COMBINED OPTICAL AND ELECTRICAL STIMULATION OF NEURAL TISSUE *IN VIVO*

By

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CHAPTER I

INTRODUCTION AND BACKGROUND

Introduction

The activation of neural tissue *in vivo* is a technique with numerous, diverse applications both in the research and clinical arenas. Since the 1800s, electrical techniques have been the gold standard for stimulating and monitoring neural networks and their behavior [1-3]. Electrical stimulation is well suited for evoking action potentials in neurons. Not only is it easily controllable and quantifiable, but it is also reliable and energy efficient. There are, however, limitations to this technique. The size of electrodes and the spread of electrical current pose challenges for achieving spatial precision [4]. The presence of a stimulation artifact negates simultaneous stimulation and recording from nearby sites. There is also an inherent risk of damaging the neural tissue due to the necessity of contacting or penetrating the nerve with an electrode [5-8].

While electrical stimulation is the current standard for nerve activation, there are other methods of action potential initiation [9-14]. Ultrasound waves have been shown to activate neural structures. Gavrilov et al. demonstrated that focused ultrasound stimulates both superficial and deep neural receptor structures [10]. The mechanical effect attributed to ultrasound is proposed to result in changes in the neuron's permeability to ions, resulting in a depolarization of the membrane potential. Pulsed magnetic fields have also long been used to stimulate both the brain and peripheral nerves [11]. While it was originally thought that the induced electric field parallel to the nerve was responsible for activating peripheral nerves, it has been suggested that it is actually

the electric field component that is perpendicular to the nerve fiber that is ultimately responsible for inducing an action potential in a peripheral nerve [12]. It is also widely known that neurons may be activated and inhibited by chemical means [13]. While reports of action potential modulation using light date back to the 1950s, it was not until recently that the potential of this methodology began to be realized [14].

As early as 1994, studies showed that nerve excitation would occur for short ultraviolet pulses from an excimer laser with excitation energies near the photoablation threshold [15]. Recently, Wells et al. have demonstrated that compound action potentials may be stimulated in peripheral nerves by pulsed infrared light without causing thermal damage to the neural tissue [16-18]. In addition, infrared neural stimulation (INS) does not include many of the inherent limitations of electrical stimulation. Where electrical stimulation is limited by a stimulation artifact, the necessity of contact and limited spatial precision *in vivo*, INS is demonstrated to be an artifact-free, contact-free and spatially precise nerve stimulation modality [19]. However, INS suffers from a limited range of radiant energies for safe stimulation. The radiant exposure (laser energy per unit area) required for onset of irreversible thermal tissue damage is only about two times greater than that to achieve stimulation [18].

The motivation for this work is to demonstrate a new stimulation paradigm that combines the most attractive properties of electrical nerve stimulation and INS. Our hypothesis is that nerve excitability may be enhanced by applying a subthreshold electrical stimulus concomitantly with INS, while simultaneously mitigating the risk of tissue damage due to INS and maintaining spatial precision. This combined approach will also increase the efficiency of INS. The power requirements needed to produce

sufficient light for neural stimulation inhibit the implantation of a laser source for a neural prosthetic. By reducing the requisite light energy necessary for stimulation, the power requirements for INS will be reduced but the spatial precision will be maintained. Improved efficiency will enhance opportunities for implantable applications of this technology that may otherwise have been impossible to achieve.

Background

The Nervous System

The nervous system is what gives each human a unique personality and many other characteristic behaviors and traits, in addition to the ability to carry out coordinated voluntary and involuntary movements and biological processes. The body gathers sensory information from the environment. This information is then propagated to the brain or spinal cord, where all of the sensory information is processed and integrated so the correct response can be initiated [20]. The nervous system is divided into two main systems (Figure I.1), the central nervous system (CNS) and the peripheral nervous system (PNS). There also exists a visceromotor nervous system (VNS) which contains parts of both the CNS and PNS. The CNS consists of the brain and spinal cord, which are encompassed and protected by the brain and vertebral column, respectively.

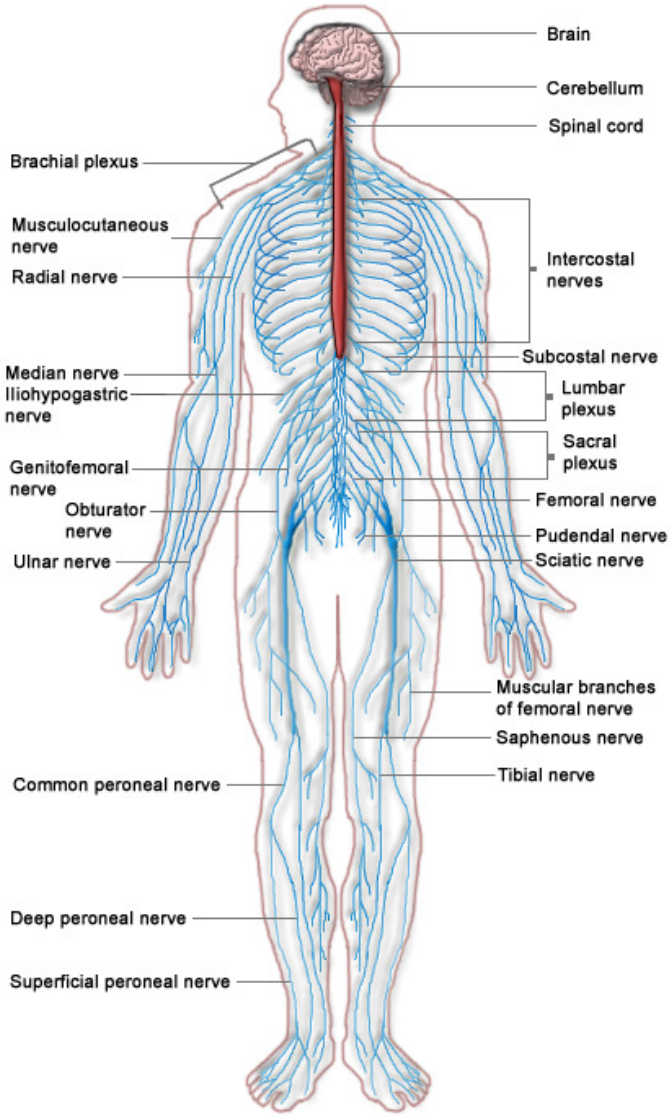

Figure I.1. General depiction of CNS and PNS. The CNS is shown in red and the PNS is shown in blue.

The function of the PNS is to connect the elements of the CNS with peripheral structures, such as muscle and epithelium. It is the PNS that transmits sensations from the sensory receptors back to the CNS, in addition to delivering signals prescribing motor functions to effectors in the musculature. Motor fibers of the PNS leave via the ventral

root of the spinal cord and carry efferent (away from the CNS) signals to effectors in the peripheral structures. Sensory PNS fibers enter the spinal cord via the dorsal root and bring afferent (towards the CNS) information such as pain, temperature, proprioception, etc. to the spinal cord and brain. The basic structure of both the CNS and PNS is the neuron. Neurons consist of a cell body with emanating structures known as dendrites, a single, longer process known as an axon, and synapses where the axon terminates.

