runs near the midline from a point near the hip joint to the spinal column. The overlying membranes on the sciatic nerve were cut through,uncovering the nerve and the nearby blood vessels. The nerve was uncaged from the hip joint. Once the nerve was visualized on both sides of the hip joint it was dissected through the hip joint and was cut parallel to the nerve. The nerve was placed gently on the electrodes. A few drops of ringer solution was added on the nerve at each electrode to ensure good electrical contact.

2.4. Measurement and recording setup

The sciatic nerve was located on grids of nerve chamber which is made of plexiglass and has 15 stainless steel pins for recording and stimulating a variety of different nerve

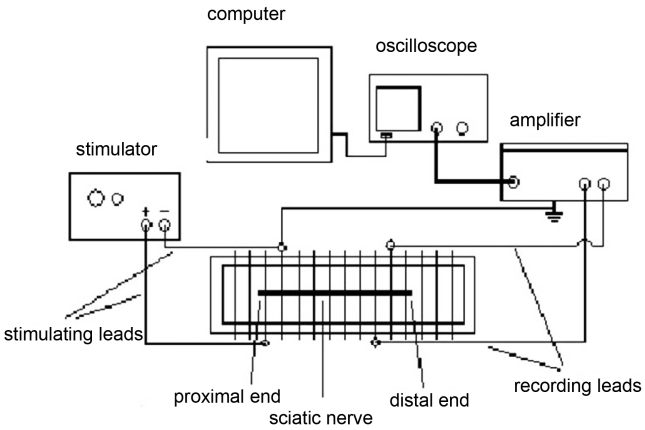

Fig. 2. Displays the setup for the detection of an action potential.

preparations. Each stainless-steel pin was spaced 5 mm apart to provide a variety of recording and stimulating configurations (Fig. 2). The stimulation and recording leads were connected to the pins of the nerve chamber with alligator clips. The reservoir capacity was 50 mL.

For the stimulation and monitoring of the compound action potential, the stimulation and recording cables were connected as follows:

As shown in Fig. 3 the positive lead of the stimulator was connected to one side of the chamber and the negative lead was connected to the next terminal on the other side of the chamber. To display the CAP of frog sciatic nerve and store it in digital form Tektronix TDS 1002B model oscilloscope was used. The data was stored on the oscilloscope USB drive and after the experiment, it was transferred to the computer where it was analyzed.

A positive and ground cable is connected next to the proximal end of the nerve placed along electrodes, over a bath of Ringer‟s solution within a nerve chamber. Negative and positive cables transmit the potential from the distal end of the nerve through the recorder to the computer.

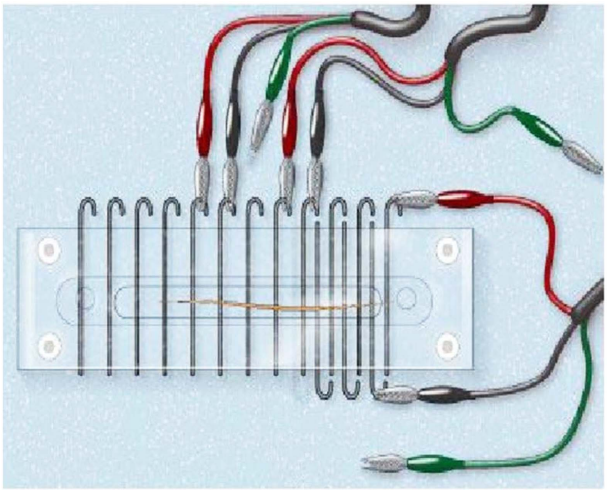

Fig. 3. (Color online) The nerve chamber.

