EFFECTS OF MAGNETIC FIELD STIMULATION ON THE ACTION POTENTIAL OF A FROG SCIATIC NERVE

A Report of a Senior Thesis

by

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Major: Biology

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Spring, 2004

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ABSTRACT

An increasing amount of research has been conducted involving possible effects of magnetic field exposure on the human body. As to the possible mechanism by which magnets could relieve pain, very little research has been conducted. In this experiment, the nervous system is targeted as a possible mechanism through which magnetic fields may relieve pain. More specifically, this experiment is designed to test the possible effects of a quadrapole static magnet on the action potentials of a frog, *Rana catesbeiana*, sciatic nerve. Using an AD instruments Power Lab, the duration, activation time, amplitude, and threshold potential of several action potentials were measured for a nerve prior to and after fifteen minutes of exposure to a quadrapole magnet. Another experiment was designed to test the effects of an extended period of time (3 hr.) of magnetic field exposure; however, an unusual action potential for the control nerve at time three hours prevented any conclusions from being made for this experiment. In the initial experiment, the results show a significant decrease in duration (p=0.00656), significant increase in amplitude (p=0.0015) and activation time (p=3.9E-06), and the lack of a significant change in threshold potential (p=0.148) following the application of the magnets. These results suggest the presence of several uncontrolled variables including the moisture and temperature of the nerve used. Better instruments, more action potentials recorded, and

changes in magnetic field strength and gradient could help to elucidate a possible mechanism by which magnetic fields could influence voltage-gated channels, the action potential of a nerve, and potentially one's ability to perceive pain.

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to those who were most helpful throughout this year-long research project. More specifically, I would like to thank Ms. Debbie Threadgill for her assistance in ordering and creating some of the instruments needed to perform this experiment. I greatly appreciate the assistance of Drs. Roger Miller, Terry Bunde, and Kristi Kneas while trying to correct the outdated instruments used early on in my experimentation process. All three individuals spent a great deal of time and effort trying to generate an action potential. I would also like to thank Dr. Miller and Dr. Bunde for allowing me to carry out an intelligent conversation involving possible mechanisms by which magnetic fields could influence an action potential.

Finally I would like to thank Dr. Drew Crain for all of the time, effort, trust, patience, and care that he has shown me while carrying out this research. Dr. Crain demonstrated a great deal of trust in purchasing an expensive piece of equipment for this project. Throughout the research, I felt comfortable discussing a wide array of possible mechanisms by which magnets could relieve pain or affect an action potential. His knowledge in applying physiological tools to possibly explain the effects of magnetic fields on the human body was greatly appreciated. I also can't express in words my appreciation for his patience and support throughout the difficult times in this process. I again would like to thank all of those who helped me throughout these past fifteen months.

CHAPTER I

INTRODUCTION

At its most basic level, the human body is nothing more than a very complex electrical machine. The human body consists of cells, which are polarized. All cells contain various concentrations of positively and negatively charged ions. Furthermore, all molecules are made up of atoms consisting of electrons and protons. This basic understanding of the cellular and atomic structure of the human body suggests that the human body is an electrical machine. When considering the structure of the human body and the laws of physics, one can also assume that electricity and magnetism can alter the function of the human body. This simple relationship between the basic structure of the human body and the power of electricity and magnetism has provided a great deal of excitement in the fields of biomagnetic and bioelectromagnetic research. Many scientists believe that electromagnetic energy is a very legitimate and powerful form of medicine. According to Robert O. Becker, M.D., there is “one fundamental force that heals: electromagnetic energy” (Philpott, 1990, p.5). As Dr. Zimmerman reports " The healing potential of magnets is possible because the body's nervous system is governed, in part, by various patterns of ionic currents and electromagnetic fields" (Hawkins, n.d., ¶ 3). Many scientists state that

electromagnets can be used to treat cancer. In his book Cross Currents, Becker (1990) describes how Dr. Kenneth McLean gathered data suggesting that rats with cancerous cells survived under high strength DC magnetic fields. One book produced in Kansas argues that by hanging a neodymium magnet around one's head, one will be cured of cancer (Livingston, 1998). The likelihood that magnets can cure cancer is very slim; however, this example illustrates the excitement and potential benefits of further research in the field of bioelectromagnetism.

History of Magnetic Field Therapy

During the past thirty years, magnet therapy has become one of the more popular new forms of alternative medicine. Many companies are taking advantage of the new market known as magnetotherapy. Magnetic necklaces, bracelets, earrings, and belts are being sold in large quantities. Magnets are also being isolated in specific parts of the body to relieve pain in the neck, back, knee, elbow, ankles, feet, and hands (Livingston, 1998).

Magnets have been used to relieve pain for thousands of years. The ancient Chinese and ancient Egyptians were the first to notice the healing power of lodestones (natural magnets). During the scientific revolution, magnet therapy became a fad due to the emphasis on navigation and the idea of magic or mysticism. The first individual to publicly suggest the effects of magnets on the human body was Anton Mesmer. In his doctoral thesis, Mesmer "proposed that a subtle 'universal fluid,' a kind of invisible magnetic energy, or gas, permeated the universe as well as all bodily fluids" (Whitaker & Adderly,1998, p.27). In 1775, Mesmer published the report "On the Medicinal Uses of the Magnet." In the

article, Mesmer mentions how an individual with uncontrollable seizures did not experience any more seizures following the application of magnets. Shortly after publishing the article, Mesmer hypothesized that two forms of magnetism existed: animal magnetism and mineral magnetism. Mesmer then used his understanding of "animal magnetism" to cure people experiencing chest pain and stomach pain and even restored the hearing of a deaf person. Anton Mesmer was the first scientist to staunchly advocate the use of magnets as a form of medicine (Whitaker & Adderly,1998).

The rise of the electricity in the late 19th century initiated research in electromagnetism. Doctors began to investigate the effects of applying electric fields on patients. The medical community reported treatments of emotional disorders, arthritis, sleep disorders, and headaches following the use of electromagnetic therapy. Research in the field of bioelectromagnetism has expanded with increasing availability of powerful magnets such as the neodymium magnets. Today, magnets are being used to overcome brain disorders, pain, and arthritis.

Biological Effects of Magnetic Field Stimulation

Due to the extensive use of magnets throughout history, many physiological and psychological changes have been observed. Magnets have been used to encourage sleep, relieve pain, reduce inflammation, kill bacteria, and fight infection. Furthermore, magnets have been shown to promote blood and oxygen circulation throughout the blood, normalize acid/base balance, speed up migration of calcium ions to facilitate the healing of nervous tissue and bones, increase

enzyme activity, and increase the production and release of hormones. Magnets promote the healing process, increase energy, reduce stress, and alter transcription.

A thorough review of the literature reveals that magnets tend to have four major effects on the human body. Magnets have been shown to cause vasodilation, increase energy, and alter hormone levels and sensory reception. Speculative scientists usually argue that magnetic fields relieve pain by way of one of these four effects.

Vasodilation

Many of the aforementioned effects result from a change in the autonomic nervous system. More specifically, the magnetic field activates the parasympathetic nervous system. As a result, the circulatory system, immune system, and the brain undergo changes. As seen in Table 1, magnetic field application has been shown to vasodilate blood vessels and cause an increase in cellular oxygen and the removal of fluids and gasses from the circulatory system. Vasodilation alters the immune system by fighting infection, reducing inflammation, and removing the toxic byproducts of inflammation. Changes to the parasympathetic nerves in the brain cause happiness, reasonableness, and mental acuity.

Table 1. Detailed vasodilator effects on several systems throughout the human body.