The electrical signals transmitted within the nervous system propagate via axons (Figure I.2A). Electrical signals arrive at the dendrites and cell body of a neuron, where the signal is subsequently propagated down the axon. Most axons are wrapped in myelin, which acts as an insulator to allow the signals to propagate much faster. In general, the axon diameter (0.1-20 μm), the thickness of the myelin (up to 70% of total fiber diameter), and the distance between the nodes of the myelin sheath (nodes of Ranvier) (200-2000 μm) are directly related to the conduction velocity of the axon [21]. Conduction velocities of nerve fibers vary by fiber type. A-type fibers are myelinated, larger diameter fibers (2-20 μm) with conduction velocities ranging from 12-120 m/s. B-type fibers are also myelinated, but with smaller diameters than A-type fibers (< 3 μm). The conduction velocities of B-type fibers range from 3-15 m/s. C-type fibers are unmyelinated, small diameter fibers (0.3-1.3 μm) with conduction velocities ranging from 0.5-2.3 m/s [22]. The nodes of Ranvier play an important role in increasing the conduction velocity of myelinated fibers, by allowing the wave of depolarization to "jump" in discrete steps from node to node.

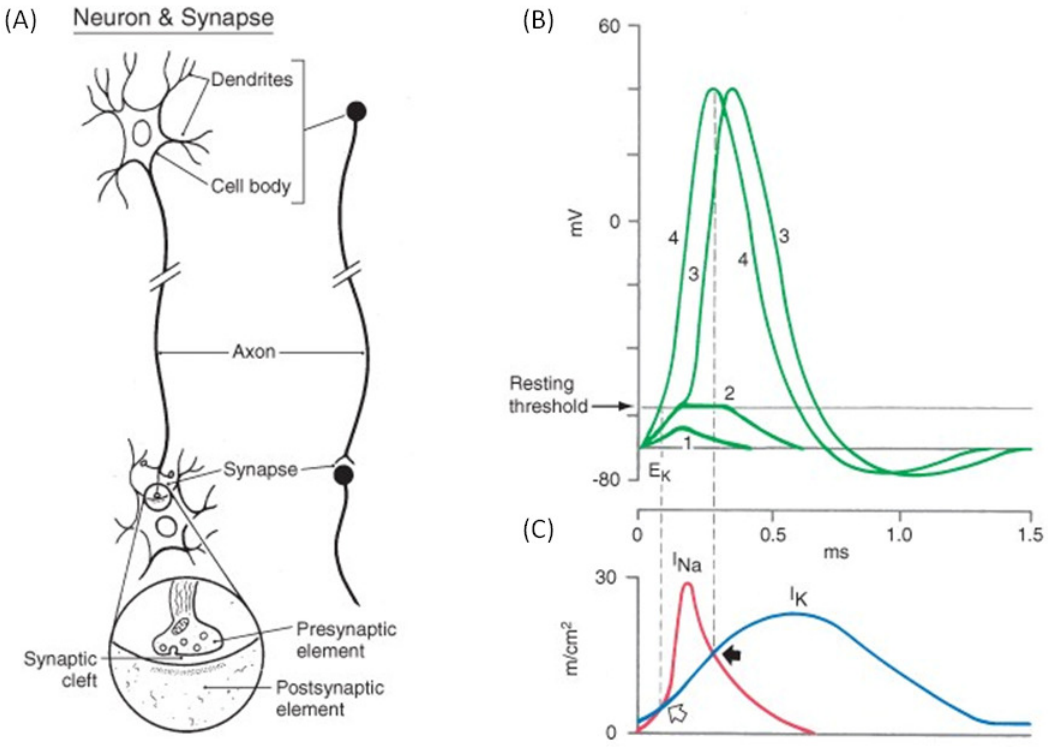

Figure I.2. Diagram of neuron with responses to various stimuli. (A) Neuron cell body, axon and synapses; (B) Responses due to (1) subthreshold, (2) just below threshold, (3) above threshold and (4) superthreshold stimuli; (C) Timing of sodium and potassium currents. From [20].

At rest, a neuron exists at a steady state, with osmotic forces across the cell membrane balanced and the concentration gradients of ions to which the membrane is permeable offset by a resting voltage. Na^+/K^+-ATPase (or Na^+/K^+ pump) also plays a role in the balance of osmotic forces and stabilization of membrane voltage by actively pumping three Na^+ ions out of the cell and 2 K^+ ions into the cell. The membrane is more permeable to K^+ than Na^+, so K^+ tends to diffuse out of the cell while Na^+ stays in the cell. This creates a net loss of ions out of the cell, which the Na^+/K^+ pump counteracts.

This helps to maintain the cell's resting potential by keeping a high concentration of K^+ and a low concentration of Na^+ inside the cell. This balance results in a transmembrane potential ranging from -70 to -90mv, which is described by the Goldman equation:

$$E_m = \frac{RT}{F} ln \left(\frac{\sum_i^N P_{ion_i^+} [ion_i^+]_{out} + \sum_j^M P_{ion_j^-} [ion_j^-]_{in}}{\sum_i^N P_{ion_i^+} [ion_i^+]_{in} + \sum_j^M P_{ion_j^-} [ion_j^-]_{out}} \right) \quad (1)$$

where E_m is the membrane potential (V), P_{ion} is the permeability for a given ion (M/s), $[ion]_{out}$ is the extracellular concentration of a given ion (mols/m^3), $[ion]_{in}$ is the intracellular concentration of a given ion (mols/m^3), R is the ideal gas constant, T is absolute temperature and F is Faraday's constant [20]. When this potential is depolarized beyond a given threshold (Figure I.2B), the voltage-gated Na^+ and K^+ channels in the plasma membrane will open. Following their chemical gradients, Na^+ rushes into the cell and K^+ flows out. At first, the Na^+ influx is greater than the K^+ efflux, which results in a positive-feedback mechanism where the Na^+ current opens more Na^+ channels. The newly opened Na^+ channels do not stay open indefinitely (Figure I.2C), as they undergo inactivation, where they cannot be opened again for a finite duration of time (several milliseconds). The time that the Na^+ channels are inactive is known as the refractory period and serves the purpose of promoting unidirectional propagation of the depolarizing wave. The membrane K^+ and Cl^- conductances peak after the Na^+ conductance and will result in a dampening and limited hyperpolarization of the transmembrane potential.

This propagating wave of depolarization and repolarization across the plasma membrane of a neuron is known as an action potential. Action potentials propagate along nerve fibers to synapses, relaying pertinent information and commands from sensory receptors and the CNS. Synapses are the specialized structures where axons

communicate with other neurons, muscles and cells. An arriving action potential causes the release of a neurotransmitter such as acetylcholine, norepinephrine and dopamine into a gap between the axon and the postsynaptic element. The neurotransmitter diffuses rapidly across this gap and binds to receptors on the postsynaptic element. Depending on the neurotransmitter, this process will have either an excitatory or inhibitory effect on the postsynaptic neuron, muscle or cell [20]. In an unmyelinated axon, the action potential will propagate continuously, whereas the propagation of an action potential in a myelinated axon occurs quickly due to the discreet "jumps" between the nodes of Ranvier. Peripheral nerve fiber bundles consist of numerous axons sending efferent and/or afferent signals via the propagation of these action potentials.

