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Pulsed laser versus electrical energy for peripheral nerve stimulation

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Abstract

Transient optical neural stimulation has previously been shown to elicit highly controlled, artifact-free potentials within the nervous system in a non-contact fashion without resulting in damage to tissue. This paper presents the physiologic validity of elicited nerve and muscle potentials from pulsed laser induced stimulation of the peripheral nerve in a comparative study with the standard method of electrically evoked potentials. Herein, the fundamental physical properties underlying the two techniques are contrasted. Key laser parameters for efficient optical stimulation of the peripheral nerve are detailed. Strength response curves are shown to be linear for each stimulation modality, although fewer axons can be recruited with optically evoked potentials. Results compare the relative transient energy requirements for stimulation using each technique and demonstrate that optical methods can selectively excite functional nerve stimulation. Adjacent stimulation and recording of compound nerve potentials in their entirety from optical and electrical stimulation are presented, with optical responses shown to be free of any stimulation artifact. Thus, use of a pulsed laser exhibits some advantages when compared to standard electrical means for excitation of muscle potentials in the peripheral nerve in the research domain and possibly for clinical diagnostics in the future.

Keywords

Optical stimulation; peripheral nerve; nerve; laser stimulation; laser; nerve stimulation method

1. Introduction

Neural Stimulation Techniques and Limitations

Neural stimulation has traditionally involved application of a transient electrical impulse to excitable tissues within the nervous system and observation of the associated physiological response. Soon after the discovery of electricity, scientists and clinicians investigated both

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the mechanism as well as its clinical utility as a technique for studying, diagnosing, and treating the nervous system (Fritsch 1870; Geddes and Bourland 1985; Konrad and Tacker 1990; Devinsky 1993; Pudenz 1993). The advantages of using electrical stimulation are well characterized; it is a controllable, quantifiable, reliable and relatively precise technique when compared to chemical, mechanical, or magnetic stimulation methods. Furthermore, through the use of electrodes, delivery of the stimulus can be precise (e.g. microelectrodes) and accurate (e.g. through the use of constant current stimulation source) when compared with most neural excitation modalities. The technique provides a dependable excitation method in which stimulation parameters – namely current (milliamperes), voltage (Volts), pulse duration (milliseconds), and repetition rate (Hz) - can be easily controlled and measured (Geddes 1987). While significant advances have been made in electrode design and stimulation approaches since the early days of electrical neural stimulation, this method remains fundamentally unchanged and in some cases limited.

Limitations of electrical stimulation include; 1) the necessity of a tissue-electrode interface and possible toxicity related to the electrode material (Geddes and Roeder 2003; Geddes 2004), 2) dependence of effective stimulation upon tissue impedance and coupling (Ragheb and Geddes 1990; Geddes and Roeder 2003), 3) spread of electrical current in a graded fashion beyond the electrode (Popovic, Gordon et al. 1991; Liang, Lusted et al. 1999; Testerman 2005). Since many applications of neural stimulation require limited dispersion of the stimulus with respect to a small target tissue, electrodes designed to deliver precise stimulation have inherently high impedance characteristics, which in turn, necessitates higher voltage to deliver the same charge (Ohm's law). In addition, to selectively stimulate an individual neuron or axon requires that the investigator physically contact or impale the neuron, and thereby introduce trauma to the tissue being studied. Moreover, in many applications where electrophysiological recordings are performed to observe the response to stimulation, an inescapable 'stimulation artifact' exists due to the fact that the stimulation technique occurs in the same domain as the recording technique. In other words, recordings of minute electrical potentials immediately adjacent to the point of electrical stimulation are contaminated by the electric field involved in the stimulation (related to voltage applied to deliver the current). This has prompted many to apply sophisticated and complex techniques for reducing stimulation artifact in the recordings to allow for spatial precision (Fiore, Corsini et al. 1996; Miller, Abbas et al. 2000; Wagenaar and Potter 2002; Andreasen and Struijk 2003). Although electrical stimulation remains the gold standard for neural excitation, these limitations have lead researchers to look for new ways to stimulate.

Scientists have attempted stimulation by chemical, mechanical, thermal, magnetic, and optical means in search of a modality that could overcome the fundamental limits imposed by electrical stimulation (Booth, von et al. 1950; Orchardson 1978; Cruccu and Romaniello 1998). The exploration of these modalities has yielded a variety of techniques. In this paper, optical neural stimulation is defined as the direct induction of an evoked potential in response to a *transient* targeted deposition of optical energy. We have previously reported studies demonstrating that one can effectively stimulate neural tissue with low energy, pulsed laser light resulting in evoked action potentials in amphibian and mammalian peripheral nerves without causing histological tissue damage using laser parameters reported therein (Wells 2005; Wells 2005).

1.2. Experimental goals

The primary objective of this paper then is to provide a systematic comparison of this new modality of nerve stimulation to the standard method of electrical stimulation in peripheral nerves. Towards this objective, the apparatus and methodology for using pulsed lasers to apply a transient laser stimulus (defined as an optical stimulus) to a peripheral nerve preparation (sciatic nerve) for comparison with a standard method of electrical stimulation

of the nerve is described. Results that determine key criteria for laser selection and setup and the effect of varying optical stimulus wavelength on threshold action potential generation based on tissue geometry and morphology are presented. Threshold intensity of optical (J/cm^2) versus electrical (J/cm^2 or A/cm^2) stimuli to initiate action potentials (threshold energy of stimulation) in the sciatic nerve of the rat is determined and compared. The measured responses include the compound nerve and muscle action potentials (CNAPs and CMAPs, respectively) and any associated noise and signal artifacts. An optical strength-response curve is described and compared to published strength response curves generated using electrical stimulation. A practical example where optical stimulation provides a distinct benefit is presented.

1.3. Optical Energy – Terms and Concepts

There are significant differences between electrical stimulation and the application of pulsed laser energy to stimulate neural tissue. In some ways, analogies can be drawn with regard to electrode/light-tissue interaction and transport theory; however, the energy types and physical properties of this interaction with tissue are fundamentally different. Similar to the history of electrical stimulation, lasers have been used in medicine since shortly after the discovery in the early nineteen sixties. The use of lasers for these processes is based on the potential for high spatial precision and the ability to couple laser light into fiber optics for easy and minimally invasive delivery to the tissue. By design, laser radiant exposure associated with therapeutic procedures results in non-reversible thermal or mechanical alteration of the tissue as photon energy is converted to heat (for review see: (Thomsen 1991; Jacques 1992)). The laser-tissue interaction is mediated by a thermal or thermo-mechanical process depending on operational parameters of the laser. The key parameter in these interactions is the wavelength, λ . It determines light penetration and distribution in the tissue and is dependent upon optical properties (refractive index $[n]$, absorption coefficient $[\mu_a]$, scattering coefficient $[\mu_s]$, in units of cm^{-1} , and anisotropy factor $[g]$) of tissue (for details see: (Welch and Gemert 1995; Jansen 2004)).