Major Effect	Body System	Detailed effect	Reference
Vasodilation	Circulatory system	Increases cellular oxygen	Philpott, 1990
		Pulls fluids and gasses	Philpott, 1990
		Reduces fluid retention	Philpott, 1990
	Immune system	Fights infection	Philpott, 1990
		Supports biological healing	Philpott, 1990
		Reduces inflammation	Philpott, 1990
		Remove the toxic byproducts of inflammation	Philpott, 1990
		Increases immune function	Null, 1998
		Lowers the acidity	Null, 1998
	Brain	Promotes reasonableness and mental acuity	Philpott, 1990
		Promotes happiness	Philpott, 1990

Source: (Compiled from Null, 1998; Philpott, 1990).

Sensory Reception

Many researchers involved in the studies of magnetic therapy have noticed biological effects on the nervous system. More specifically, magnetic fields have been shown to decrease the sensory reception of the nerves. As seen in Table 2,

experiments suggest that magnets can relieve pain by blocking the action potential from pain receptors and by increasing the depolarization potential of cells in an

Table 2.

Observed changes in the sensory receptors following the application of electromagnetic energy.

Major Effect	Body System	Detailed Effect	Reference
Sensory Reception	Nerves	Blocks action potential Relieves / stops pain Raise the depolarization potential of the cell	Cavopol et al. 1995 Philpott, 1990 Null, 1998

axon. Both of these methods prevent messages indicating pain from reaching the brain and as a result, pain is not sensed.

Changes in Hormonal Secretion

Another one of the major effects that magnetic fields have on the human body is the alteration in the secretion of hormones. Researchers believe that magnetic fields primarily act on the pineal gland. This tiny gland, located in the forebrain, is many times referred to as the "third eye" (Guyton & Hall, 2000, p.927). Light has inhibitory effects on the function of the pineal gland. In fact, the pineal gland functions best at night when no light is present and when a

substantial amount of melatonin, the chief hormone of the pineal gland, is produced. Some research has shown that the pineal gland also responds to

Table 3.

Observed changes in the secretion of hormones following the use of magnets.

Major Effect	Body System	Detailed Effect	Reference
Change in Hormonal Secretion	Pineal Gland	Increases quality of sleep	Null, 1998
		Increases melatonin production	Null, 1998
		Reduces stress	Null, 1998
	Hypothalamus	Increase the production and release of hormones	Santwani, 1994

Source: (Compiled from Null, 1998; Santwani, 1994)

electromagnetic radiation. Furthermore, the research has suggested that static magnetic fields can decrease the production of melatonin during the night. As seen in Table 3, this change in the secretion of the melatonin has been shown to increase the quality of sleep and have anti-stress and anti-aging effects on the human body. Studies have also shown that magnetic fields can also stimulate secretion of hormones from the hypothalamus.

Increase in Cellular Energy

Energy enhancement is another way in which many of the biological effects of magnetic fields can be classified. Many studies have shown that

magnetic field application causes an increase in enzymatic function. As seen in Table 4, this increase in enzymatic function causes a reduction of fatty deposits, an increase in metabolism, heat formation, and cell division. Null (1998) suggests

Table 4.

Observed changes in the amount of energy at the cellular level following the use of magnets.

Major Effect	Body System	Detailed Effect	Reference
Increase Energy	All Cells	Reduces fatty deposits	Philpott, 1990
		Increase enzyme activity	Santwani, 1994
		Increase metabolism	Becker & Marino 1982
		Causes heat formation	Santwani, 1994
		Promotes cell division	Santwani, 1994
	Pineal Gland	Increase production of melatonin	Null, 1998

Source: (Compiled from Becker & Marino, 1982; Null, 1998; Philpott, 1990; Santwani, 1994)

that the anabolic hormone produced by the pineal gland, melatonin, is partially responsible for the increase in energy found in all the cells throughout the body, following the use of magnets.

Mechanism by Which Magnets Relieve Pain

Perhaps the biggest question in the field of magnetic therapy involves a lack of an understanding how magnetic fields affect the human body. Many scientists dispute the mechanism of magnetic therapy. Currently, there are very few heavily supported theories concerning the mechanisms by which magnets relieve pain. Most advocates avoid any discussion regarding possible physical and physiological mechanisms. Instead, supporters for biomagnetism tend to highlight the many reported benefits from the scientific community. Many scientists vaguely say that magnets relieve pain by increasing blood flow. This simple explanation illustrates the lack of an understanding concerning the mechanism by which magnets affect the body. Few scientists discuss how magnets increase blood flow. Some researchers believe that the pain is relieved because magnets increase the depolarization potential of the nerve cells. Once again, the researchers avoid discussing how magnets increase the depolarization potential. Other mechanisms that scientists suggest include the increase in enzymatic function and the effect on the endocrine system.

Vasodilation

The most often proposed mechanism by which magnets relieve pain concerns the observed increase in blood flow through the capillaries. Most of the research suggests that magnetic fields increase blood flow. More specifically, magnetic fields have been shown to increase the microcirculation within various mammals prior to a gradual decrease in blood flow. According to Gmitrov, Ohkubo, & Okano (2002), “after magnetic field exposure we observed increased

blood flow. . . which gradually decreased after exposure cessation" (p.229). Studies by Okano, Gmitrov, & Ohkubo (1999) suggest that the blood flow is influenced by acetylcholine, noradrenaline, and magnetic fields: "the results demonstrated that SMF significantly enhanced vasodilatation, with increased vasomotion under noradrenaline-induced high vascular tone as well as vasoconstriction with reduced vasomotion under acetylcholine-induced low vascular tone" (p.170). This research suggests that magnetic fields do in fact increase blood flow.

However, many scientists believe that the increased blood flow does not explain the mechanism by which pain is relieved. Conventional thought suggests that increased blood flow and vasodilation can cause wholesale changes to the function of the brain and especially the immune system. White blood cells provide a great deal of the immune defense for the body. Magnetic fields have been shown to significantly increase the white blood cell count (Becker & Marino, 1982). Vasodilatory effects also tend to increase the concentration of oxygen in the blood stream. This increase in blood oxygen levels leads to reduced inflammation and infection, and an increase in alkalinity and biological healing. Many proponents of magnetic therapy believe that this increase in immune function is responsible for the relief of pain. Despite this logical approach, many believe that it is impossible for all of these immune functions to relieve pain in such a short period of time. As stated by Lara Owen in her book, Discover the Healing Power of Magnets, "the theory that the magnet enhances the blood flow and therefore diminishes inflammation and pain, resulting in less pain,

is not a sufficient explanation for the very dramatic pain relief that is often felt in too short a time to be caused by increased blood flow" (2000, p.203). More than likely, vasodilation is one of many mechanisms by which magnetic fields can relieve pain.

This particular proposed mechanism contains a contradicting observation. Based on the effects found in Tables 1 and 2, the magnetic fields deactivate sensory neurons but activate the parasympathetic nervous system. Traditional physiology suggests that deactivated sensory neurons tend to deactivate the autonomic nervous system. This relationship confuses many scientists studying the mechanism by which magnets relieve pain.

Changes in Hormonal Secretion

The endocrine system is viewed by many physiologists as the master regulator of the body's function. The endocrine system consists of many glands and organs that produce hormones. Some of the more important endocrine organs include the pituitary, thyroid, adrenal medulla, adrenal cortex, pineal gland, ovary, and testis. Hormones are sent to various parts of the body where they initiate a specific reaction. Hormones can alter the metabolic rate, heart rate, blood pressure, sperm and egg production, and blood glucose level (Raven & Johnson 1999).

Because of the power of the endocrine system, many scientists believe that this system could play a major role in relieving pain. Specifically, many researchers believe that the aforementioned pineal gland plays a vital role in the relief of pain. Research suggests that the pineal gland is responsive to

electromagnetic fields. The pineal gland secretes a hormone, melatonin, that "has been shown to be antistressful, antiaging, antiinfectious, anticancerous, and to have control over respiration and production of free radicals" ("Magnetic Field Therapy," 1994, 333). These reported effects from high melatonin synthesis suggest that the hormone could be used to relieve pain.