Electrical Stimulation

Electrode-based techniques have long been the prominent method for activating, modifying, recording and studying neurons and their behavior [1-3]. While depolarization of a neuron is usually accomplished via synaptic currents, a propagating action potential may also be generated following artificially induced depolarization of the transmembrane potential via an electrical current. To induce an action potential in an axon, an electrode must be placed in contact with the axon or nerve fiber bundle. In order for an action potential to be generated, the transmembrane potential of a neuron must be depolarized beyond a threshold. This threshold may also be described as the minimum amplitude or pulse duration of the electrical stimulus necessary to induce an action potential, which directly determines the amount of charge transferred at the cathode [23]. The amplitude of the electrical stimulus may either be a controlled voltage or controlled current. Both controlled-voltage and controlled-current are commonly used

for various applications. Current-controlled stimuli do present several advantages, however, as the stimulation thresholds remain more or less constant and the injected charge can be accurately calculated. The accurate calculation of the injected charge is significant as it determines the electrode voltage across the phase boundary. Regulating this voltage allows for stimulation to be conducted without electrochemical reactions and tissue damage occurring at the electrode-tissue interface [24]. The pulse duration of the electrical stimulus is also important as it is known that shorter duration pulses require a greater amplitude stimulus to start an action potential [25, 26]. Although longer pulse durations limit the current amplitude, the total charge transfer may lead to tissue damage [27]. The charge threshold also increases with increasing pulse duration. Pulse durations on the order of 10-100 μs are generally used in most neuroprosthetic applications [23].

Electrical stimulation has rightly served as the gold standard for neural activation. It is an easily controlled modality having a wide range of parameters that may be tailored for a given application. Current, voltage, pulse duration and repetition rate are all parameters that are easily varied and measured. The waveform of stimulation and the electrode configuration are also important parameters to be considered. Monophasic stimulation is very effective at stimulating neural tissue, but results in tissue damage due to electrochemical reactions (i.e. corrosion). Biphasic waveforms are often employed in an effort to mitigate tissue damage and are primarily used for long-term stimulation. The first phase of the waveform evokes stimulation while the second phase reverses the electrochemical reactions. Biphasic waveforms may also be altered such that the phases are charge balanced or charge imbalanced, delayed, or have fast or slow reversal. Each of these waveforms has its own set of advantages and disadvantages. Merrill et al. have

conducted an excellent comparison of stimulating waveforms, ranking their effectiveness in action potential initiation, tissue damage and corrosion [28]. These waveforms may be delivered via various electrode configurations, primarily monopolar and bipolar electrodes. Monopolar electrodes exhibit lower stimulation thresholds, while bipolar electrodes reduce current spread. The preferred electrode configuration is completely dependent on the application [29-31]. In addition to being easily controlled and finely tuned, electrical stimulation is generally accurate, reliable, reproducible and relatively precise when compared to other stimulation modalities such as chemical, mechanical or magnetic. Electrical stimulation is also clinically used in various applications including, the restoration of motor function, seizure control and deep brain stimulation [23, 32, 33].

While electrical stimulation has served as the gold standard of neural stimulation, both in research and in the clinic, there are several inherent limitations to its effectiveness. One such limitation is the necessity of contact between the electrode and the neurons. While it is intuitive that intracellular electrodes that impale the neuron will cause damage, extracellular electrodes also pose risks of tissue damage. Damage due to electrical stimulation is of two primary types: mechanical damage due to the mere presence of the electrode and damage due to electrical stimulation [5]. Mechanical damage includes abrasion due to tension on the nerve or movement between the nerve and electrode. Damage due to electrical stimulation includes interstitial edema and early axonal degeneration and may result from electrochemical reactions at the electrode-tissue interface. In chronic stimulation scenarios, interstitial edema may occur at two days and early axonal degeneration after one week if parameters are not properly optimized. The presence of a stimulation artifact also limits the utility of electrical stimulation in

particular research applications, as it makes recording the evoked potentials much more difficult and prohibits recording from a site in the vicinity of the site of stimulation [9]. Perhaps the most restrictive aspect of electrical stimulation is its limited spatial precision due to current spread [34, 35]. Although the threshold for stimulation increases as the square of the distance from the tip of the electrode, there are many adverse effects stemming from unwanted current spread [36-38]. Two examples are the small but significant number of cochlear implant patients who experience stimulation of their facial nerve by current originating from their cochlear implant and the limited number of independent frequency bands that can be encoded in state of the art cochlear implants [39, 40]. Although it has long served as the gold standard of artificial neural activation, the inherent limitations of electrical stimulation open the door for the development of improved stimulation modalities.

Optical Stimulation

The ability to modulate and even evoke action potentials in neurons using light has been known for decades, but recently this method of neural stimulation has become much more promising with the discovery of caged compounds, Channelrhodopsin-2 and infrared neural stimulation (INS) [14, 15, 41, 42]. Caged compounds are introduced into the cytoplasm of cells or tissues and render biomolecules inert until they are released at high yield and sufficient speed following photolysis at a wavelength of light that will not damage the biological tissue [42]. Photostimulation of caged glutamate is a popular method of studying and mapping functional circuitry; however it is limited by its spatiotemporal resolution [43]. It has been demonstrated that introducing microbial light-

sensitive proteins such as *Chlamydomonas reinhardtii* Channelrhodopsin-2 (ChR2) or *Natronomonas pharaonis* halorhodopsin (NpHR) into neurons allows for neuronal depolarization at millisecond-timescale temporal resolution [41, 44]. Whereas stimulation of caged compounds and microbial opsins inherently require exogenous additives and/or engineered neurons, INS is a neural stimulation modality where pulsed, low-intensity infrared light focused on a nerve will generate a propagating action potential within an endogenous neural system.

The significance of INS is that it is not subject to the limitations of electrical stimulation. Specifically, INS provides a contact-free, artifact-free and spatially precise means of neural stimulation with radiant exposures (J/cm^2) below the threshold for laser-induced damage [16-19]. Figure I.3 summarizes the advantages of INS in comparison to electrical stimulation. First, electrical stimulation requires contact with the nerve bundle (Figure I.3A), whereas the delivery of light to the nerve from the optical fiber is contact free, as illustrated in the cartoon in Figure I.3B. Second, the compound nerve action potentials (CNAPs) in each of the nerve fasicles stimulated electrically contain a stimulation artifact (Figure I.3A), while INS lacks any stimulation artifact (Figure I.3B). Finally, the overall emphasis of the diagram is the superior spatial resolution of INS compared to electrical stimulation. It can be seen that INS recruits a significantly smaller population of axons when compared to electrical stimulation. This is exhibited not only by the smaller magnitude of the CNAP from the nerve fasicle stimulated by INS, but also by the fact that only the gastrocnemius fasicle is stimulated by INS, whereas both the gastrocnemius and the biceps femoris fasicles are stimulated electrically. The underlying mechanism of INS is yet to be elucidated, but preliminary investigations suggest that it is

a phenomenon mediated by thermal gradients arising when tissues absorbs infrared light [45].