In a broad sense, the optical properties of the tissue impact the efficiency at which (laser) energy transfer occurs in tissue, much like electrical impedance impacts the flow of current into the tissue with electrical stimulation. Without optical absorption from specific molecules (*chromophores*), there is no energy transfer to the tissue and the tissue is left unaffected by the light. In the infrared, water is the primary absorber in soft tissues including the peripheral nerve. The absorption of light results in an exponential decay of the radiant exposure (defined as the energy per unit area at any depth) in a material as a function of depth, predicted by Beer's law. The penetration depth of light, defined as the depth in the medium at which the radiant exposure, is reduced to $1/e$ times ($\sim 37\%$), the incident irradiance (energy per unit area at the surface) is inversely proportional to the attenuation coefficient (measure of light lost due to absorption and scattering).

For example, the penetration depth of the Holmium:YAG (Ho:YAG) laser at $2.12 \mu\text{m}$, a wavelength shown to be optimal for peripheral nerve stimulation (Wells 2005), can be calculated to be about $330 \mu\text{m}$, assuming that absorption in the peripheral nerve is only determined by water. The relationship between radiant exposure as a function of tissue depth with a pulsed Ho:YAG laser source in soft tissue is shown in Figure 1. Thus by optimizing laser parameters based on tissue morphology and optical properties, the irradiated tissue volume can be selectively targeted to some degree to match the goal of a particular procedure (for review see: (Vogel and Venugopalan 2003)); in this case activation of a spatially limited set of nerve fibers lying directly below the irradiated tissue surface. Laser energy absorbed by tissue is typically converted to thermal energy and the amount of energy absorbed per unit volume of tissue can directly be related to the temperature rise in the

tissue, is dependent on the density [kg/m³] and the specific heat [J/kg K] of the irradiated material.

The radiant exposure yields information about the amount of light *distributed* through the depth of tissue, but no information about the amount of light *absorbed* at that point. We define a new term called the energy density, Q , as the number of photons absorbed per unit volume [J/m³]. The energy density is the product of the radiant exposure at some point in the tissue and the probability of absorption of that light at that point:

$$Q(z) = \mu_a H_0 e^{-\mu_a z} = \mu_a H(z) \quad (2)$$

Once the energy density $Q(z)$ [J/m³] is known, then the laser induced temperature rise is given by:

$$\Delta T(z) = \frac{Q(z)}{\rho c} \quad (3)$$

Where ρ is the density [kg/m³] and c is the specific heat [J/kg K] of the irradiated material. Note this equation assumes instantaneous delivery of pulsed light, such that the pulse width (Ho:YAG = 350 μ sec) is much shorter than the thermal diffusion time in tissue (soft tissue = 100 msec). The predicted *average* increase in surface tissue temperature across the laser spot from a single pulse using 0.4 J/cm² radiant exposure delivered to peripheral nerve tissue with the Ho:YAG laser is roughly 2.87°C. Assuming water dominated absorption through the epineurium and perineurium with a thickness of 150 μ m, the *average* temperature rise within the irradiated zone at the actual surface of the axonal layer is calculated to be roughly 1.86 °C. The time course for this temperature increase through internal conversion of photon energy to heat can be assumed to be instantaneous. Thermal denaturation of structural proteins in fresh tissues occurs at temperatures greater than 45 °C while birefringence changes in collagen (i.e. epineurium) occur between 55 – 90 °C, each of which are greater than the temperatures implicated in transient optical nerve stimulation using threshold radiant exposures (Thomsen 1991). Note that the microstructure of myelin and connective tissues overlying neural axons is unique compared to the bulk properties of the nerve tissue, dominated by water absorption, used in many of our assumptions. While the microstructure of the tightly packed lipids comprising myelin sheaths presents a morphologically distinct layer of tissue, at 2.1 μ m (4600cm⁻¹) there are no selective absorption bands for lipids and cholesterol, the main constituents of myelin (Horrocks 1967), (Fukami 2003). Therefore we have no reason to suspect that myelin or underlying axons will selectively absorb additional light other than that predicted from the bulk absorption properties of nerve tissue.

To contextualize these and other terms related to optical energy the following comparisons are presented in the context of peripheral nerve stimulation:

1. Light is described as a packet of photons traveling through a medium whose energy is proportional to its wavelength (Hecht 2002). Electrical current is described by the movement of electrical charge, its strength determined by the charge separation and electric field strength (Halliday 1978).
2. The measure of energy delivered to the tissue is typically in Joules (J). In electrical terms, a Joule reflects the amount of energy transferred to tissue by the localized net movement of charge, whereas in optical terms it reflects the energy of the photons as they are scattered, absorbed, reflected, or transmitted by the tissue.

Therefore, although the unit of Joules is common between these two phenomenon, it is important to understand that a Joule or Watt of electricity ($1 \text{ J} = 1 \text{ C} \cdot \text{V}$) represents a different form of energy than a Joule or Watt of light (Joule 1965).

3. Damage to neural tissue can be directly related to energy per unit area transferred to the tissue over time (W/cm^2). In electrical terms, the stimulus parameters related to neural damage are well defined by Agnew and McCreery; citing that the current density (current flow per unit area via surface stimulation in Amperes / cm^2) relates to the neural damage by both the charge transfer across the electrode-tissue interface and the passage of current through the tissue (Agnew and McCreery 1987). In optical terms tissue damage relates to the laser irradiance, defined as radiant energy per unit area delivered in a given time ($[\text{Joules} / \text{cm}^2 - \text{sec}]$ or $[\text{Watts} / \text{cm}^2]$), which is determined by the spot size of the laser beam, the total laser energy deposited, and the penetration depth of the light in tissue (Jacques and Prahl 1987).
4. Transfer of laser energy at this interface is dependent upon the index of refraction between the two media, governed by laws of reflection and refraction (Pedrotti 1993). One might liken this to the electrode-tissue interface that governs the amount of current flow into tissue (Nilsson 1996).
5. The penetration depth of light is governed by tissue optical properties, which are in turn a function of wavelength (Chandrasekhar 1960) where light intensity decays exponentially as a function of depth through absorption of photons. In contrast, electrical current (dependent upon tissue impedance) spread through the tissue volume is related to electrical field gradients. In this case the magnitude of the field gradient in tissue is inversely proportional to the distance from the site of stimulation because the current density spreads out in the volume conductor (Plonsey 2000), not due to the energy being absorbed as with lasers.
6. The mechanism by which electrical energy is transduced into activation of action potentials is well understood (Geddes 1972). Specificity is determined by the type, shape, size, and position of the electrode in relation to the axonal layers of the nerve (Geddes 1989) and the details of the electrode-tissue interface. The mechanism by which laser energy results in action potential generation is unknown at this time. Possible mechanisms, while not validated, are presented as plausible hypotheses later in the discussion section. Specificity is determined by the laser spot size and penetration depth.