Research into the effects of electromagnetic fields on the release of melatonin has revealed many different results. Some research suggests that electromagnetic fields increase melatonin production (Lerchl, Zachmann, Ali, & Reiter, 1998) and other experiments report no significant change in melatonin production (Rogers, Reiter, Smith, & Barlow-Walden, 1995). One of the biggest reasons why many scientists disregard the pineal gland as a possible mechanism for relieving pain is because of some of the recent research suggesting that electromagnetic fields can actually suppress the production of melatonin (Tripp, Warman, & Arendt, 2003). These experimental results suggest that electromagnetic fields would have none of the positive effects needed to relieve pain.

Increases Energy in the Cells

Most of the aforementioned mechanisms suggest that magnets affect the body from a biochemical perspective. Other scientists suggest that proper analysis of the mechanism involves analyzing the effects of magnetic energy at an atomic and physical level. Many biophysicists believe that magnetic fields target the cell membrane where ions are exchanged between cells (Owen, 2000). It is the passage of sodium and potassium ions across the cell membrane that creates

an electric potential within the cell. Some scientists believe that the electric potential within each cell changes as a function of energy. When energized, the cells have a negative electric potential. As an individual gets tired, the negative electric potential becomes neutral (depolarizes). According to Gary Null, "as the cell performs its daily functions, it becomes depolarized. Depolarized cells equal a tired person. It is believed that magnetic energy has the ability to penetrate all facets of the human body and reach every cell. That translates to greater energy and vitality throughout the body as a whole" (1998, ¶ 77). This novel theory could explain the observed reduction in fatty deposits and increase in metabolism, heat production, cell division, and enzyme activity. Since this particular idea is relatively new, very little research has been conducted to prove the possible mechanism: "There's no proof for any of this, but it all makes for interesting speculation" (Owen, 2000, p.207).

Sensory Reception

Pain is detected by the activation of pain receptors throughout the body. When an external force alters the pain receptor, an action potential is sent through afferent nerves to the brain. The brain then interprets the action potential and notifies the body of pain. Some studies, discussed later, suggest that pain can be relieved by blocking the action potential to prevent communication with the brain.

Action Potential

Messages are transmitted throughout the body by action potentials (for review see Guyton & Hall 2000). All cells have a resting membrane potential. In large nerves, a negative electric potential of 90 mV is created due to the removal

of positive potassium ions from the inside of the cells. Following a stimulus (chemical, physical, electromagnetic, or thermal), an action potential is created due to a change in the resting membrane potential(see Figure 1). An action potential is simply a wave of depolarization down an axon. Depolarization occurs when the electric potential within the nerve increases from –90 mV to approximately 60 mV. Repolarization, the decrease in electric potential from 60 mV to –95 mV, follows the depolarization. Finally the electric potential of the nerve returns to its typical resting membrane potential of approximately –90 mV.

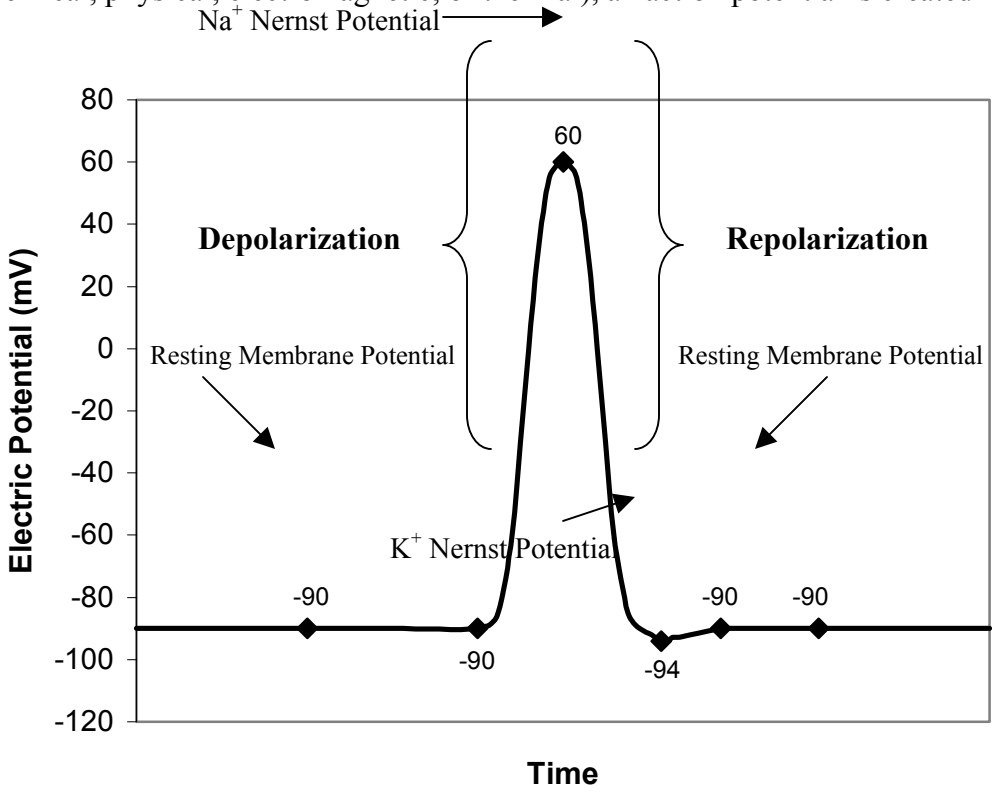

Figure 1. The typical change in electric potential within a cell when undergoing an action potential(Guyton & Hall, 2000).

This change in electric potential within the cell can be explained by the opening of the sodium and potassium voltage-gated channels. The increase in electric potential is due to the opening of the sodium voltage-gated channel,

which allows positive sodium molecules to enter and depolarize the cell. Shortly before the electric potential reaches its maximum of 60 mV, the potassium voltage-gated channels are opened and allow positive ions to leave and repolarize the cell. The changes in electric potential are due to the opening of sodium and potassium voltage-gated channels.

This alteration in polarization is transferred down the axon and eventually to the brain. Once the action potential, wave of depolarization, reaches the brain, it is interpreted by the brain. If the action potential is sent from a pain receptor, the individual does not feel the pain until it reaches the brain. The brain interprets the action potential to be pain. The brain then responds to the message by whichever mechanism is necessary.

Blockade of Action Potential

In this study, the effects of magnetic fields on the action potential of a frog sciatic nerve will be determined. Several studies conducted by Cavopol, Wamil, Holcomb, & McLean at Vanderbilt University show that magnets can block the action potential by as much as eighty percent. Two studies conducted by the aforementioned authors were published with in two months in the journal Bioelectromagnetics. In both experiments, cultured adult mouse dorsal root ganglion neurons were grown and stimulated using a unique magnet. These scientists used the MAG-4A magnet, four permanent center-charged neodymium magnets of alternating polarity. These two studies contained some interesting results concerning various variables of magnetic fields and how they affect the blockade of action potentials. The studies revealed that the magnitude of the

magnetic field did not influence the blockade of action potential. Instead, the gradient greatly affected the firings of action potential in the cultured neurons. The magnitude of the magnetic field is essentially the number of magnetic field lines that pass through a given area. The gradient is produced over a given area when the magnitude of the magnetic field changes across the given area. This change in magnetic field of over the applied region (the gradient) caused the greatest blockade of action potential.