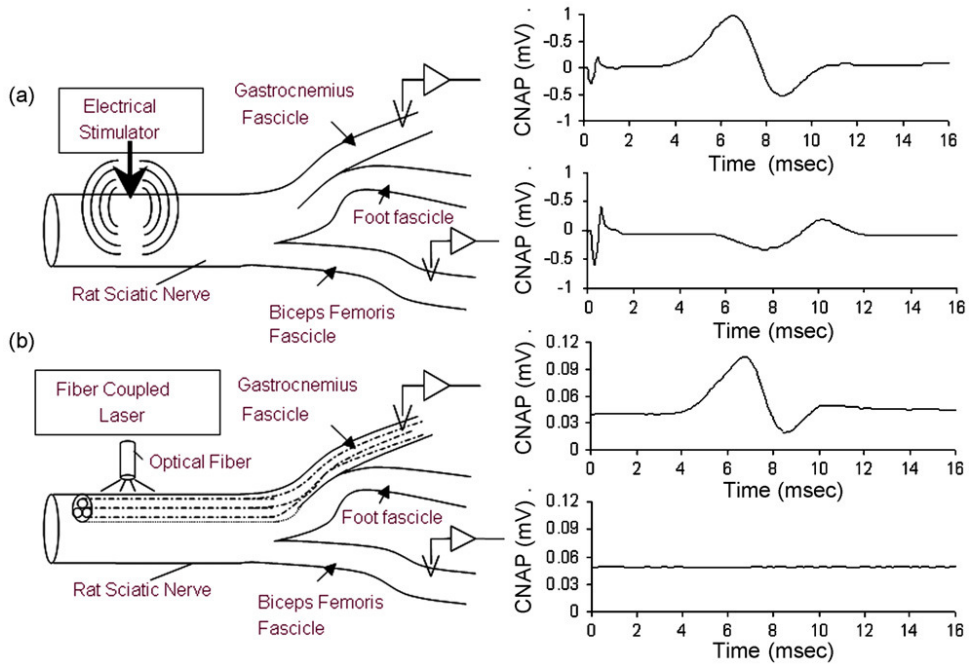

Figure I.3. Illustration of the limitations of electrical stimulation and the benefits of INS. Threshold electrical stimulation (a) necessitates contact, evokes an action potential in both the gastrocnemius and biceps femoris fasicles, and is accompanied by a stimulation artifact. Threshold INS (b) is contact free and spatially precise as only the gastrocnemius fasicle is targeted. Note the lack of stimulation artifact in (b), the contact-free nature of INS and the relative magnitudes of the CNAPs in (a) and (b). From [19].

Infrared neural stimulation is so named for the infrared light applied incident to the neural tissue, evoking an action potential. INS has been successfully demonstrated for many wavelengths including 2.1, 2.12, 3.0, 4.0, 4.5, 5.0 and 6.1μm. The rationale for the selection of these wavelengths is their effective penetration depths in tissue. As

biological soft tissue, including neural tissue, is predominantly composed of water, the effective penetration depth in tissue may be approximated from the curve showing the absorption of light in water (Figure I.4). In addition, for each of these wavelengths a safety ratio has been defined as the ratio of threshold radiant exposure (J/cm^2) for damage to threshold radiant exposures for stimulation (defined as induction of a visible muscle twitch upon stimulation in the rat sciatic nerve) [16]. For the rat sciatic nerve, the best combination of penetration depth and safety ratio is achieved using a holmium:YAG laser (λ =2.12 μm). At this wavelength, the optical penetration depth in neural tissue is ~ 400 μm with the onset of irreversible damage at 2 Hz stimulation occurring for radiant exposures that are twice that needed for stimulation (0.66-0.70 J/cm^2 for damage as opposed to 0.34-0.48 J/cm^2 for stimulation). The probability of damage was also shown to increase for greater repetition rates (5 and 8 Hz) [16, 18].

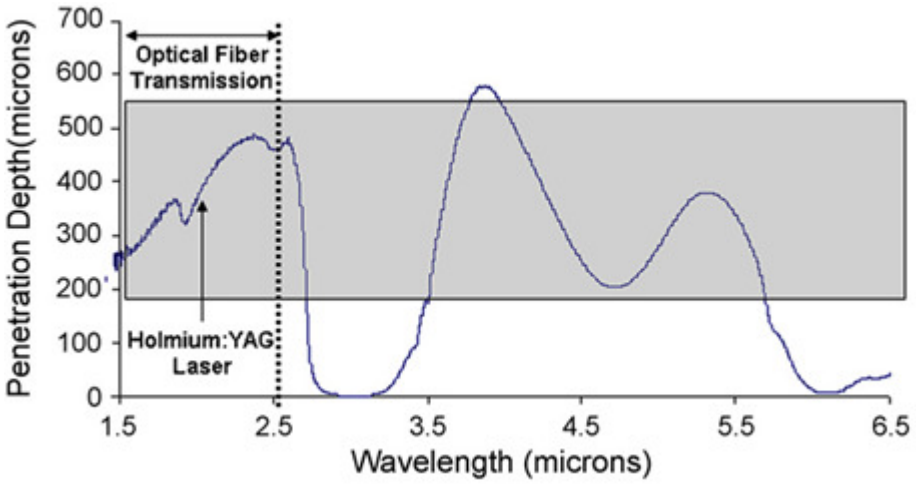

Figure I.4. Penetration depth in the rat sciatic nerve as a function of wavelength. The shaded area represents the theoretically optimal window for INS of the sciatic nerve. Optical fibers may be employed for transmitting wavelengths of light within the specified region. From [19].

Excitement for INS is growing in situations where spatially precise nerve stimulation is necessary. Several niches for INS have successfully been found both in therapeutic and diagnostic applications. One of the most promising applications of INS is stimulation of the cochlea and auditory nerve for improved cochlear implants [46, 47]. Current spread from cochlear implants is a significant problem limiting the spatial selectivity of electrical stimulation and the accurate reproduction of auditory signals. Izzo et al. have shown that INS stimulates the auditory neurons directly (as opposed to the inner hair cells) in a spatially precise manner unmatched by electrical methods of stimulation [47]. At short pulse widths (35μs), spiral ganglion cells require very low radiant exposures to achieve stimulation (mJ/cm^2) and may be safely stimulated for hours at high repetition rates (10^1-10^2 Hz) without inducing damage [48].

Along with auditory prosthetics, cavernous nerve monitoring is an application well suited for INS. The cavernous nerves on the surface of the prostate are responsible for erectile function. Therefore it is imperative that these nerves be located during prostate resections in order to maintain sexual function. Electrical stimulation has been used intra-operatively for mapping the cavernous nerves, but has proven to be inconsistent and unreliable due to the necessity of contact, limited spatial precision and stimulation artifact. Recently, Fried et al. have demonstrated that INS provides a contact-free and spatially precise viable alternative for mapping the locations of these nerves [49, 50]. A significant increase in intracavernosal pressure was found to accompany INS of the cavernous nerve and return to baseline following cessation of stimulation. The optimal INS parameters for non-damaging cavernous nerve mapping were reported at wavelengths of 1860-1870 nm, radiant exposures greater than 0.35 J/cm^2 at a pulse rate

of 10 Hz [51]. Fried et al. are also currently developing a laparoscopic probe for clinical use [52]. Cavernous nerve mapping and cochlear stimulation are but two of the applications of INS currently pursued. Other applications arising from stimulation of nerves such as the facial nerve, vagus nerve and the central nervous system are being developed [47, 50, 53, 54].

Motivation and Significance

While INS provides solutions to many of the problems limiting electrical methods of neural stimulation, it is not a perfect answer. There are two main drawbacks of INS which limit its application. First and foremost is the limited range of radiant exposures which can safely stimulate nerves. As previously mentioned, the radiant exposure required for the onset of irreversible laser-induced damage to the rat sciatic nerve is only about twice that of the radiant exposure needed to achieve stimulation [18]. Secondly, high laser power requirements for INS are impractical. In order to enhance the applicability and clinical utility of INS, both of these limitations must be addressed.

By either decreasing radiant exposure needed for neural stimulation or increasing the radiant exposure which will cause irreversible laser-induced damage, the window for safe and effective INS may be markedly enhanced. This will not only improve the attractiveness of INS in the eyes of researchers and clinicians alike, but it could potentially increase the functionality of INS. With a greater window of safe and effective stimulation, INS will pose less of a risk for tissue damage *in vivo*, particularly in chronic applications. The risk of damage to neural tissue is a common concern shared by experts and skeptics alike. By demonstrating that the radiant exposures required for stimulation

can be significantly reduced below those required to cause damage, most, if not all, of the concern may be alleviated.