2.5. Experimental Procedure

45 mT static magnetic field was applied on the sciatic nerve via a pair of ceramic magnets with dimensions 100×100×10 mm. Firstly two control recordings were taken with two minute successions. Subsequently, ringer solution was added on the sciatic nerve and immediately two recordings were taken with two minute successions. Thereafter, through a period of six minutes, the sciatic nerve was exposed to a 45 mT static magnetic field. During this time four recordings were taken in two minute successions from the beginning to the end of the exposure. Finally, after the static magnetic field exposure period ended, additional four recordings were taken within six minuteswith two minute successions. The above-mentioned procedure was repeated twice without the two-minute control periods due to the survival time of the nerve.

The sciatic nerve bundle was stimulated from the proximal end of the nerve with a single impulse. The stimulus impulse duration was 0.2 ms and the amplitude 1.4 volts. The recordings were taken from near to the distal end of the nerve. The length of isolated nerves was in the range of 3.4-4.0 cm. Both right and left sciatic nerves were used in the measurement. Therefore, a total of twenty-four recordings were obtained. In some experiments, total experiment time was not long enough to compare to the other recordings and in some other experiments proper recordings could not be taken due to temporary setup problems, therefore a total of sixteen experiments were evaluated for statistical analysis.

The investigated parameters associated with CAP are shown in Fig. 1. The symbols P1, P2 and P3 indicate the first negative peak, first positive peak and the second negative peak. The positive peak and the negative peak before P1 are not related to biological signal but they comprise of stimulus artifact.

When the analysis was performed, the four parameters as shown in Fig. 1 were evaluated:

PP1: Indicates an absolute difference between the values of P1 and P2

PP2: Indicates an absolute difference between the values of P2 and P3

Latency 1: Time duration beginning from stimulus artifact initial rise to P1

Latency 2: Time duration beginning from stimulus artifact initial rise to P2.

2.6. Statistical analyses

Statistical analyses were performed in IBM SPSS for Windows 22.0 software (IBM Corporation, Armonk, NY, ABD). The normality of the continuous numerical variables was tested by the Shapiro-Wilks test. Numerical values of test results were presented with average ± standard deviation. Since the groups comparison didnot have a normal distribution, non-parametric tests were used. Mann-Whitney U test was used to evaluate between group-importance for amplitude and latency variables. Wilcoxon test was used to analyze the meaningful relationship between the pre and post-treatment values. The statistical significance level was determined $p < 0.05$ for all statistical analyses.

3. Results

The study was performed on twelve female Rana Ridibundas. After three successive applications of SMF, significant increase in PP-1 and PP-2 of CAP in all "pre-exposure", "exposure" and "post-exposure" periods were observed (Table 1-2). These increases were also significant statistically. Also, when "pre-exposure", "exposure" and "post-exposure" measurements were compared there was a higher increase in PP-2 variable after the 3rd application of SMF.

Similarly, after three successive applications of SMF significant increase in Latency-1 of CAP in all "pre-exposure", "exposure" and "post-exposure" periods were observed (Table 3). For the Latency-2 parameter, there was not a statistical difference between "pre-exposure" and "exposure". An increase in "post-exposure" compared to "exposure" measurement was observed for three successive applications.

4. Discussion

This study is an experimental study to evaluate the effect of SMF on nerve conduction (CAP) on animal peripheral nerves. We aimed to address the effects of SMF application on frog sciatic nerve Compound Action Potential. We detected a significant rise in both CAP peak

Table 1. The changes in PP-1 parameter of frog sciatic nerve CAP with SMF application.

	Pretreatment (PrT) Mean ± SD	During (D) Mean ± SD	Post treatment (PoT) Mean ± SD	P*
1st application	1,719 ± 0,719	1,924 ± 0,829§	2,008 ± 0,843†	< 0.001
2rd application	1,978 ± 0,842	2,195 ± 0,915§	2,26 ± 0,869†	< 0.001
3nd application	2,172 ± 0,701	2,625 ± 0,981§	2,626 ± 0,974†	< 0.001*
P*	0,006	0,002	0,002	

*: Friedman Test, †,§,‡: Wilcoxon Signed Ranks Test; †: There is a significant change when PoT and PrT are compared, §: There is a significant change when D and PrT are compared, ‡: There is a significant change when PoT and D are compared

Table 2. The changes in PP-2 parameter of frog sciatic nerve CAP with SMF application.