2. Materials + Methods

All experiments were conducted at the Vanderbilt University W.M. Keck Free Electron Laser Center and Vanderbilt Biomedical Optics Laboratory in accordance with protocols approved by the Institutional Animal Care and Use Committee were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

2.1. Animal preparation

Sprague-Dawley rats (300-400 g) were implemented as a mammalian model for *in vivo* sciatic nerve experiments. In preparation for surgery, rats were anesthetized with intraperitoneal injection of ketamine (80mg/kg) and xylazine (10mg/kg) solution and maintained under sedation with additional boluses of ketamine for the duration of each individual experiment. Once anesthetized, animals were placed in the prone position and the right and left sciatic nerve exposed over the length of the femur. An incision was made

posterior-laterally extending from the gluteus muscles to the popliteal region. This allowed access to the sciatic nerve from its pelvic cavity exit to the level of the knee and visualization of specific motor branches (*n. fibularis* and *n. tibialis*) to the biceps femoris, gastrocnemius, and distal muscles. This surgical procedure served to expose sufficient area for electrical and optical stimulation and electrical recording of CNAPs along the nerve and CMAPs from the biceps femoris and gastrocnemius muscles. The muscle fascia overlying the nerve was carefully removed to expose the nerve surface with its epineurial covering maintained intact. Nerves were continually moistened with normal saline to avoid desiccation during the acute study. Rats were euthanized with carbon dioxide gas following each individual acute experiment.

2.2. Experimental Design and Electrophysiological Evaluation

Electrical stimulation energy (J/cm^2 or A/cm^2) was used as the standard method to compare with pulsed laser energy (J/cm^2). Figure 2 illustrates the setup for performing optical as well as electrical stimulation of the sciatic nerve. Each nerve tested was set up so that both electrical and optical stimulation could be performed at the same location, at a point proximal to recording electrodes in the main trunk of the sciatic nerve. Furthermore, the motor branch of the sciatic nerve leading to the biceps femoris muscle was identified and utilized for recording potentials induced from stimulation (proximal to the branch point) of motor fibers in the sciatic nerve. Additional recordings were made as needed from other muscle groups distal to the knee.

At the beginning of each experiment, the viability of the sciatic nerve and its threshold to electrical stimulation were tested (roughly $0.1 \text{ A}/\text{cm}^2$) with a single 350 μsec pulse stimulus. Recordings of CNAPs were made by placing a bipolar electrode in contact with the nerve distal to the point of stimulation. To calculate CNAP conduction velocity, the distance between the two pairs of bipolar recording electrodes was measured. Muscle potential recordings were made by placing needle electrodes (Grass E-2 electrodes; Grass Telefactor, Inc.; West Warwick, RI) into the muscle belly in a bipolar fashion (belly-to-belly placement). Both optical and electrical responses were recorded with a modular data acquisition system (MP100, Biopac Systems Inc. Santa Barbara, CA) controlled using a laptop computer and Acknowledge® software (Biopac Systems Inc.). All recordings were captured digitally after differentially amplifying the signal and sampling the response at 6877 Hz. Data recordings were pre-triggered 2 ms prior to the stimulation pulse, so as to gather a baseline signal prior to the stimulation. Nerve potential responses were amplified 5000 times and filtered using a high pass filter ($> 20 \text{ Hz}$) and low pass filter ($< 3 \text{ KHz}$). Muscle potential responses were amplified 1000 times and filtered using a high pass filter ($> .05 \text{ Hz}$) and low pass filter ($< 5 \text{ KHz}$).

2.3. Laser Selection and Setup

A portable Holmium:YAG laser (Ho:YAG Model 1-2-3 laser, Schwartz Electro Optics, Inc.) was used in these studies. This laser operates at a wavelength of $2.12 \mu\text{m}$ with pulse duration of 350 μs (Full Width Half Maximum - FWHM) and has been shown to be an efficient source for optical stimulation of the peripheral nerve (Wells 2005). The Ho:YAG laser beam was coupled directly to a 600 μm optical fiber with a numerical aperture equal to 0.39 (3M Optical Fiber Power Core, FT-600-DMT), which was mounted on a three dimensional micromanipulator and precisely positioned over the nerve at the site of electrical stimulation (Figure 2). The size of optical fiber was chosen based on the relative size of the typical rat sciatic nerve fascicle. Light departs the optical fiber in a diverging manner such that light incident on tissue will not converge to a smaller spot than the fiber output diameter, which prevents large energy densities within the tissue and resulting thermal damage. The intensity of radiant exposure used to stimulate the nerve optically was controlled using filters placed

in the path of the beam. Measurement of the optical stimulus intensity was facilitated by placing a beam splitter coupled to a laser energy/power meter (Molelectron EPM 2000, Portland, OR) and detector (Molelectron, LP50 head) in the laser path to sample and measure 10% of the energy delivered for each pulse. The remaining 90% was transmitted through a focusing lens to be fiber coupled at a coupling efficiency of about 70%. The correlation factor between the measured energy and the energy delivered to the tissue was calculated before each set of experiments to accurately document the laser energy for each individual pulse during stimulation experiments. Note, maintaining homeostatic water content by saline perfusion of tissue is an important variable to control in optical stimulation of tissue to avoid dehydration from heating upon multiple laser pulses.

3. Results

3.1. Physiologic validity of optical stimulation and comparison of nerve and muscle responses to standard electrical methods for nerve stimulation

Figure 3 illustrates a typical CMAP response obtained when the sciatic nerve in a rat was stimulated with the Ho:YAG laser. A solution of the neuromuscular transmission blocking agent, succinylcholine, was dripped on the muscle at the region of innervation and its effect on subsequent stimuli noted in Figure 3b. Figure 3c contains the potential recorded 30 minutes following drug washout. These results serve to validate the physiological basis of optical stimulation of the nerve and corroborate our previous data published in amphibians and mammals.

Stimulation threshold of the peripheral nerve motor axons was defined as the minimum energy required to initiate a visible muscle twitch. Threshold Holmium:YAG laser stimulation (n=15) occurred in the *in vivo* rat sciatic nerve with a laser radiant exposure of $0.32 \pm 0.1 \text{ J/cm}^2$. Electrical stimulation threshold at the same site as the optical stimulus required $0.95 \pm 0.58 \text{ A/cm}^2$. Both forms of energy were measured at the nerve surface. For these studies, the pulse width was held constant at 350 μsec and the area of stimulation (i.e. laser spot size or tissue-electrode interface surface area) for each modality was accurately measured and kept near $0.5 - 1.0 \text{ mm}^2$.