The significance of the efficiency of INS is pertinent to applications requiring an implantable laser source. Currently, the power requirements to generate the amount of infrared light needed to reach stimulation thresholds prohibit the development of implantable laser sources, as typical diode lasers operate at 30% efficiency and require tens of amps of input current. However, vertical-cavity surface-emitting lasers (VCSELs) may provide a viable option for implanting INS technology. VCSELs revolutionized the data communication industry in the 1990's as their performance characteristics, reliability and performance/cost ratio allowed for low cost, high data rate communication over short distances. Recently VCSEL technology has been expanded to include a wider range of available wavelengths as well as varieties of arrays and package form factors. VCSELs emitting at appropriate wavelengths for neural stimulation will allow INS to be implanted in arrays of more than 100 hundred lasers on a single chip. These VCSELs have narrow spectral width, low power consumption, and packaging flexibility making them attractive for INS applications [55]. VCSELs operating in pulsed mode have been shown to only provide milliwatts of average power, whereas the optical power currently needed for INS in the peripheral nerve is on the order of watts [56]. Although excitement for INS is growing as each new application is investigated, increasing the breadth of safe stimulation as well as reducing the power requirements of INS systems will greatly expand interest in the field.

Hypothesis and Objectives

Our research group comprised of members of the Biomedical Engineering and Neurosurgery departments at Vanderbilt University have recently discovered the potential of using pulsed, low-intensity infrared light to evoke action potentials in frog and rat sciatic nerves [16, 17]. Since this discovery, interest in INS has quickly grown and numerous, well-suited applications are currently under investigation including the development of improved cochlear implants, the identification of the cavernous nerve during prostate resection, stimulation in the CNS, vagus nerve stimulation and dorsal root rhizotomies [47, 50, 53]. Although INS provides a contact-free, artifact-free and spatially precise alternative to electrical stimulation, it is limited by the ratio of damaging to safe radiant exposures, as well as the impractical power requirements necessary for implantable neural prosthetics. By mitigating or removing these limitations, the attractiveness and effectiveness of INS will be greatly enhanced. Fortunately, both limitations are not completely independent and may thus be addressed simultaneously.

While INS improves upon the drawbacks of electrical stimulation, there is no fundamental reason to believe that the two modalities are mutually exclusive. We hypothesize that infrared light applied in conjunction with subthreshold electrical stimulation will increase the window of safe INS, while maintaining its superior spatial precision characteristics. Specifically, we hypothesize that a subthreshold pulse of electrical stimulation applied concomitantly with infrared light will lower the threshold for optical stimulation. This will be accomplished as the electrical pulse will enhance the excitability of the neural tissue to infrared light by transiently depolarizing the membrane potential to just below the threshold for an action potential. The additional optical energy

bringing the membrane potential above threshold will result in stimulation having the same spatial precision associated with INS alone but requiring significantly less optical energy. By lowering the threshold for INS, a larger ratio of damaging to safe radiant exposures will become available and the reduced amount of infrared light needed will lower diode power requirements for INS.

There are three main objectives to this study. The first is to demonstrate feasibility of the combined optical and electrical stimulation modality. Once feasibility is established, the subthreshold electrical stimulus will be varied to determine how much optical energy (reported as a percentage of INS threshold) is necessary to achieve stimulation when applied simultaneously with a given subthreshold electrical stimulus. The relationship between electrical stimulus and additional optical energy required will then be evaluated and any implications to the mechanism of INS will be considered. Finally, the pulse of infrared light will be delayed in relation to the electrical pulse to determine if there exists an optimal pulse synchronization. The results of this final objective may also lead to a better understanding of the mechanism of INS.

The overall goal of this thesis is to provide the framework by which a combined optical and electrical stimulation modality may be developed. The results of the study are expected to prove feasibility of this stimulation paradigm, provide a relationship for the relative contributions of each individual modality and determine the optimal synchronization between the times at which the pulses are delivered. In addition, the results are expected to provide focus to the investigation into the mechanism of INS.

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CHAPTER II

COMBINED OPTICAL AND ELECTRICAL STIMULATION OF NEURAL TISSUE *IN VIVO*

Introduction

Driven by recent research demonstrating the advantages of stimulating neural tissue with infrared light, applications of infrared neural stimulation (INS) ranging from stimulation of the auditory system for improved cochlear implants to cavernous nerve mapping during prostate resections are generating significant interest [1-8]. While electrical stimulation has long been the method of choice for stimulating and monitoring neural activity, INS is a capable alternative unhindered by several fundamental limitations inherent to electrical stimulation. Specifically, INS provides a contact-free, artifact-free and spatially precise neural stimulation modality [6, 7]. While INS is a superior modality for spatially selective *in vivo* applications, it is limited by a relatively narrow window for safe stimulation. Wells et al. have shown that radiant exposures (J/cm^2) generating <1% probability of laser-induced thermal damage are only a factor of 2 greater than radiant exposures needed for stimulation (defined as the induction of a visible muscle twitch following stimulation of the sciatic nerve in the rat) [9]. In order for INS to be applied at higher repetition rates or radiant exposures much greater than threshold, it is imperative that the range of radiant exposures for safe, yet effective stimulation be extended. Additionally, implantable INS stimulators may be limited by the laser power necessary for stimulation. Reducing the power requirements of the INS stimulator will facilitate the transition of INS technology into an implantable device.

To avoid these limitations, we hypothesize that the nerve excitability to INS may be enhanced by applying a subthreshold electrical stimulus concomitantly with the delivery of pulsed infrared light, thus lowering the threshold for optical stimulation while maintaining spatial precision and mitigating the risk of laser-induced tissue damage. By reducing the threshold radiant exposure of infrared light needed to achieve stimulation by nearly 4-fold, we have greatly increased the safe and effective range of INS. Also, implicit in lowering the required optical energy per pulse is the reduction in the power required by the INS stimulator. In order to prove this concept, we first varied the magnitude of the subthreshold electrical stimulus to determine the relationship between the electrical stimulus magnitude and the requisite amount of additional optical energy to achieve stimulation. We then investigated the relative delay of the infrared pulse relative to the electrical pulse to determine the optimal pulse synchronization for minimizing the optical energy required. Finally, it was confirmed that the spatial precision of INS is maintained for this combined optical and electrical stimulation modality.

Methods

Male Spraque-Dawley rats (276-300g) were anesthetized with 50% urethane (1.5g/kg IP), and the sciatic nerve was exposed from the pelvic cavity to the knee. Saline was continuously applied for the duration of the experiments to prevent dehydration of the nerve.

The system diagram used for these experiments is shown in Figure II.1. An electrical stimulator (Grass S44; Grass Medical Instruments, Quincy, MA) was connected to a bipolar hook electrode placed under the main trunk of the sciatic nerve. A pulsed

infrared diode laser source (Lockheed Martin Aculight Capella^{TM}) was coupled to a 400 μm diameter-optical fiber (Ocean Optics). The distal end of the fiber was cut, flat-polished and positioned directly above the nerve and approximately 700 μm from the surface of the nerve, in the same location as the electrode. Using the knife edge technique, the laser spot size on the nerve was determined to be 0.3584 cm^2 [10]. The wavelength of the diode laser was set to 1.875 μm. The pulse duration for both electrical and optical stimulation were 2 ms, which was dictated by the minimum pulse duration needed to get sufficient pulse energy out of the 5W laser diode. Pulses were delivered at a repetition rate of 2 Hz for all experiments. The electrical stimulator and the diode laser were synchronized by a digital delay generator (Stanford Research Systems, DGD-535) and both pulse waveforms were displayed on a digital oscilloscope to monitor the relative time of the pulses. The Nicolet Spirit^{TM} Evoked Potentials System was used for electrophysiological evaluations. Needle electrodes were paired and inserted into both the biceps femoris and gastrocnemius in a bipolar configuration.