Hypothesis

Over the past ten years, much research has been conducted testing the relief of pain due to the application of magnets. Studies have been conducted on pain relief due to magnets in individuals with various ailments: fibromyalgia, postpolio, lumbar radiculopathy, low back pain, chronic knee pain, pudendal neuralgia and sciatica nerve pain, and carpal tunnel syndrome. Different types of magnetic field stimulation have been applied to relieve pain. Typically, pulsed magnetic stimulation, transcranial magnetic stimulation, has been applied to illnesses within the brain. Recently, more research has been conducted on the effects of static magnetic field stimulation on muscular and skeletal pain.

Several experiments involving magnetic field therapy have received a great deal of attention in the medical community. In 1997, Vallbona, Hazlewood, and Juricle completed a double-blind pilot study testing how postpolio patients with low back pain respond to the application of static magnetic fields. The results showed a significant reduction in pain in the group with the magnets compared to the control group. Three years later in March of 2000, Collacott,

Zimmerman, White, and Rindone published a similar double-blind pilot study in the Journal of the American Medical Association in which bipolar magnets were applied to patients with low back pain. The experimental results suggest that there was no significant relief of pain.

Despite this most recent study, the interest regarding magnetic field therapy continues to increase and receive considerable research. As previously mentioned, many studies have been conducted involving various types of pain. A majority (approximately 65%) of these studies report a significant decrease in pain. Experiments have been performed in which patients with chronic knee pain, carpal tunnel syndrome, pudendal neuralgia and sciatica nerve pain, pelvic pain, and lumbar radiculopathy notice a significant decrease in pain.

These recent studies and the potential benefits associated with further research in bioelectromagnetics are intriguing. As the number of studies involving magnetic field therapy increases, the major question in the field of study is not do magnets relieve pain, rather the question involves the mechanism by which magnetic fields can relieve pain. Of the aforementioned proposed mechanisms, the mechanism with the least amount of contradictory information and the most promising published research is the change in sensory reception. There have been several studies suggesting that the action potential sent to the brain to detect pain is blocked under the presence of static magnetic fields. In the current experiment, changes in the action potential of a frog sciatic nerve will be investigated following the application of static magnetic fields with varying magnitudes and gradients. Based on the recent studies involving the relief of pain and the

blockade of action potential, I hypothesize that the frog sciatic nerve will elicit fewer action potentials due to the presence of static magnetic fields.

CHAPTER II

MATERIALS AND METHODS

Setup of Materials

To test the possible effects of magnetic fields on the action potential of a nerve, a pair of bullfrogs, *Rana catesbeiana*, was purchased from Amphibians of North America, Charles D. Sullivan Company (Nashville). Four neodymium, iron, boron magnets with a diameter of 7 mm, height of 3.5 mm were purchased from scientificsonline.com. These four magnets were drilled into a block of wood. As seen in Figure 2, the magnets were aligned in quadrapole in which the magnets were alternated north and south. The pole of each magnet was determined using a standardized bar magnet with labeled north and south poles as the north and south poles of magnets are attracted to one another. However, biomagnetic literature refers to the geomagnetic north pole as the south pole and the geomagnetic south pole as the north pole ("How Biomagnetic Therapy Works"). To clarify this discrepancy, in this experiment the pole of the magnet which is attracted to the north pole of the bar magnet is termed the north pole. The same reasoning can be used for determining south pole.

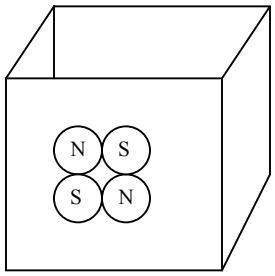

Figure 2. Representation of four magnets of alternating polarity drilled into a piece of wood.

Amphibian Ringer's solution was prepared before the experiment to preserve the sciatic nerve extracted from the bullfrog. The ingredients for creating this solution are seen in Table 5. To isolate the sciatic nerves, the bullfrog was chilled and decapitated. Both nerves were removed between the knee and hip. The nerves were handled with glass probes due to the electrical nature of the nerves. The nerves were stored in the Ringer's solution prior to transferring the nerve to the nerve chamber.

Table 5.

Materials needed to prepare a two-liter solution of Frog's Ringer's Solution.

Ingredient Added	**Amount Added**
Distilled Water	1604.2 mL
HEPES (buffer)	2.383 g
1 M NaCl	133 mL
1 M Glucose	11.1 mL
0.1 M Sodium Bicarbonate	23.8 mL
0.1 M Calcium Chloride	11.0 mL
0.1 M Potassium Chloride	19.0 mL

Instrumental Setup

To measure the action potential of a frog sciatic nerve, an AD instruments Power Lab 2/25 (Colorado Springs) was used with an attached MLT012 nerve chamber. Figure 3 displays the setup for the detection of an action potential. A positive and ground cable connect the stimulator 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

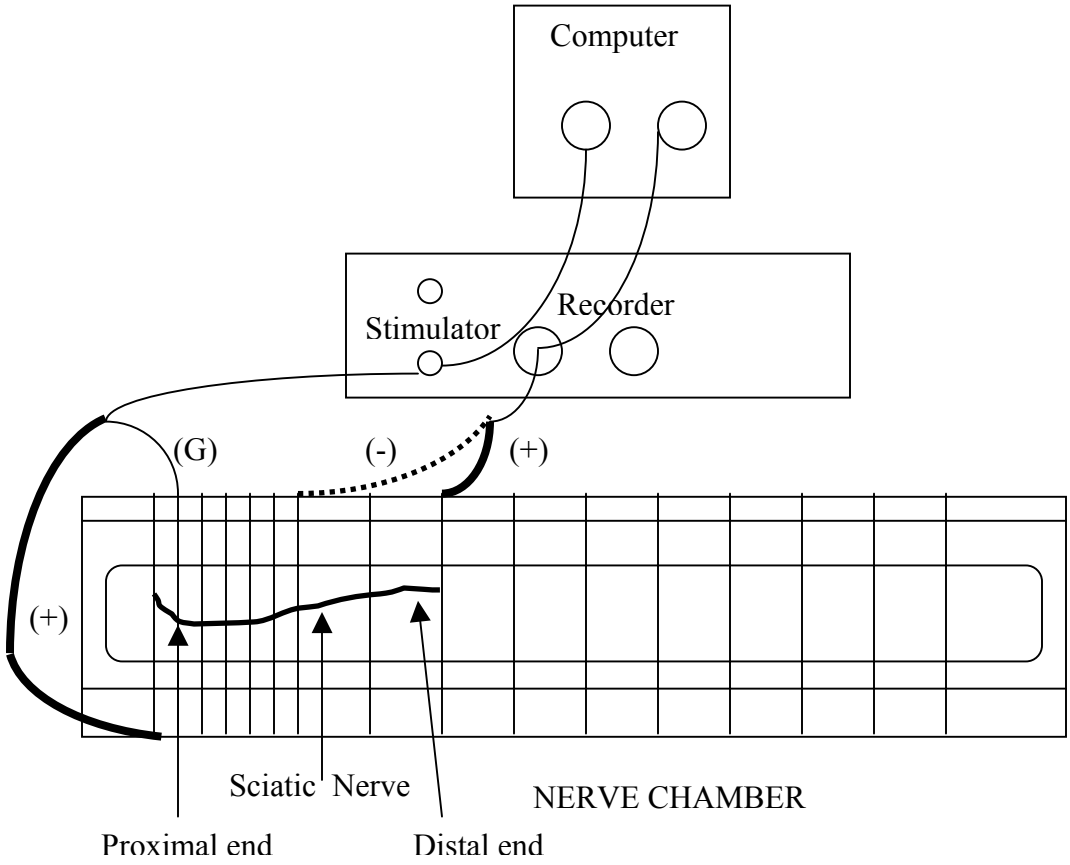

Figure 3. Proper setup of a nerve chamber with associated electrical cables, stimulator, and recorder. The proximal end receives the stimulus and the action potential is recorded at the distal end of the nerve.