Figure 4 shows CNAP recordings with both optical and electrical stimulation from a rat sciatic nerve. The distance between recording and stimulating locations was 10 mm. The electrical stimulus intensity used was 1.4 A/cm^2 (pulse width = 350 μsec), which is roughly 1.5 times threshold. Within the same nerve, recordings from optical stimulation at the same site using an intensity 1.5 times stimulation threshold, 0.55 J/cm^2 , is shown in Figure 4b. Stimulation for each modality occurs at $t = 0 \text{ msec}$. The general shape and timing of the waveforms from each peripheral nerve excitation modality are very similar with the exception of the electrical stimulation artifact. Onset time for a recorded response in the nerve from electrical stimulation and initial peak time and amplitude within this waveform are lost within the signal artifact, in this case the stimulation to recording distance was 1 cm. Peaks from the optically excited nerve potential recordings occur 0.87, 1.16 and 2.18 msec after stimulation, while the onset time for CNAP waveform is 0.29 msec. The nerve conduction velocity for fast conduction motor axons (A_α) resulting from optical stimulation is 34.5 m/sec. Peaks from slower conducting fibers are also evident from optical stimulation tracings, most likely from A_δ and C motor fibers, with conduction velocities of 11.5 and 4.58 m/sec, respectively. Note that the electrical stimulation artifact prevents accurate measurement of the onset time for stimulation in nerve potentials, and therefore the nerve conduction velocity.

3.2. Strength-response relationship in optical stimulation

A well-known relationship, the strength-response curve, exists between the excitation intensity using electrical stimulation and the intensity of the evoked CNAP response. The minimum CMAP recorded from bipolar surface electrical stimulation using energies required for threshold excitation for this peripheral nerve prep was 0.2 V. To determine if a similar relationship exists for optical stimulation the laser intensity was varied from 0.7 to 3.0 J/cm² within the same rat sciatic nerve and the evoked muscle potential from optical stimulation was recorded. The peak voltage from each recording was plotted with the corresponding laser intensity used to create the optical equivalent of the strength-response curve (Figure 5). The minimum recorded CMAP from laser stimulation of this peripheral nerve is 0.05 V, which is 4 times smaller than the minimum CNAP initiated from electrical stimulation using bipolar surface electrodes. A linear relationship between the strength of optical stimulation and the peak voltage of the recorded signal (corresponding to the number of axons stimulated) exists between 0 to 2 J/cm². Excitation energies outside of this range result in loss of a normal physiologic response, most likely due to thermal damage to the irradiated tissue. Excitation energies less than 1 J/cm² are shown to selectively stimulate nerve axons with greater spatial precision than seen with threshold electrical stimulation.

3.3. Spatial selectivity in activation

Upon investigation, a difference exists in the selectivity in muscular activation associated with each modality. We define 'spatial selectivity' of stimulation as the end-point response, which is isolated muscle activation from stimulation within the main sciatic nerve proximal to the first branch point. As a demonstration of the spatial specificity inherent to optical stimulation, CMAP recordings from electrical and optical stimulation were compared within the rat sciatic nerve. Muscle recording electrodes were located approximately 40 and 55 mm from the site of stimulation within the gastrocnemius and biceps femoris, respectively. Figure 6 depicts selective activation for optical stimulation with respect to electrical stimulation. Electrical stimulation with stimulation threshold energy (1.02 A/cm²), was delivered proximal to the first nerve branch point and the muscular responses within gastrocnemius and biceps femoris were simultaneously recorded. The range of voltage change in these waveforms is 1.495 and 0.492 V, respectively, as seen in Figure 6a. Optical stimulation at threshold (0.4 J/cm²) is shown for comparison with a voltage change 0.102 V recorded in the gastrocnemius and no response observed in the biceps femoris (Figure 6b). The shape and timing of these signals are nearly identical, however, the magnitude of the responses are larger with electrical excitation.

3.4. Topographic peripheral nerve mapping as a practical example of selectivity

To illustrate the relative precision of stimulation with optical methods, the sciatic nerve was functionally mapped by translating a fiber optic probe across the surface of the main nerve trunk using optical stimulation. The location of nerve fascicles leading to various muscles were identified in 12 sciatic nerves. The results from all 12 maps are overlaid in one diagram of the proximal sciatic nerve. Radiant exposures above stimulation threshold (0.4 – 0.7 J/cm²) were used for stimulation just proximal to the first major branch point of the sciatic nerve. Both 400 and 600 µm diameter fiber optical probes were utilized for delivery of the laser pulse and translated across the nerve to selectively target the tissue and functionally evaluate the width of each motor site. Individual trial results as well as a generalized map based on these data are shown in Figure 7. The following isolated muscle response were mapped; semitendinosus, gastrocnemius (upper and lower), vastis lateralis, biceps femoris (upper and lower), semimembranosus, and the 5 hindpaw digits. In figure 7, each column on the left of the sciatic nerve diagram represents one trial from one sciatic nerve mapping experiment, unless indicated above the trial results with n=2 or n=3 if the exact same results were produced on different nerves more than one time. Although there is some variation

among trials, general trends concerning the location of muscle groups can be seen as a general topographic representation of the distribution of fascicles innervating distal musculature of the hind limb to create a generic functional map of the rat sciatic nerve. The three main branches leading to specific muscles are represented with black horizontal lines diverging to the right.

4. Discussion

4.1. Comparison of low versus high energy optical irradiation of tissue

Results presented here demonstrate that a fundamentally novel approach to precise, noise-free stimulation of neural tissue can be performed with low-level, pulsed infrared laser energy. Optical stimulation was first reported by Fork (Fork 1971), as action potentials were generated in *Aplysia* neurons through a reversible mechanism. This was the first indication that optical irradiance of nerve cells could perhaps induce neural stimulation by way of an elicited action potential resulting in a provoked response. When a short pulse, ultra-violet excimer laser was used for ablation of rat CNS fibers in the medial lemniscus and cuneate bundle in the spinal cord, evoked potentials were recorded from the thalamus in the region of the ventralis posterior nucleus. The results were reported as a side effect to the intended delivery of ablative laser energy (Allegre, Avriillier et al. 1994). However, no further reports were found that followed up on this observation. Depolarization and subsequent action potential firing was reported from transiently irradiated pyramidal neurons with a high intensity mode-locked infrared femtosecond laser, reported by Hirase et al, during multi-photon experiments (Hirase, Nikolenko et al. 2002). These studies differ from previously published reports because of the emphasis on transient low energy (non-ablative and non-damaging) pulses that produce direct stimulation of the peripheral nerve. With such low energy pulses, we feel that this technique might be suitable for medical applications in the future.

Electrical stimulation requires contact between the electrode and the tissue being stimulated. It is susceptible to all the properties of the electrode-tissue interface; namely, impedance, current shunting, and field distortion around the area of contact between the electrode and the tissue (Branner and Normann 2000). For example, current cochlear implants presently use a maximum of 6-10 channels/electrodes. Along the basilar membrane of the cochlea, each electrode stimulates an area of approximately 4-8 mm (van den Honert 1987). After implantation, issues of half-cell potential differences, metal toxicity and tissue reaction to the implanted electrodes significantly limit the materials and sizes of materials used for chronic electrode implants to achieve stable function over years of use. While many engineering challenges remain, it has recently been shown by others that optical stimulation using small fiber optics (50-100 μm) results spatial resolution of stimulation that is comparable to normal acoustic stimulation and superior to electrode stimulation (Richter CP 2005). Moreover since the reported stimulation thresholds in the cochlea are more than 100 $\times$ lower than those reported in the sciatic nerve, the overall heat load to the tissue is reduced and stimulation at repetition rates of several hundreds Hz for up to 6 hours showed no functional damage to the tissue (Izzo AD 2006).