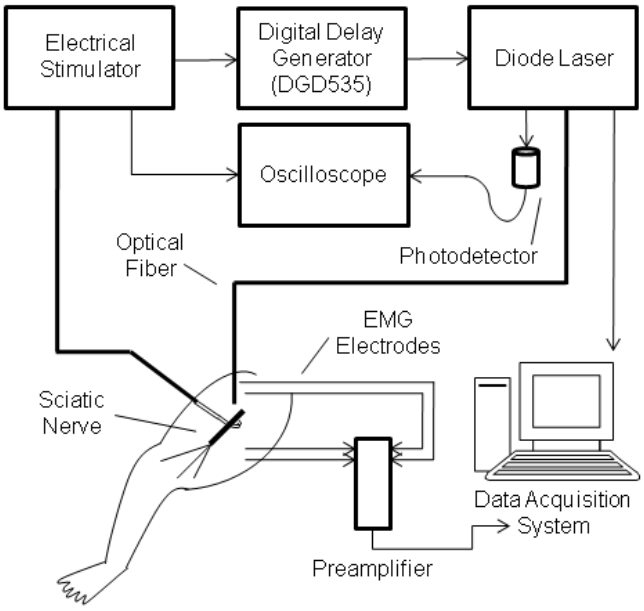

Figure II.1. Schematic representation of the experimental setup used for all experiments in this study.

For the first experiment, the electrical and optical pulses were delivered with no relative delay. After finding the electrical stimulation threshold, the electrical stimulus was reduced below threshold to a known amount (i.e. 90% of threshold). An optical stimulus was then applied concomitantly with the electrical stimulus, and its magnitude was increased until reaching threshold. Finally, the electrical stimulus was removed and stimulation threshold was found using only INS. This process was repeated in order to establish a relationship describing the relative amounts of electrical and optical energies needed to reach threshold. In the second experiment, electrical stimulation threshold was determined and subsequently reduced to 90% of threshold. Using the digital delay generator, the arrival of the optical stimulus was delayed relative to the electrical stimulus

and the amount of additional optical energy needed to achieve stimulation was found as before.

Results

Figure II.2A demonstrates the effects of combining electrical and optical stimulation into a single stimulation modality. Individual data points reflect the amount of optical energy (expressed as a percentage of INS threshold) required to reach the stimulation threshold when applied concurrently with a given electrical stimulus (expressed as a percentage of electrical stimulation threshold). The best-fit line models the data incorporating the known endpoints where 100% of either modality alone is required to reach stimulation threshold. Interestingly, the data does not fit a linear relationship. Rather, the required optical energy can be predicted by

$$O = 0.24 \ln(1 - E) + 1 \quad (2)$$

with $R^2 = 0.54$, where O is the optical energy expressed as a percentage of INS threshold and E is the magnitude of the electrical stimulus expressed as a percentage of electrical stimulation threshold. Inter- and intra-animal variability is expected in this type of *in vivo* experiment. Also, as the electrical stimulus approaches the electrical stimulation threshold, additional variability may be added due to small fluctuations in electrical stimulation threshold resulting in action potential firing. The results indicate that if the electrical stimulus is applied at 95% of the electric threshold, then the optical threshold will be reduced by a factor of nearly 4 according to equation (2). For 80% or 90% of threshold, the optical threshold is reduced by 1.64 fold and 2.22 fold, respectively. This reduction in optical threshold significantly increases the window for safe INS as less

energy is required to stimulate, thereby reducing the heat load in the tissue. If the ratio of damage threshold to stimulation threshold for INS alone is assumed to be approximately 2:1, as reported by Wells et al., then we can predict that applying an electrical stimulus at 90% of electrical stimulation with INS will increase this ratio to almost 4.5:1 [9]. For electrical stimuli at 80% and 95%, the ratio is predicted to be approximately 3.25:1 and 7.1:1, respectively. Threshold radiant exposures for INS alone ranged from 1.24 - 2.33 J/cm^2. Combined with a subthreshold depolarizing electrical stimulus, radiant exposures were reduced to 0.1 – 2.1 J/cm^2.

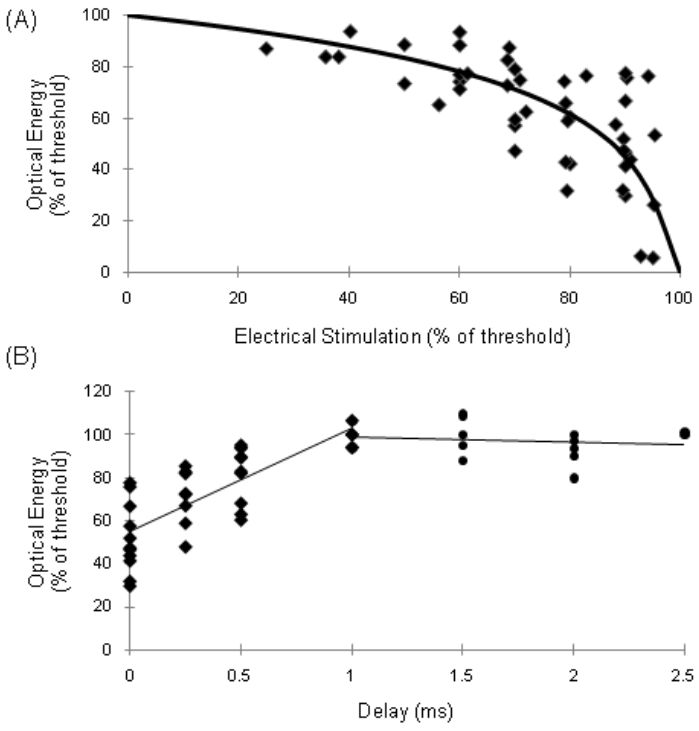

Figure II.2. Results of combining optical stimulation with electrical stimulation; (A) Additional optical energy (% of threshold) required to reach threshold as a function of subthreshold electrical stimulus; and (B) Additional optical energy (% of threshold) required to reach threshold as a function of delay between electrical (90% of threshold) and optical stimuli.

Figure II.2B demonstrates the effects of delaying the arrival of the optical stimulus relative to the electrical stimulus. The results indicate that the greatest benefit is achieved when the pulses are delivered at the same time. For delays up to 1 ms, there are still benefits of applying an electrical stimulus with INS; however the amount of radiant exposure necessary for stimulation increases linearly. For delay times > 1 ms, there are no benefits of combining the modalities as 100% of the optical threshold is needed to achieve stimulation. Figure II.3 illustrates that the spatial selectivity of INS is preserved in this combined stimulation modality. Note how the stimulated compound muscle action potential (CMAP) is only present in one muscle group.