	Pretreatment (PrT) Mean ± SD	During (D) Mean ± SD	Post treatment (PoT) Mean ± SD	P*
1st application	0,937 ± 0,356	1,119 ± 0,464§	1,144 ± 0,468†	< 0.001
2rd application	1,121 ± 0,479	1,319 ± 0,582§	1,339 ± 0,521†	< 0.001
3nd application	1,223 ± 0,386	1,519 ± 0,536§	1,531 ± 0,51†	< 0.001
P*	< 0.001	0.001	0,003	

*: Friedman Test, †,§,‡: Wilcoxon Signed Ranks Test; †: There is a significant change when PoT and PrT are compared, §: There is a significant change when D and PrT are compared, ‡: There is a significant change when PoT and D are compared

Table 3. The changes in Latency-1 parameter of frog sciatic nerve CAP with SMF application.

	Pretreatment (PrT) Mean ± SD	During (D) Mean ± SD	Post treatment (PoT) Mean ± SD	P*
1st application	0,000819 ± 0,000108	0,000824 ± 0,00011	0,000864 ± 0,00011†‡	< 0.001
2rd application	0,000853 ± 0,000106	0,000869 ± 0,000106§	0,000939 ± 0,000139†‡	< 0.001
3nd application	0,000921 ± 0,000124	0,000928 ± 0,000109	0,000962 ± 0,000124†‡	0,008
P*	< 0.001	< 0.001	< 0.001	

*: Friedman Test, †,§,‡: Wilcoxon Signed Ranks Test; †: There is a significant change when PoT and PrT are compared, §: There is a significant change when D and PrT are compared, ‡: There is a significant change when PoT and D are compared

Table 4. The changes in Latency-2 parameter of frog sciatic nerve CAP with SMF application.

	Pretreatment (PrT) Mean ± SD	During (D) Mean ± SD	Post treatment (PoT) Mean ± SD	P*
1st application	0,00138 ± 0,000251	0,00138 ± 0,000235	0,00144 ± 0,000229†‡	< 0.001
2rd application	0,001418 ± 0,000222	0,00147 ± 0,000236§	0,001578 ± 0,000285†‡	< 0.001
3 nd application	0,001418 ± 0,000222	0,00147 ± 0,000236	0,001578 ± 0,000285‡	0,185
P*	0,003	< 0.001	< 0.001	

*: Friedman Test, †,§,‡: Wilcoxon Signed Ranks Test; †: There is a significant change when PoT and PrT are compared, §: There is a significant change when D and PrT are compared, ‡: There is a significant change when PoT and D are compared

amplitude and latency values after Magnetic Field Therapy (MFT). The CAP is a cumulative response of fibers of the sciatic nerve. The rise in the PP1 and PP2 parameters may stem from the contribution of fibers which were not firing without SMF exposure. It may be suggested that SMF manifest its effect on lowering the excitation threshold of the individual nerve fibers.

For the convenience of research, static magnetic fields can be classified into four ranges. These ranges are weak (< 1 mT), moderate (1 mT to 1 T), strong (1 T to 5 T), and ultrastrong (> 5 T) [6-8]. In contrast to studies with high static magnetic field values; significant neuronal conduction block and an increase in inactivation time constant are closely related to the activation gate of Na channels, which were observed in studies performed under the moderate static magnetic range [9-11]. The increase in time activation constant implies a decrease in the depolarization rate of the cell and a decrease in conduction velocity.