4.2. Factors determining optical penetration and laser selection

The typical rat sciatic nerve stimulated in this study was approximately 2 mm in diameter, with a 100 - 200 μm epineurial and perineurial sheath between the actual axons and the nerve surface. The typical fascicle thickness is constant across all mammalian species (although the number of fascicles per nerve varies greatly) and tends to be between 200 and 400 μm (Paxinos 2004). Thus, to theoretically achieve selective stimulation of individual fascicles within the main nerve, the penetration depth of the laser must be greater than the

thickness of the outer protective tissue (200 μm) and in between the thickness of the underlying fascicle. The optimal zone for laser penetration into the peripheral nerve is depicted in Figure 8 within the shaded area. This is a graph of the penetration depth, calculated using the reciprocal of absorption coefficient spectrum of the rat sciatic nerve measured using Fourier Transform Infrared Spectroscopy (FTIR)(Wells 2005). Generally speaking, ultraviolet wavelengths ($\lambda = 1 \text{ nm} - 0.45 \mu\text{m}$) are strongly absorbed by tissue constituents such as amino acids, fats, proteins, and nucleic acids, while in the visible part of the spectrum ($\lambda = 0.40 - 0.70\mu\text{m}$), absorption is dominated by (oxy)hemoglobin and melanin. The near-infrared part of the spectrum ($0.70 - 1.3 \mu\text{m}$) represents an area where light is relatively poorly absorbed (allowing deep penetration) while in the mid- to far-infrared ($\lambda > 1.4 \mu\text{m}$) absorption in tissue is dominated by water resulting in shallow penetration (Vogel and Venugopalan 2003). The mid-IR portion of the soft tissue absorption spectrum, depicted in Figure 8, contains wavelengths with ideal tissue penetration properties. Thus by adjusting the wavelength one can vary the penetration depth of the light delivered, which allows targeting of nerve fascicles of varying thicknesses near the nerve surface with this method. In this respect optical stimulation of neural tissue share no similarities with electrical stimulation. Implementing these wavelengths with lower penetration depths, the calculated temperature increase per laser pulse in tissue is relatively low and theoretically well below tissue temperature required for thermal changes in nerve tissue or resulting damage. By irradiating the nerve surface overlying the target fascicle for stimulation within the main branch, infrared laser light may provide profound selectivity (in terms of spot size and nerve tissue depth) in excitation of individual fascicles within a nerve bundle resulting in isolated muscle contraction. Adequate laser energy may be deposited within the sciatic nerve by considering tissue structure and properties to tailor the laser parameters to the desired physiologic effect with a high degree of spatial discrimination.

4.3. Physiological validity of the technique and energy comparison to electrical stimulation

It is evident from Figure 3 that a CMAP is produced upon optical stimulation prior to addition of neuromuscular transmission blocking agent, succinylcholine. This muscle potential disappeared within 1 minute of application of succinylcholine, and reappeared again upon drug washout. The use of the blocking agent as well as optically isolating the underlying muscle during stimulation implies that, as seen in electrical stimulation, muscle recordings elicited by optical stimulation require neuromuscular transmission mediated by acetylcholine and that this nerve excitation is exclusively due to laser irradiation of the sciatic nerve.

A direct comparison of the energetics needed for stimulation is difficult since optical and electrical interaction in tissue rely on different forms of energy to activate nerve potentials. Typically electrical nerve stimulation is far more energy efficient than optical stimulation of neural tissue based on a first order approximation calculation. This conclusion is expected since electrical energy (electrons with a potential energy) is the inherent method by which action potential propagation is achieved through voltage sensitive membrane channels. Optical methods appear to lose energy through an intermediate energy transfer step resulting in lower laser energy (photon absorption in tissue) available to activate the membrane and produce nerve potentials.

A unique advantage with optical stimulation is the lack of stimulation artifact intrinsic to traditional electrical methods for neural stimulation. The artifact associated with electrical stimulation complicates recording neural potentials near the site of stimulation. As the stimulus intensity is increased this electrical noise gains in magnitude. For these reasons, it is very difficult to quantify excitability characteristics of tissue with recording electrodes in close proximity to the stimuli. Figure 4(a) demonstrates this fundamental limitation of adjacent electrical stimulation and recording processes. A large stimulation artifact conceals

the nerve response for nearly 2 msec following stimulation. Thus, the onset time and in some cases peak amplitude of the response is very difficult to distinguish from the noise, obscuring the determination of the signal peaks.

In contrast, in response to optical stimulation the nerve conduction velocities from the fast conducting motor fibers within the sciatic nerve are measured to be 34.5 m/sec with slower conduction fiber velocities of 11.5 and 4.58 m/sec. Peak amplitudes of the CNAP response from all three fiber types are clearly quantified. It is worth noting that the velocity of conduction within the nerve subsequent to optical stimulation falls within the normal range for the rat sciatic nerve fast conducting A_α motor neurons and slower conducting A_δ and C motor neurons. Thus, this new modality for nerve excitation enables simultaneous stimulation and recording from adjacent portions of a nerve; a phenomenon that is problematic using electrical means for activation. These results also imply that multiple motor fiber types are excitable with pulsed laser irradiation using optimal laser parameters.

4.4. Strength-response curve

Although individual axons within the nerve bundle follow an all or none response to a stimulus, the compound action potential is continuously graded. The relationship between optical radiant exposure, or laser energy per unit area [J/cm^2] and the amplitude of the evoked CNAP (Figure 5) is comparable to the strength-response curves observed in electrical excitation, where the amplitude of the CNAP is proportional to the magnitude of stimulation (Mountcastle 1974; Geddes and Bourland 1985; Geddes and Bourland 1985). From data obtained with electrical stimulation the greater the energy applied, the more fibers recruited, resulting in larger amplitude compound potentials. Electrical stimulation has an exponentially decaying spread of charge radiating from the electrode leading to a graded response when stimulating excitable tissue (For review see: (Palanker, Vankov et al. 2005)). Increasing optical energy results in a linear increase in recruitment of axons up to a point, suggesting that the energy is transduced immediately beneath the laser spot and has limited diffusion to surrounding tissue. Laser spot size incident on the nerve can be decreased to an extremely small area. As a result of a small spot and lack of radial diffusion in tissue, optical stimulation allows for more selective excitation of fascicles, resulting in isolated, specific muscle contraction; an advantage associated with this modality. It is clear from the CMAP threshold response from each modality in Figure 5 that threshold for eliciting a response with electrical stimulation results in more muscle fibers recruited than with optical stimulation. This illustrates the idea that the current required for threshold electrical stimulation is significant and that precise stimulation is limited by the spread of charge in tissue resulting in recruitment of a relatively large population of axons. Note that optical stimulation with less than $1 \text{ J}/\text{cm}^2$ can produce extremely precise stimulation of individual fascicles in a volume of axons considerably smaller than that attainable with threshold electrical stimulation with bipolar surface electrodes. An upper limit to this excitation does exist at about $2 \text{ J}/\text{cm}^2$ stimulation radiant exposures, where a decrease in the physiologic response occurs most likely from thermal axonal damage, affecting the tissue's ability to initiate and propagate action potentials. One can expect the slope of this line to shift up and down based on the thickness of the epineurial covering surrounding the irradiated tissue, as more or less photon energy may be required to obtain a stimulatory effect.