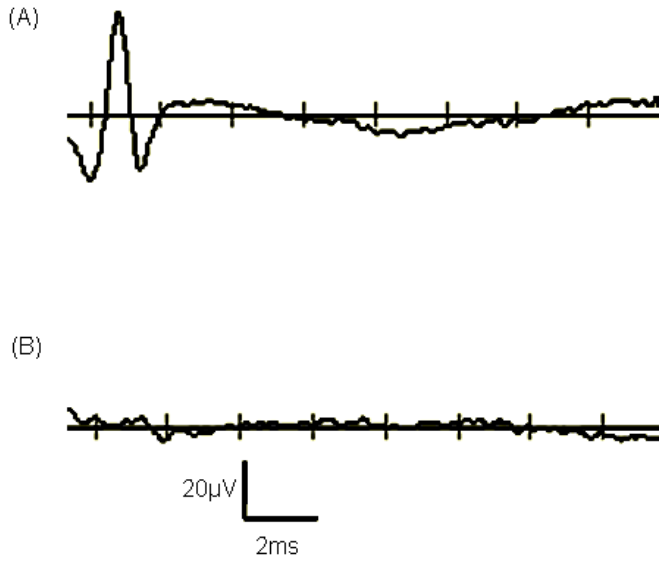

Figure II.3. Spatial selectivity is maintained with combined optical and electrical stimulation. (A) Average of 20 consecutive recordings of CMAPs from electrodes placed in gastrocnemius; and (B) the same recordings as (A) for electrodes placed in biceps femoris.

Discussion and Conclusions

The results of this study demonstrate proof of concept for a combined optical and electrical nerve stimulation modality. The results confirm the hypothesis that delivery of a subthreshold electrical stimulus concurrently with INS will lower the required optical energy per pulse to achieve stimulation. This suggests that the ratio of safe to damaging radiant exposures of INS may be increased by the simultaneous delivery of a subthreshold electrical stimulus. Not only are these findings practical for the further developments of INS technology, they may also shed light on the underlying mechanism of INS. A particularly significant aspect of these results is that the reduction of INS threshold for a given subthreshold electrical stimulus does not follow a linear trend (Figure II.2A). This implies that electrical stimulation and INS do not function by the same mechanism. Otherwise, one would expect that a linear superposition would achieve stimulation as is seen when two simultaneous electrical pulses are combined [11]. A number of scenarios may explain this data, one of which is the presence of a mediator between the transient temperature gradient induced by INS and the opening of ion channels that operate independently of the direct stimulation of voltage-gated Na^+ channels [12].

The concept of delivering a subthreshold electrical stimulus to enhance the excitability of neural tissue to an added electrical stimulus is not a foreign concept [11, 13, 14]. The mechanism by which threshold changes occur as a result of a subthreshold stimulus has been explained by a mathematical model of induced ionic currents with enhanced excitability primarily following membrane potential. Persistent and transient Na^+ currents initiate "superexcitability;" Na^+ channel inactivation, decay in the leakage

current and activation of outward K^+ currents (primarily slow K^+ channels) cause the decline in excitability over time [14, 15]. However, the change in threshold for subsequent electrical stimuli is not linear whereas our data shows a linear change in optical stimulation threshold with increasing delay time [11, 13, 14].

The linear decrease in the change in optical threshold we see with increasing pulse delays is not entirely unexpected and may be explained in light of published results. Pulse durations used to study the effects of latent addition on thresholds for CMAPs are much shorter than what we have used. Bostock et al. have shown that as the pulse duration increases, the nonlinear decay in the threshold change slowly begins to look more linear [11]. The 2 ms pulse we used for our experiments is 10 times longer than what was used for their study. Therefore it is likely that the linear decay in the change in optical threshold would mirror that for a conditioned electrical stimulus following a 2 ms subthreshold depolarization. The justification for this hypothesis relies on the assumption that the subthreshold depolarizing stimulus contributes to the optical and electrical stimuli according to the same mechanism. It is hypothesized that this contribution will vary similarly in time for a given subthreshold electrical stimulus.

While the results of this study are very encouraging and highlight the advantages of a combined optical and electrical stimulation modality, a better fundamental understanding of this new stimulation paradigm is necessary for further development. Primarily it must be determined whether a combination of a subthreshold electrical stimulus with a subthreshold optical stimulus will result in less tissue damage than INS alone. The effects of laser-induced tissue damage from INS are well characterized, but the damaging effects of electrical stimulation are not as clear – especially in the context

of a combined stimulation modality [9]. It remains to be determined whether there exists an optimal combination of optical and electrical stimuli parameters which will minimize tissue damage.

Our data emphasize the obvious and practical benefits of combined optical and electrical stimulation. Further evaluation of the available parameters is necessary, but the proof of concept is evident. The subthreshold electrical stimulus clearly reduces the potential risks of laser-induced damage without interfering with the spatial precision inherent to INS (Figure II.3). These results will facilitate the development of implantable INS stimulators by reducing required laser power, as well as benefit researchers needing a safe, spatially precise stimulation modality. While the benefits of applying INS concurrently with electrical stimulation suggest that ionic currents contribute to the mechanism of INS, the differences in excitability between delayed optical and electrical stimuli following an initial subthreshold depolarizing stimulus indicate that the mechanism of INS is more involved.

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CHAPTER III

CONCLUSIONS AND FUTURE DIRECTIONS

The results of this study demonstrate proof of concept for a combined optical and electrical nerve stimulation modality. The results confirm the hypothesis that delivery of a subthreshold electrical stimulus concurrently with INS will lower the required optical energy per pulse to achieve stimulation. We have also shown that by delaying the optical pulse relative to the electrical pulse, the change in threshold for INS follows a linear trend. These findings will be instrumental in the further development of implantable INS stimulators, as well as targeting the underlying mechanism of optical stimulation.

As hypothesized, the pulse energy for optical stimulation may be reduced by the simultaneous application of a subthreshold electrical stimulus. It is interesting to note, however, that the summation of the electrical and optical stimuli (relative to the stimulation thresholds for each modality) does not total 100% of the sum of the individual modality thresholds. Of course if a second electrical pulse was applied concurrently with a subthreshold depolarizing electrical stimulus, then the magnitude of the second pulse (expressed as a percentage of threshold) would be equal to the percentage of threshold for the depolarizing stimulus subtracted from 100%. Indeed this intuition has been confirmed previously [1]. This is not the case for the combination of optical and electrical stimuli as Figure II.2A illustrates. Instead, equation (2) provides an empirical relationship between the INS stimulus (as percentage of INS threshold) needed in addition to a given subthreshold electrical stimulus to achieve stimulation. An

important inference from this relationship is that INS and electrical stimulation do not work by the same mechanism that simply superimpose. There are a number of potential explanations for this relationship, one of which is the presence of a mediator. This mediator may exist in the form of a thermally activated protein affecting ligand-gated ion channels or a thermally activated transmembrane ion channel akin to the TRPV family, with numerous other potential possibilities. It is difficult to make any more specific speculations as to the mechanism given our results, but these findings may help to target its further investigation.

The concept of delivering a subthreshold electrical stimulus to enhance the excitability of neural tissue to an added electrical stimulus is not a foreign concept [1-3]. The mechanism by which threshold changes occur as a result of a subthreshold stimulus was recently explained by a mathematical model of induced ionic currents. The enhanced excitability of neural tissue was shown to primarily follow the membrane potential. Persistent and transient Na^+ currents initiate the period of enhanced excitability. Na^+ channel inactivation, decay in the leakage current, and activation of outward K^+ currents (primarily slow K^+ channels) cause the decline in excitability over time [3]. This ionic response to a subthreshold stimulus is independent of the second stimulus. Therefore, we know that this response is occurring for the subthreshold depolarizing electrical stimulus in our experiments.