The signal conduction speed of a nerve fiber is also affected by extracellular resistance of the environment that the fiber lies in [12]. In a study conducted on 40 postnatal females, the effects of Pulsed Magnetic Field Therapy (PEMFT) and Ultrasound Treatment were compared in postnatal Carpal Tunnel Syndrome patients. As a result, there was a significant decline in the pain level of the median nerve, sensory and motor distal latencies. There was also a significant increase in sensory and motor conduction velocities of the median nerve [13]. In contrast to this study, there was an increase in latencies of the CAP in our study. This difference may be related to the time course, therefore, the total amount of exposure to SMF.

In one study possible changes in nerve injury after PMFT application were addressed. The study indicated PMF improved both the morphological properties and the electrophysiological function of nerves. Besides, they found PMF has a regulatory effect on sensory fibers [14]. Our results are consistent with the findings of an increase in the amplitude of the CAP.

The researchers studied the effects of the magnetic field with different amplitudes in the human median nerve and confirmed a significant increase in action potential amplitude in the post-exposure period [15]. Our finding that the amplitude of CAP increases from “during” to “post-exposure” confirms the results of this study.

In another experimental study, they studied the effect of PEMFT on nerve recovery after nerve injury on twenty-four rats. In light of functional assessment, they found improvement in the Flexor Digitorium Sublimis muscle grasping function. Besides, they reported an increase in the number of nerve fibers in the experimental group compared to the control group. Their outcomes were similar to our experimental results, that there was an increase in CAP amplitude [16].

In a study, to test possible effects of SMF on frog sciatic nerve, the researchers addressed the effects of moderate-intensity gradient static magnetic fields on excitation and response characteristics of frog sciatic nerve [17]. In the study, one control and two SMF exposure groups were-evaluated. Their results show that 0.7T SMF reduced the nerve conduction velocity of C fibers for a period of 4to 6 h exposure but 0.21T SMF did not make any change during the whole 6h period. In our study, 45 mT SMF exposure for 20 minutes increased the latency of the CAP. As opposed to Okano's study this finding points out lower duration and intensity of SMF affects latency thus nerve conduction velocity.

The effects of the magnetic field on neuropathic pain were also studied [18]. In the study, researchers applied multiple-dose MF to assess allodynia density and mechanical-thermal sensitivity to stimulus on Rat Tibial Nerve cut. They found that the use of low-frequency magnetic field reduced the pain in rats after nerve transection but there was no change in the nociceptive sensitivity of healthy rats. Histological and immune histological examination results confirmed these finding. A sciatic nerve is composed of different types of fibers that have different diameters. The diameter of an axon determines the excitation threshold of a nerve. Axons with smaller diameters have a higher excitation threshold. In our results, larger PP1 and PP2 implicates the higher contribution

coming from nerve fibers with smaller diameters. This means SMF may decrease the excitation threshold of a small diameter fiber thereby causing an increase inpain perception.

The possible effects of the strong static magnetic field (8T) on sciatic nerve bundles of frogs were also considered [19]. In the study, they measured CAP which is electrically excited by a pair of impulses with varying interpulse intervals. Nerve conduction velocity was not changed with 8T SMF but membrane excitation during the relative refractory period was enhanced by 10 %. Unlike the mentioned study, there was an increase in the latency in our work. This means a decrease in nerve conduction velocity under 45 mT SMF. The latency of the CAP was dominated by larger and faster conducting axons. Therefore decrease in the velocity can be attributed to changes on membrane Na^+ channels' opening-closing dynamics.

5. Conclusion

In conclusion, various effects of SMF therapy on the peripheral nerve system were confirmed in both clinical and experimental studies. In our experimental study, we aimed to discover a mechanism that affects the peripheral nervous system. The results of this study reveal the SMF increases both the amplitude and latency of the CAP on the peripheral nervous system. The increase in the amplitude suggests that SMF affects the transmembrane Na^+ channel dynamics and changes the excitation threshold of nerve fibers with different diameters. The increase in the latency points SMF has an impact on the conduction velocity of fibers.

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