4.5. Spatial selectivity comparison

These preliminary data with optical nerve stimulation in rat sciatic nerves confirm that optical stimulation exhibits spatial specificity, or lack of spread of stimulus to neurons not in direct contact with the stimulus source. The precision and spatial specificity with optical activation demonstrates selective recruitment of nerve fibers as indicated by comparing the relative magnitudes of nerve and muscle potentials (Figure 6) elicited from optical and

electrical stimulation. Thus, individual nerve fibers near the nerve surface and innervated muscles can be selectively stimulated for a controlled, explicit response by simply translating the stimulation spot across the tissue to target relevant nerve fibers. In fact, for all optical stimulation experiments it was observed that a specific muscle or group of muscles contract depending on the location of the incident laser upon the main nerve bundle. Figure 6 illustrates the capability of optical stimulation to selectively activate specific fascicles within the main rat sciatic nerve to target specific muscle groups such as the gastrocnemius while yielding no response from adjacent fibers that innervate the biceps femoris. This is unlike electrical stimulation where using the minimum energy required for muscle stimulation via hook electrodes applied to the surface of the nerve still results in excitation of more than one fascicle and a response from more than one muscle. Techniques in intraneural electroneurography (Lee, Chae et al. 2006) and nerve stimulation via microelectrodes can be precise and are routinely used to recruit individual and small numbers of adjacent axons; however, this often requires direct contact and placement of a small, fragile electrode near the site and may require impaling the nerve. In theory, optical stimulation can be delivered with a selectivity dependant only on the spot size generated from the laser optical fiber used, which can be as small as 4 μm in diameter in a non-contact manner or the diffraction limit of light. Future experiments will elucidate the minimum spot size required to reliably activate nerve tissue. The similarity in the shape and timing of the signals from optical and electrical stimulus, as illustrated in this figure indicate that conduction velocities, represented by the time between the stimulus and CMAP, are identical. These traces imply that measured action potentials are indistinguishable in terms of velocity regardless of the mechanism of activation.

4.6. Potential mechanisms for optical stimulation

It is clear that some process of transduction of light energy is required as an intermediate step before depolarization, which delivers energy required to activate the membrane or channel proteins and cause action potential propagation. There are several hypotheses for the mechanism of optical stimulation that we can offer based on existing literature, which mostly pertains to electrical stimulation and signal transduction. However, none of these have been validated and as such the mechanism by which optical energy causes axonal signal transduction is currently unknown. The interaction may be a direct thermal effect, where heat is transferred to tissue through a process called internal conversion, thus activating heat sensitive channels in the membrane. Experiments conducted thus far demonstrate that this temperature effect if causative relies on a transient thermal gradient sustained in the tissue with requirements on the peak temperature rise and duration of the laser pulse. Changes in the open probability for a sodium channel may occur from light (heat) energy by changing the rate constants governing the closed to open channel states (temperature dependence mediated by the channel Q10 value), thus generating an additional hypothesis for optical stimulation. Creation of micropores in the membrane (Hirase, Nikolenko et al. 2002) has been proposed as a possible mechanism; however, since the amount of radiant energy delivered to tissue at the wavelengths studied is well below the energies required to overcome the tensile strength of the lipid bilayer, it is believed to be unlikely. Other alterations in the tissue such as light electric field effect, photochemical interaction (caused by absorption of a specific activating chromophore) and photomechanical interactions (resulting in a pressure wave or mechanical displacement) are a possibility, but initial experiments indicate otherwise (Wells et al, 2006). Experiments are currently in progress to elucidate the exact mechanism of action.

4.7. Creation of a functional map with optical stimulation

Despite the lack of understanding of the mechanism involved, optical stimulation has nevertheless been shown to exhibit advantages in some peripheral nerve applications in

neuroscience. In order to evaluate the efficacy of optical nerve stimulation in the peripheral nerve and to validate the claim of spatial specificity, a detailed spatial map of the rat sciatic nerve was obtained, as seen in Figure 7. Here, it is clear that optical stimulation can be used to functionally map the entire nerve surface with a high degree of spatial selectivity, a process that requires physical contact with microelectrodes and is more difficult to achieve selectivity via electrical methods involving surface stimulation with hook electrodes. These results are consistent with the motor neuron topography reported using fluorescent dye (DiI tracers) to map the topographical projections of motor neurons of the rat sciatic nerve (Kobbert and Thanos 2000). Thus, in this initial assessment of the spatial precision of optical stimulation versus surface electrical stimulation, optical excitation has been shown to provide high spatial selectivity without the use of extrinsic agents or tissue contact.

4.8. Clinical value of optical stimulation

This paper demonstrates that optical stimulation provides a method of non-contact, noise-free, precise stimulation of exposed nerves. Examples of limitations in electrical stimulation techniques arise when precise stimulation of neural structures are required such as for peripheral nerve surgery during which small clusters of nerve fibers are stimulated to determine their viability in peripheral nerve repair. Electrical stimulation is utilized to identify the connectivity and functionality of specific nerve roots to be selectively avoided or resected (Ueno, Kaneko et al. 2001). This usually requires separating the nerve bundles apart to avoid volume conduction of the stimulation field and restrict the testing to specific fascicles. The optical method confines the stimulation field to the spot size and as a result only stimulates small segments of a nerve without requiring separation of the nerve bundles. As a clinical example, acoustic neuromas are common benign but difficult tumors to remove in the central pontine angle due to multiple cranial nerves juxtaposed with the tumor. To remove the tumor without resecting the adjacent facial or vestibular nerve, electrical stimulation and ABR are used for intraoperative monitoring and to distinguish between tumor and normal cranial nerve. Lack of real time monitoring and the inherent spread of charge from large stimulation currents may in some cases cause surgeons to resect the facial nerve although it appears intact on monitoring (Martin, Sethi et al. 2001). The precision of optical stimulation could facilitate partial nerve resection and improved outcome for these debilitating long-term effects. Other clinically useful applications may include sub-fascicular neural mapping for peripheral nerve reconstruction, such as repair of brachial nerve plexus, or applications in otolaryngology.