Experimental Design

Two separate experiments were performed to test a magnet's ability to alter the action potential of a sciatic nerve. The first experiment involved isolating a sciatic nerve and calculating the amplitude, time of activation, and

duration of the action potential. The settings used for testing these variables are shown in Table 6. The threshold potential of the sciatic nerve was also determined; the settings are recorded in Table 7. The quadrapole magnet depicted in Figure 2 was then applied to the sciatic nerve in the chamber for approximately fifteen minutes. Shortly after, the amplitude, the duration of the action potential, and the threshold potential were measured using the same variables used in determining the initial readings of the nerve. The mean amplitude, duration, and activation time were determined by averaging three trials in which each trial contains five action potentials. The threshold potential was recorded in three separate trials.

Table 6.

Default settings used in measuring the amplitude, time of activation, and duration of the action potential.

SETTINGS FOR AMPLITUDE AND DURATION CALCULATIONS

Settings	Stimulator:	**Mode**	Step
		Output	Repetitive
		Marker channel	OFF
		# of steps	1
		Start	When recording starts
		Delay	0 ms
		Range	20 ms
		Step width (ms)	100
		Pulse duration (ms)	0.15
		Output Range (mV)	500
		Start Level (mV)	0
		End Level (mV)	150
	Trigger	**Source**	User
		Stop	Fixed duration, 1s
	Input Amplifier	**Range (mV)**	500
		Positive checkbox	Checked
		Negative checkbox	Checked
		Low pass filter	OFF
		Mains filter checkbox	Unchecked
		AC checkbox	Checked
	Basic settings	**One channel**	

		View ratio	1:50
		Sampling rate (kHz)	40

Table 7.

Default settings used in measuring the threshold potential of the action potential.

		SETTINGS FOR THRESHOLD DETERMINATIONS	
Settings	Stimulator:	**Mode**	Step
		Output	Once only
		Marker channel	OFF
		# of steps	51
		Start	When recording starts
		Delay	0 ms
		Range	20 ms
		Step width (ms)	25
		Pulse duration (ms)	0.15
		Output Range (mV)	500
		Start Level (mV)	0
		End Level (mV)	100
	Trigger	**Source**	User
		Stop	Fixed duration, 1s
	Input Amplifier	**Range (mV)**	500
		Positive checkbox	Checked
		Negative checkbox	Checked
		Low pass filter	OFF
		Mains filter checkbox	Unchecked
		AC checkbox	Checked
	Basic settings	**One channel**	
		View ratio	1:50
		Sampling rate (kHz)	40

In the second experiment, the magnets were applied to the nerve for approximately three hours. Both sciatic nerves from a bullfrog were extracted and the amplitude, time of activation, and duration of the action potential, and threshold potential were determined. After determining the initial readings for both nerves, one nerve was placed in a petri dish containing amphibian Ringer's solution without a magnet while the other nerve was placed in a similar

environment with the quadrapole magnet hovering over the nerve. Because of the time between readings, both nerve bundles required higher voltages and a longer duration to witness an action potential and to calculate the threshold potential. During the second experiment, several settings, summarized in Table 8, were changed to initiate an action potential. The mean duration, amplitude, and time of activation was determined by averaging four trials in which five action potentials were elicited for each trial. The threshold potential was determined during four different trials.

Table 8.

Settings changed to initiate an action potential from the nerves with and without exposure to magnetic fields.

Time	Variables Tested	Instrument Used	W/ Magnets	W/O Magnets
			Settings Changed	
T= 0 hrs	Threshold Potential	Stimulator Trigger	**# of steps** = 76 **End level** = 150mV **Stop**: fixed duration, 4s	**# of steps** = 84 **End level** = 185mV **Stop**: fixed duration, 4s
T= 3 hrs	Amplitude, time of activation, and duration	Stimulator	**End level** = 300mV	**End level** = 300mV
	Threshold Potential	Stimulator Trigger	**# of steps** = 101 **End level** = 300mV **Stop**: fixed duration, 4s	**# of steps** = 101 **End level** = 300mV **Stop**: fixed duration, 4s

CHAPTER III

RESULTS

To test the effect of magnetic fields on the action potential of a compound action potential, the duration, the time of activation, the amplitude of the action potential and the threshold potential were determined in two experiments. In the first experiment, a bullfrog sciatic nerve was isolated and the aforementioned variables were tested and measured for a magnet-exposed nerve and a control nerve. A quadrapole arrangement of magnets was aligned over the sciatic nerve resting in the nerve chamber. After ten to fifteen minutes of magnetic field stimulation, the action potential was recorded for further analysis.

The duration of the action potential was recorded as the amount of time between the initial increase in potential, shortly after the opening of the Na^+ channels, and the instant in which the potential, following hyperpolarization, matches the value recorded for the initial depolarization. As seen in Figure 4a, the difference between the duration of the action potential prior to and during the application of the magnets is significant ($p = 0.000656$). The amplitude measured for each action potential was determined by identifying the highest electrical potential reached following stimulation. In comparing the amplitude of the nerve

prior to and during magnetic field stimulation, one can observe significantly higher amplitudes (p=0.001534) during the application of magnets (see Figure 4b).