Along with a nonlinear relationship between the optical and electrical stimuli needed to reach stimulation threshold, it was also found that delaying the optical pulse relative to the electrical stimulus produced a linear decrease in the change in optical threshold (Figure II.2B). This is particularly noteworthy when compared to reports in the

literature of similar experiments where a sub(or supra-)threshold conditioning stimulus was delivered in combination with an additional test electrical stimulus [1-3]. The conditioned threshold current was shown to decay nonlinearly as a function of delay between conditioned and test stimulus for short pulse durations (Figure III.1A).

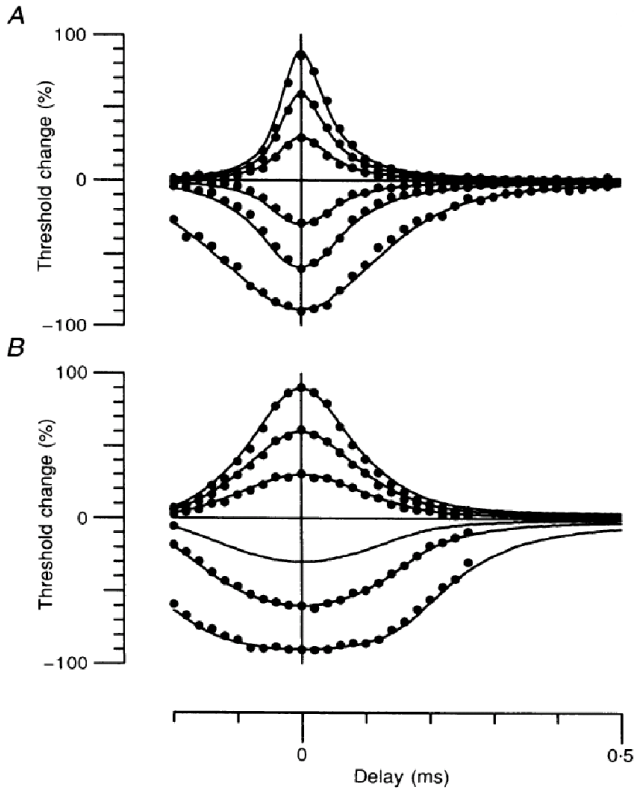

Figure III.1. Estimated conditioned threshold current (expressed as percentage change from unconditioned threshold) needed to elicit a compound motor action potentials as a function of delay between conditioned and test electrical stimuli. (A) Conditioned stimuli were delivered as 90, 60, 30, 0, -30, -60 and -90% of threshold with a pulse duration of 60 μs. (B) Same as (A) with a pulse duration of 200 μs. From [1].

For purposes of comparing with our data, the bottom traces of Figure III.1 are the threshold conditioned current given a conditioned stimuli at 90% of threshold.

Interestingly, when the pulse durations were extended as shown in Figure III.1B, the rate of decay for the change in threshold was decreased. Also, at these longer pulse durations, a linear fit may approximate the data over a substantial range of delays. Our pulse duration was set at 10 times those used to produce the results shown in Figure III.1B. Therefore, the linear trend seen in our results (Figure II.2B) is consistent with trends shown in Figure III.1B. At shorter pulse durations, it is expected that the optical stimulus needed to reach threshold will be more closely approximated by a nonlinear fit.

There is a fairly simple hypothesis as to why the optical stimulus needed to reach threshold will follow the same trend as the conditioned threshold current. In both situations there is a definite contribution by the "conditioning," or subthreshold depolarizing stimulus. If we assume that the contribution of this subthreshold stimulus to either a subsequent electrical or optical stimulus is due to the induced ionic currents as described above (but with different relative contributions to each modality), then it may be hypothesized that this contribution will vary similarly in time [3]. Therefore, one would expect the threshold change for an added electrical or optical stimulus to vary the same with delay between the conditioning and added pulse with other parameters held constant.

In order for a combined optical and electrical stimulation modality to be effective, not only must the ratio of damaging to safe radiant exposures be significantly increased, but stimulation must also maintain the selective precision inherent to INS. We have demonstrated that this combined modality does indeed maintain spatially precise stimulation (Figure II.3). While the subthreshold depolarizing stimulus was delivered to

a broad region and thus reaching all fasicles within the nerve, only axons receiving laser light were given the additional optical energy needed to reach threshold.

While our results indicate that the ratio of damaging to safe radiant exposures for INS may be significantly increased by the concurrent delivery of a subthreshold depolarizing electrical stimulus, further development and application of this new stimulation paradigm rely on a better fundamental understanding of the relationship between these two modalities. The foremost question that must be answered is whether there exists a combination of electrical and optical stimuli which will maintain spatial selectivity without damage induced by either the laser or the electrical stimulus. The application of this combined modality is moot if the combination raises the risk of tissue damage when compared to INS alone. The effects of laser-induced tissue damage from INS are well characterized, but the damaging effects of electrical stimulation are not as clear – especially in the context of a combined stimulation modality [4]. There are typically three main mechanisms by which damage may occur – mechanical tension or abrasion, neuronal hyperactivity, and irreversible reactions at the electrode [5, 6]. Research has suggested numerous factors that may be adjusted to mitigate and/or avoid damage as a result of electrical stimulation. These parameters include charge per pulse/phase, charge density, electrode size and material, stimulation duty cycle incorporating "on" and "off" periods of stimulation, and stimulation waveform among others [6-8]. We plan to take these factors into account in an effort to optimize the safety of combined optical and electrical stimulation.

There are additional questions we would like to answer in an effort to better understand and advance this concept. How the relative pulse durations of optical and

electrical stimuli will affect the change in INS threshold is an intriguing question we are interested in answering. Our current results do not provide any indications as to whether it is important for the optical and electrical stimuli to be of the same duration. We are anxious to investigate whether one pulse should be much shorter or much longer than the other for maximal reduction in INS threshold.

Along with varying the relative pulse durations, we would also like to further investigate the effects of delaying the optical stimulus relative to the electrical stimulus. By decreasing both the electrical pulse duration and delay step size, we expect to see results mirroring those of Figure III.1; although this is not known for certain. We would also like to deliver the optical stimulus prior to the electrical stimulus to find out whether the effects are symmetrical.

One of the most interesting and significant questions that must be answered is the spatial dependence between the optical and electrical stimuli. All of our experiments were conducted with the bipolar hook electrode placed under the nerve trunk and the laser spot focused on top of the nerve, directly above the electrode. Spatial dependence of the stimuli will depend significantly on the effects of current spread over a broad range. We would like to investigate whether the laser spot must be located in the exact region of the electrode, or whether there is some graded increase in INS threshold as the two stimuli are spatially offset.

Once we have fully parameterized this modality we would then like to translate our results into the development and testing of a combined INS and electrical stimulation probe. The probe will be capable of delivering various combinations of optical and electrical stimuli based on the results from this and future studies. Various electrode

configurations will be considered. It is hypothesized that a nerve-cuff electrode will exhibit the most desirable characteristics as cuff electrodes have been shown to demonstrate selective stimulation of nerve fasicles through shaping of the electric field [9, 10]. The use of a nerve-cuff electrode will also facilitate the true integration of the two stimulation modalities for a hybrid probe. Effectiveness of the probe will be evaluated using experiments similar to those described in this study. While the probe's design will be targeted towards research applications rather than chronic implantation, it is the goal of this technology to facilitate the implantation of INS. The results of this and future studies should make this goal possible.

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