4.9. Advantages and Limitations

We have systematically compared and contrasted two very different methods for stimulation of nerves. Regardless of the underlying mechanism, this new stimulation modality relying on pulsed laser light has both potential advantages and drawbacks when compared to electrical stimulation. Optical stimulation yields artifact-free signals, whereas electrical methods usually result in stimulation artifact in the recorded signal when recording the signal close to the stimulus, preventing the adjacent placement of stimulating and recording electrodes. This new methodology results in neural potentials that can be more easily recorded near the stimulation site. In addition, lower stimulus energy need to be applied due to reduced need for signal averaging (typically requiring hundreds of stimuli pulses), which in turn facilitates higher throughput of mapping. The ease of precise stimulation makes sub-fascicular mapping possible with this new technology. Light can be delivered in a non-contact manner, which may be advantageous in certain procedures. It should be noted that advantages of this method have only been presented in the context of peripheral nerves. However, the full extent of its capability including its limitations has yet to be discovered. Optical stimulation is not an advantageous method for procedures requiring non-selective, whole nerve stimulation due to limits in penetration depth and exponential absorption of

radiant exposure. A non-tunable penetration depth may confine the use of this technique to smaller nerve branches in human subjects. This technique is limited to procedures in which the nerve preparation is exposed and clear from debris, such as saline, blood, or tissue, covering the target irradiation zone for stimulation. Furthermore, if the mechanism is thermal, long term tissue tolerance with optical stimulation may be a potential obstacle for implantable implementation. Future safety studies are required to assess the full clinical potential of this technique. Current studies have been limited to spatial targeting of a few hundred microns (a single nerve fascicle). The validity of the technique to stimulate at a resolution of a few microns has yet to be proven. Thus while this paper presents one successful application of optical stimulation, the full extent of the phenomenon has yet to be elucidated. While optical stimulation is unlikely to replace electrical stimulation, the method has the potential to impact neural stimulation and is therefore worthwhile pursuit as an alternative to electrical stimulation in certain situations.

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References

- Agnew WF, McCreery DB. Considerations for safety in the use of extracranial stimulation for motor evoked potentials. *Neurosurgery* 1987;20(1):143–7. [PubMed: 3808255]
- Allegre G, Avriillier S, et al. Stimulation in the rat of a nerve fiber bundle by a short UV pulse from an excimer laser. *Neurosci Lett* 1994;180(2):261–4. [PubMed: 7700591]
- Andreasen LN, Struijk JJ. Artefact reduction with alternative cuff configurations. *IEEE Trans Biomed Eng* 2003;50(10):1160–6. [PubMed: 14560769]
- Booth J, von MA, et al. The photochemical action of ultra-violet light on isolated single nerve fibres. *Helv Physiol Pharmacol Acta* 1950;8(2):110–27. [PubMed: 15435963]
- Branner A, Normann RA. A multielectrode array for intrafascicular recording and stimulation in sciatic nerve of cats. *Brain Res Bull* 2000;51(4):293–306. [PubMed: 10704779]
- Chandrasekhar, S. Radiative Transfer. Oxford University Press; Oxford: 1960.
- Cruccu G, Romaniello A. Jaw-opening reflex after CO₂ laser stimulation of the perioral region in man. *Exp Brain Res* 1998;118(4):564–8. [PubMed: 9504851]
- Devinsky O. Electrical and magnetic stimulation of the central nervous system. Historical overview. *Adv Neurol* 1993;63:1–16. [PubMed: 8279294]
- Fiore L, Corsini G, et al. Application of non-linear filters based on the median filter to experimental and simulated multiunit neural recordings. *J Neurosci Methods* 1996;70(2):177–84. [PubMed: 9007757]
- Fork RL. Laser stimulation of nerve cells in *Aplysia*. *Science* 1971;171(974):907–8. [PubMed: 5541653]
- Fritsch G, H E. Ueber die elektrische Erregbarkeit der Grosshirns. *Archiv Anatomie, Physiologie, und Wissenschaftliche Medicin* 1870;37:300–332.
- Fukami, Y.; A, K. The thermal dissociation of cholesterol esters using a 5.75micron-free electron laser; SPIE; 2003.
- Geddes, LA. Electrodes and the measurement of bioelectric events. New York: Wiley-Interscience; 1972.
- Geddes LA. Optimal stimulus duration for extracranial cortical stimulation. *Neurosurgery* 1987;20(1): 94–9. [PubMed: 3808283]
- Geddes LA. Accuracy limitations of chronaxie values. *IEEE Trans Biomed Eng* 2004;51(1):176–81. [PubMed: 14723507]
- Geddes LA, Bourland JD. The strength-duration curve. *IEEE Trans Biomed Eng* 1985;32(6):458–9. [PubMed: 4007910]

- Geddes LA, Bourland JD. Tissue stimulation: theoretical considerations and practical applications. *Med Biol Eng Comput* 1985;23(2):131–7. [PubMed: 3982092]
- Geddes LA, Roeder R. Criteria for the selection of materials for implanted electrodes. *Ann Biomed Eng* 2003;31(7):879–90. [PubMed: 12971619]
- Geddes, LA.; B, LE. *Principles of Applied Biomedical Instrumentation*. New York: John Wiley & Sons; 1989.
- Halliday, D.; R, R., editors. *Physics*. New York: John Wiley & Sons; 1978.
- Hecht, E. *Optics*. Reading MA: Addison Wesley Longman Inc.; 2002.
- Hirase H, Nikolenko V, et al. Multiphoton stimulation of neurons. *J Neurobiol* 2002;51(3):237–47. [PubMed: 11984845]
- Horrocks LA. Composition of myelin from peripheral and central nervous systems of the squirrel monkey. *J Lipid Res* 1967;8(6):569–76. [PubMed: 4964432]
- Izzo, AD.; P, J.; Suh, E.; Walsh, JT.; Jansen, ED.; Richter, CP. *Selectivity of optical stimulation in the auditory system*; SPIE; San Jose, CA. 2006.
- Jacques SL. Laser-tissue interactions. Photochemical, photothermal, and photomechanical. *Surg Clin North Am* 1992;72(3):531–58. [PubMed: 1589829]
- Jacques SL, Prahl SA. Modeling optical and thermal distributions in tissue during laser irradiation. *Lasers Surg Med* 1987;6(6):494–503. [PubMed: 3573921]
- Jansen, E. *Laser Tissue Interactions*. Marcel Dekker Publishing; 2004.
- Joule, JP. *The Scientific Papers of James Prescott Joule*. London: Dawson's; 1965.
- Kobbert C, Thanos S. Topographic representation of the sciatic nerve motor neurons in the spinal cord of the adult rat correlates to region-specific activation patterns of microglia. *J Neurocytol* 2000;29(4):271–83. [PubMed: 11276179]
- Konrad PE, Tacker WA Jr. Suprathreshold brain stimulation activates non-corticospinal motor evoked potentials in cats. *Brain Res* 1990;522(1):14–29. [PubMed: 2224506]
- Lee DH, Chae SY, et al. Prognostic value of electroneurography in Bell's palsy and Ramsay-Hunt's syndrome. *Clin Otolaryngol* 2006;31(2):144–8. [PubMed: 16620335]
- Liang DH, Lusted HS, et al. The nerve-electrode interface of the cochlear implant: current spread. *IEEE Trans Biomed Eng* 1999;46(1):35–43. [PubMed: 9919824]
- Martin HC, Sethi J, et al. Patient-assessed outcomes after excision of acoustic neuroma: postoperative symptoms and quality of life. *J Neurosurg* 2001;94(2):211–6. [PubMed: 11213956]
- Miller CA, Abbas PJ, et al. An improved method of reducing stimulus artifact in the electrically evoked whole-nerve potential. *Ear Hear* 2000;21(4):280–90. [PubMed: 10981604]
- Mountcastle, VB. *Medical physiology*. Saint Louis: C. V. Mosby Co.; 1974.
- Nilsson, J.; R, SA. *Electric Circuits*. Reading MA: Addison-Wesley Publishing Company; 1996.
- Orchardson R. The generation of nerve impulses in mammalian axons by changing the concentrations of the normal constituents of extracellular fluid. *J Physiol* 1978;275:177–89. [PubMed: 24735]
- Palanker D, Vankov A, et al. Design of a high-resolution optoelectronic retinal prosthesis. *J Neural Eng* 2005;2(1):S105–20. [PubMed: 15876646]
- Paxinos, G. *The Rat Nervous System*. Sydney, Australia: Elsevier; 2004.
- Pedrotti, F.; P, L. *Introduction to Optics*. Upper Saddle River, NJ: Prentice Hall; 1993.
- Plonsey, R.; B, RC. *Bioelectricity: A Quantitative Approach*. New York: Kluwer Academic/Plenum Publishers; 2000.
- Popovic D, Gordon T, et al. Properties of implanted electrodes for functional electrical stimulation. *Ann Biomed Eng* 1991;19(3):303–16. [PubMed: 1928872]
- Pudenz RH. Neural stimulation: clinical and laboratory experiences. *Surg Neurol* 1993;39(3):235–42. [PubMed: 8456389]
- Ragheb T, Geddes LA. Electrical properties of metallic electrodes. *Med Biol Eng Comput* 1990;28(2):182–6. [PubMed: 2376994]
- Richter, CP.; I, A.; Jansen, ED.; Walsh, JT. *Spatial cochlear tuning obtained with optical stimuli*; Conference of Implantable Auditory Prostheses; Pacific Grove, CA. 2005.