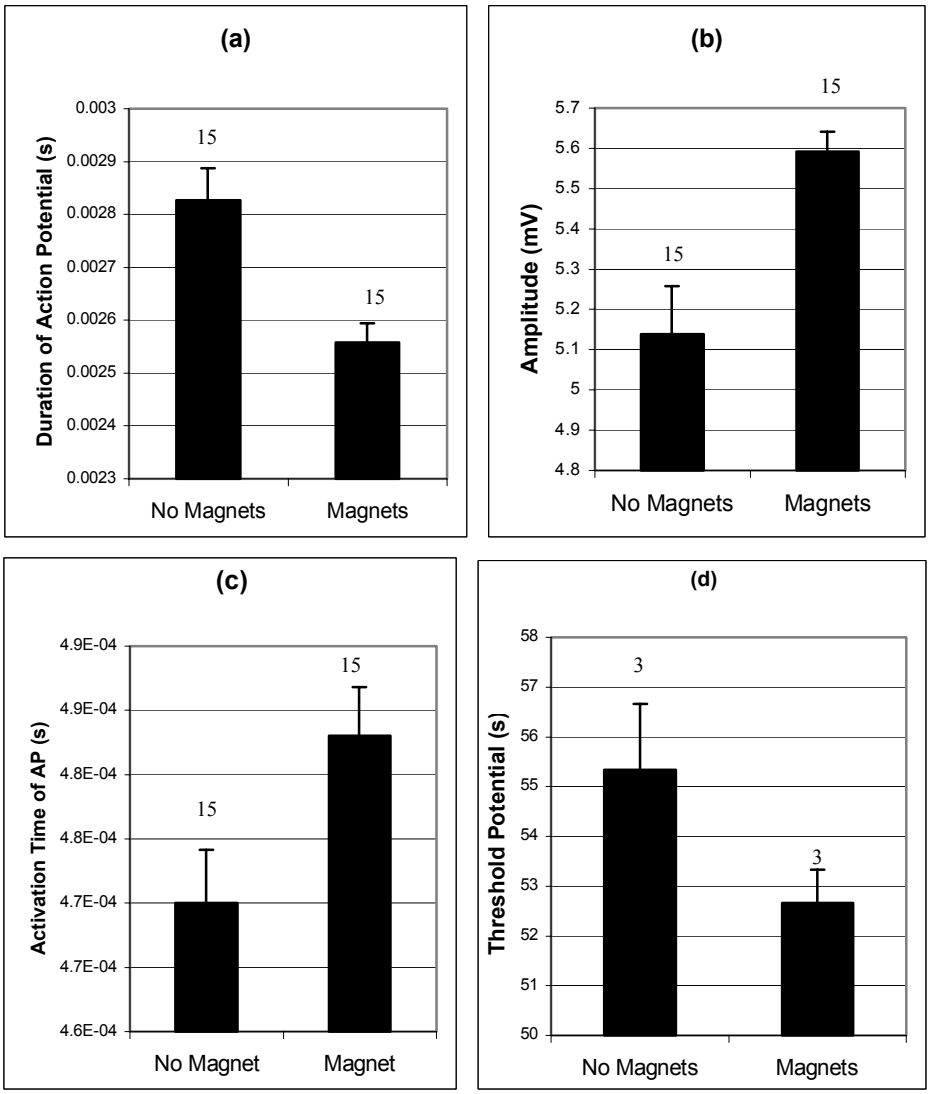

Figure 4. Mean action potential (a) duration (b) amplitude (c) activation time and (d) threshold potential (+1SE) prior to and during the application of static magnetic fields. Numbers located above the standard error bars represent the number of action potentials recorded for that particular group.

The time required to activate an action potential after a stimulus was also recorded. This variable was measured as the amount of time between the stimulus and the initial increase in the action potential. Figure 4c displays the significant difference (p=3.9E-06) between the activation times recorded prior to and during the time in which the magnets were introduced to the compound nerve. The final variable tested during the initial experiment was the threshold potential of the nerve. The determination of the threshold potential required different settings in comparison to the calculation of the aforementioned variables. After using the settings and applying the stimuli, the threshold potential was identified as the potential that initiated any slight, yet obvious increase in electric potential. As illustrated in Figure 4d, the difference in the threshold potential prior to and during the presence of magnets is not significant (p=0.14815).

During the second experiment, magnets were applied for a greater length of time, three hours, to try to identify the effects of time on the variables associated with nerve physiology. One nerve from a frog was used as a control and the action potentials were recorded at times zero and three hours. Another nerve from the same frog was introduced to magnetic field lines. The action potentials from this nerve were also recorded at times zero and three hours. In this experiment, the control frog nerve at time three hours did not produce typical action potentials. Because of the unusual action potential, only three groups were tested based on three of the four previously mentioned variables, the control nerve at t=0, the nerve with magnets at t=0, and the nerve with magnets at t=3 hours. Figures 5 a-c illustrate the results after recording the duration, amplitude, and time

of activation of an action potential. The threshold potential was determined for all four samples, including the control nerve at time three hours. As seen in Figure 5d, a significant difference ($p<0.01$) exists between the threshold potentials

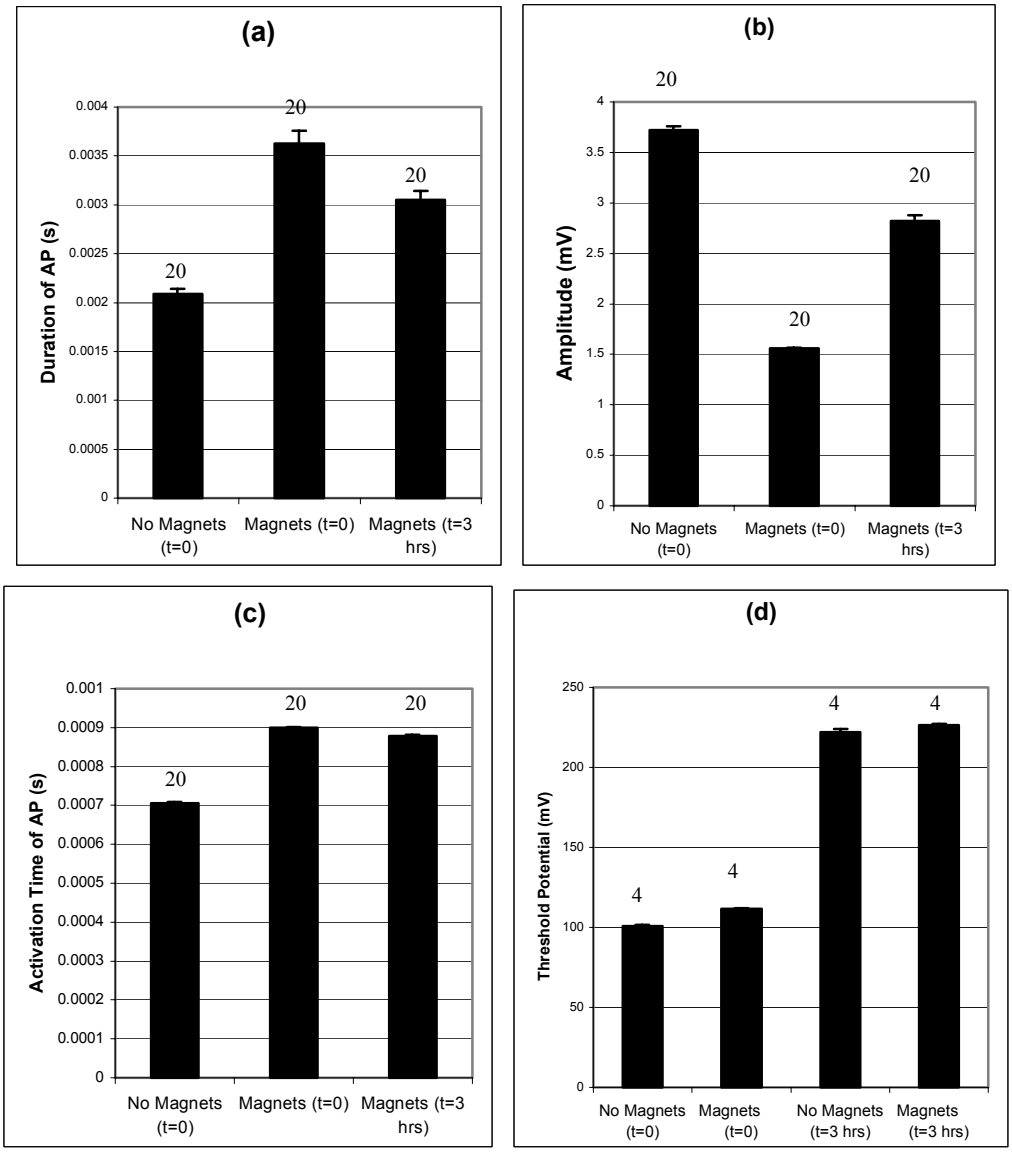

Figure 5. Mean action potential (a) duration (b) amplitude (c) activation time and (d) threshold potential (+1SE) for a magnet-exposed nerve and control nerve at times zero hours and three hours. Numbers located above the standard error bars represent the number of action potentials recorded for that particular group.

recorded at times zero and three hours. Statistical analysis also suggests that the threshold potentials for the nerve with magnets are significantly greater ($p<0.01$) than the control nerve lacking magnetic field exposure. However, the increase in threshold potential can not be attributed to the magnets, because the difference in the change in threshold potential for the two nerves over the three hour period is not significant ($p=0.21$).

As previously mentioned, the action potentials recorded for the control nerve at time three hours are unusual. The action potential found in Figure 6 is representative of a typical action potential. Figure 7 contains an unusual action potential. As seen in Figure 4, the potential following the stimulus gradually increases back to zero before slowly dipping to a potential of –1 mV. In contrast, Figure 3 shows that the potential quickly returns to zero following stimulation and then increases significantly.

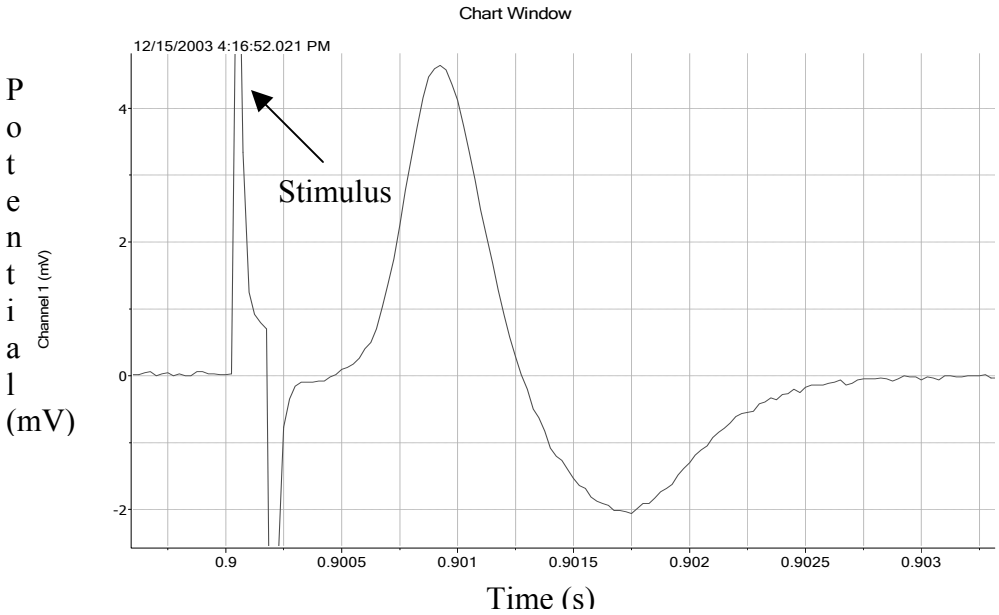

Figure 6. A typical action potential recorded during the first experiment prior to the addition of magnets.

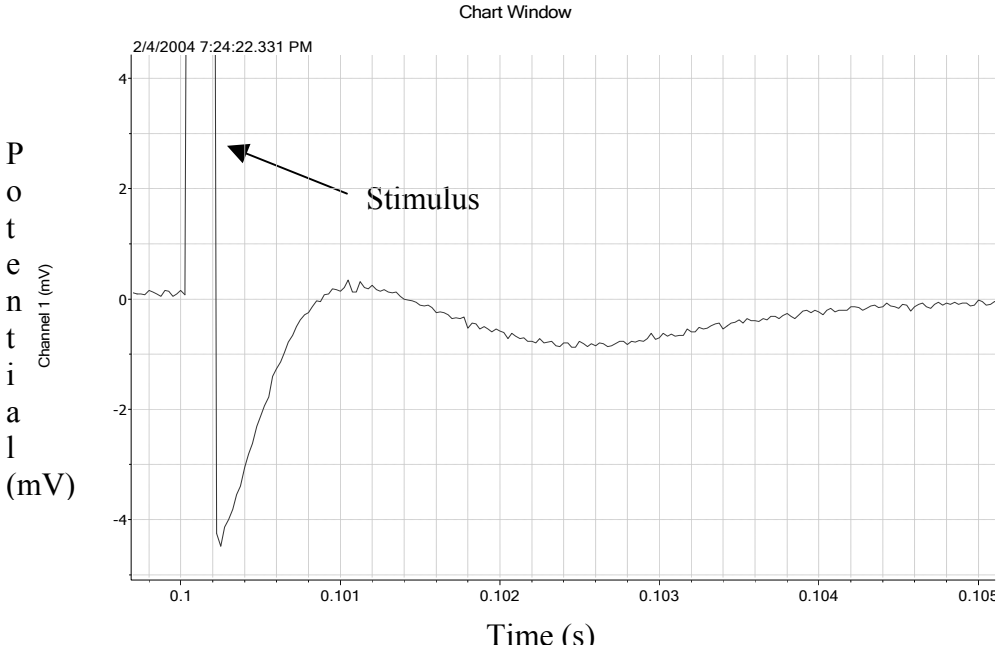

Figure 7. An action potential recorded on the control nerve at time three hours.

CHAPTER IV

DISCUSSION

First Experiment

The first experiment conducted indicates that magnets may have an impact on several aspects of an action potential. As previously mentioned, the duration, amplitude, and time required to activate an action potential all were significantly different following the addition of the quadrapole arrangement of neodymium magnets. Assuming that the magnet caused the significant differences in the duration and amplitude, the results suggest that the magnets would cause an increase in pain.

The duration of the action potential decreases due to the presence of magnetic field lines. A decrease in duration would suggest that action potentials would travel through the compound faster and thus reach the brain faster causing a quicker sensation of pain (Guyton & Hall, 2000). The decrease in duration corresponds with an article reported in the *Journal of Applied Physics*. Eguchi, Ueno, and Tatsuoka (2003, p.6742) noted that "membrane excitation during the recovery process in the relative refractory period was affected by the magnetic fields just after sodium channels were inactivated." This suggests that the

potassium channels open faster under the presence of magnetic fields, thus, decreasing the duration of the action potential.

The results from the first experiment indicate an increase in the amplitude of the action potential due to the presence of magnets. This information suggests that the amount of sodium entering the cell during an action potential increases due to an increase in the number of voltage-gated sodium channels stimulated in the compound nerve (Guyton & Hall, 2000).

Even though the difference in the threshold potential was not significant, the relationship between the threshold potentials recorded before and after the exposure to magnets suggests that magnets cause an increase in the number of receptors stimulated in the sciatic nerve. Furthermore, this would suggest that magnets would cause a decrease in the amount of force required to elicit an action potential and thus pain. More specifically, the voltage sensors on the voltage-gated sodium channels become more sensitive and react quicker to slight changes in membrane potential (Rosen, 2003, p.163).

However, a quick analysis of the results from the first experiment suggests that magnets cause an increase in the amount of time required to open the sodium channels, elicit an action potential, and thus register the feeling of pain. These results are also supported in the literature. According to Rosen (May 2003, p.517), a 150 second exposure to a 125 mT static magnetic field causes "an increase in the activation time constant, tau(m)."

Second Experiment

The second experiment was designed to test the effects of magnetic field exposure over a longer period of time. Action potentials were recorded for a control nerve and a magnet-exposed nerve at times zero and three hours. Because of the lack of a true action potential recorded on the control nerve after three hours, no conclusions can be made regarding the effectiveness of magnets in decreasing the number of action potentials elicited. Furthermore, no conclusions regarding the relationship between the three of the four measured variables (duration, activation time, and amplitude) of the magnet-exposed nerve at times zero and three hours can be made because the settings for the two times were different. However, the threshold potential for the control nerve at time three hours was recorded. The magnets did not have a significant effect on the increase in the threshold potential. This corresponds with the results seen in the first experiment concerning the effect of magnetic field exposure on threshold potential.

The change in electric potential characteristic of the control nerve at time three hours does not initially appear to be an action potential. However, when analyzing the threshold potential of the nerve at time three hours, one observes a change in the pattern of electric potential following an increase in the intensity of the stimuli applied to the sciatic nerve. The electric potential slowly increases from a large negative potential to zero. This is then followed by a slight decrease in potential before returning to a resting membrane potential. It appears as if the time required to activate the action potential and thus open the sodium-gated

channels has reduced to the point that the sodium-channels are opened as soon as the stimulus is applied. The quick opening of the sodium channels may explain why the electric potential doesn't surpass a zero potential. The slight decrease in potential could be due to repolarization and the opening of the potassium-gated channels. It is possible that these unusual action potentials are due to the lack of magnetic field lines penetrating the sciatic nerve. This information, combined with the results found in the first experiment, suggests that magnetic field lines may help to maintain the health of the sciatic nerve. A better explanation for these strange results could be due to the mishandling of the control nerve while placing the nerve in the nerve chamber.