- Testerman R. Comments on 'accuracy limitations of chronaxie values. *Trans Biomed Eng* 2005;52:750.
- Thomsen S. Pathologic analysis of photothermal and photomechanical effects of laser-tissue interactions. *Photochem Photobiol* 1991;53(6):825–35. [PubMed: 1886941]
- Ueno H, Kaneko K, et al. Endoscopic carpal tunnel release and nerve conduction studies. *Int Orthop* 2001;24(6):361–3. [PubMed: 11294432]
- van den Honert C, S PH. Single fiber mapping of spatial excitation patterns in the electrically stimulated auditory nerve. *Hear Res* 1987;29(2-3):195–206. [PubMed: 3624083]
- Vogel A, Venugopalan V. Mechanisms of pulsed laser ablation of biological tissues. *Chem Rev* 2003;103(2):577–644. [PubMed: 12580643]
- Wagenaar DA, Potter SM. Real-time multi-channel stimulus artifact suppression by local curve fitting. *J Neurosci Methods* 2002;120(2):113–20. [PubMed: 12385761]
- Welch, AJ.; v Gemert, MJC. Optical-thermal response of laser-irradiated tissue. New York: Plenum Press; 1995.
- Wells JD, Kao C, Jansen ED, Konrad P, Mahadevan-Jansen A. Application of Infrared Light for in vivo Neural Stimulation. *Journal of Biomedical Optics* 2005;10:064003. [PubMed: 16409069]
- Wells JD, Kao C, Mariappan K, Albea J, Jansen ED, Konrad P, Mahadevan-Jansen A. Optical Stimulation of Neural Tissue in vivo. *Optics Letters* 2005;30(5):504–507. [PubMed: 15789717]
- Wells, JD.; Konrad, P.; Mahadevan-Jansen, A.; Jansen, ED. Biophysical mechanisms responsible for pulsed low-level laser excitation of neural tissue. *SPIE Proceedings*; San Jose, CA. 2006.

Figure 1.

Beer's Law depicting the exponential relationship of laser radiant exposure as a function of depth of laser penetration in a homogeneous tissue. In this graph, nerve tissue optical properties with Holmium:YAG laser irradiation are modeled using the threshold laser radiant exposure required for peripheral nerve excitation.

Figure 2.
Experimental setup for optical and electrical stimulation with electrical nerve and muscle recording techniques.

Figure 3.

(a) CMAP recording from the rat gastrocnemius during optical stimulation (b) Upon topical administration of succinylcholine (c) 30 minutes following drug washout (Gain = 1).

Figure 4.

(a) Measured CNAP following adjacent high intensity electrical stimulation (1.4 A/cm²) and recording at a distance of 10 mm demonstrate presence of electrical artifact. (b) The entire measured CNAP from optical stimulation (0.55 J/cm²) at the same site shows no artifact. Note the order of magnitude difference in the scale between electrical and optical recordings (Gain = 1).

Figure 5. Relationship between the laser radiant exposure [J/cm²] and peak amplitude of the evoked CNAP. The measured optical strength-response curve is similar to that observed in electrical stimulation. Notice a linear relationship exists between this peak ENG amplitude (Gain = 1) graphed as a function of the laser radiant exposure.

Figure 6.

Spatial targeting of transient optical nerve stimulation. (a) Threshold CMAP response from electrical stimulation of the main branch of the sciatic nerve proximal to the first branch point with 1.02 A/cm². (b) Corresponding results from threshold optical stimulation (0.4 J/cm²) of specific target nerve fibers that innervates the gastrocnemius with no response from adjacent nerve fibers (quiet biceps femoris). The distance from the stimulation spot to recording electrodes was held constant for trials involving each stimulation modality (Gain = 1).

Figure 7.

Rat sciatic nerve functional map generated with optical stimulation using 400 and 600 μ m fiber diameters. Colors and shading representing the 12 muscles mapped of the hind leg are labeled to the right of the generalized functional map (n=12). The main sciatic nerve was stimulated just proximal to the branch point of the *n. fibularis superficialis*, *n. fibularis profundus*, and *n. tibialis*, while mapped muscles include the semitendinosus, gastrocnemius (upper and lower), vastus lateralis, biceps femoris (upper and lower), semimembranosus, and the 5 hindpaw digit muscles.

Figure 8. Penetration depth as a function of infrared wavelength from 2 to 6.5 μm based on the absorption spectrum of the rat sciatic nerve as measured through FTIR spectroscopy. The shaded region represents the theoretical optimal penetration depth range specific to the tissue morphology of the rat sciatic nerve. The spectral range for which optical fibers may be employed is also depicted.