Possible Mechanisms

In studying membrane potential and voltage-gated ion channels, two possible mechanisms explaining the effects of magnetic fields on action potentials seem most logical. One possible mechanism involves the effects of magnetic fields on the phosphorylation of specific sites on the voltage-gated sodium channel. Several articles have suggested that magnetic fields may influence the rate of phosphorylation of specific protein kinases (Sun, Yu, Fu, Chiang, Xie, & Lu, 2002). The presence of paramagnetic molecules such as magnesium and manganese may alter the proper functioning of the protein kinases; thus, increasing the rate of phosphorylation. As seen in Figure 8, the sites of phosphorylation in the voltage-gated sodium channels are located between the first and second domains, where some research suggests that the protein sequence controls the acceleration of slow inactivation (Goldin, 2003). According to Nosek

(n.d.), when these sites are phosphorylated, the rate of inactivation increases. Furthermore, studies have been shown that slow inactivation greatly influences the firing of action potentials (Denac, Mevissen, & Scholtysik, 2000).

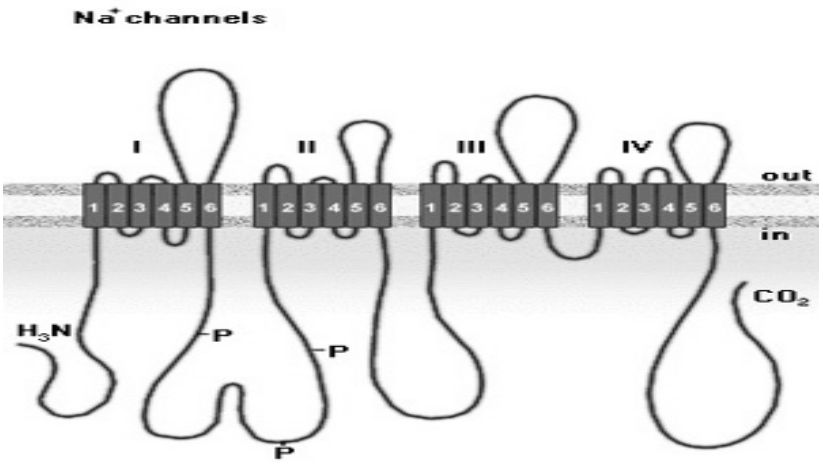

Figure 8. Two-dimensional representation of a typical sodium channel with four domains (I, II, III,& IV) of six transmembrane alpha-helices. Between domains I and II lay several sites of phosphorylation. (Picture from http://imc.gsm.com/demos/hpdemo/program/section1/1ch4/asdip10f.htm)

This could mechanistically explain the results of Cavopol, Wamil, Holcomb, and McLean (1995) that the number of action potentials elicited decreases under the presence of a quadrapole magnet. In this experiment, no conclusions regarding magnetic fields potential influence on slow inactivation can be made due to the variables measured.

The second possible mechanism by which magnetic fields may have an influence on action potentials involves the voltage sensor. There are four voltage

sensors (S4), the fourth alpha-helix in each domain, which control the pore through which sodium ions flood into the cell. Each voltage sensor contains several positively-charged amino acids including lysine and arginine. This

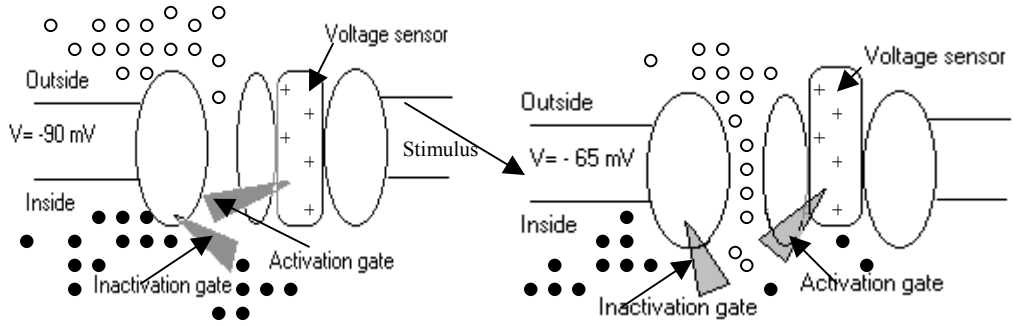

Figure 9. A two-dimensional representation of the movement of the voltage sensor and opening of the activation gate and inactivation gate following a stimulus. The white circles represent sodium ions and the black circles are potassium ions.

portion of the protein responds to changes in the electric potential across the cell membrane. As seen in Figure 9, the application of a stimulus increases the membrane potential causing the voltage sensor to move up toward the extracellular matrix. This in turn causes the opening of the activation gate. The mechanism by which the voltage sensor opens the activation gate is unclear. Furthermore, it is uncertain as to how magnetic fields may influence the voltage sensor. If indeed magnets affect the voltage sensor, all of the measured variables, except the duration of the action potential, would change due to the action of the voltage sensor.

The increase in amplitude suggests that the magnetic field exposure increased the number of voltage gates opened. In other words, the magnets may have caused more voltage sensors to respond to the applied stimulus. The decrease in threshold potential seen in the first experiment suggests that the number of gates of a given cell required to influence the initiation of an action potential of a neighboring cell is decreased. The increase in activation time suggests that the magnetic fields produce a force which prevents the movement outward of the voltage sensor and thus the opening of the activation gate. Rosen (Oct. 2003) also reports an increase in activation time due to the presence of static magnetic fields. There are currently many unanswered questions regarding the effect of magnetic fields on the action potential and on the voltage-gated sodium channel. The two previously mentioned mechanisms represent potential areas of research in trying to identify any possible effects of magnetic fields on the human nervous system.

Future Research Ideas

To gain a better understanding as to the possible effects of magnetic fields on action potentials, two areas of research must be conducted and then integrated to develop a solid understanding of the effects of magnetic fields on the transmission of action potentials. First of all, many more studies need to show consistent changes in the action potential of a nerve due to the presence of magnetic fields. More specifically, one must ask what specific changes in the action potential are due to the presence of magnetic fields. To answer this specific question, many variables must be controlled including temperature and

the moisture present on the nerve. Increasing the number of action potentials recorded and the use of better instruments in handling the sciatic nerves would greatly decrease the possible influences of external variables. These changes to the experiment would create more reliable data.

The second area of research should emphasize the molecular and physical interactions within the voltage-gated sodium, potassium, and calcium channels. Patch clamp analyses of calcium, sodium, and potassium channels have enhanced understanding of these channels' function at the molecular level. Altering of the magnetic field strength and gradient can establish a comparative approach to analyzing the voltage-gated channels. For instance, one can alter the magnetic field strength or gradient and easily determine the effects on sodium channels using patch clamp analyses. These changes in the physical nature of the magnetic field can aid in the understanding the function of various parts (including the voltage sensor) of the voltage-gated channels. Experiments designed to test the possible effects of magnetic fields on action potentials and voltage-gated channels could greatly influence how physicians view magnet therapy.

Arguably the most promising field of study involving the identification of a possible mechanism to relieving pain using magnets entails an analysis of the effects of magnetic fields on pain receptors. Several studies have suggested that magnetic fields cause analgesia, a decrease in pain reception (Thomas, Kavaliers, Prato, & Ossenkopp 1997). No mechanism regarding these analgesic effects has been elucidated. If magnetic fields influence the nervous system, the pain receptors are the first and most likely location of action. To completely

understand the effects of magnetic fields on the nervous system, studies involving pain reception and the transmission of action potentials must be investigated.

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