Published in final edited form as:

Appl Opt. 2009 April 1; 48(10): D218–D224.

Complete Optical Neurophysiology: Toward Optical Stimulation and Recording of Neural Tissue

Fred A. Wininger¹, Jennifer L. Schei², and David M. Rector^{1,*}

1Dept VCAPP, Washington State University, 205 Wegner Hall, Pullman, WA, 99164, USA

2Dept Physics and Astronomy, Washington State University, Pullman, WA, 99164, USA

Abstract

Direct optical methods to stimulate and record neural activity provide artifact free, non-invasive and non-contact neurophysiological procedures. For stimulation, focused mid-infrared light alters membrane potential and activates individual neural processes. Simultaneous intrinsic scattered light parameters, including birefringence changes, can record neural activity with signals similar to potentiometric dyes. The simultaneous combination of optical stimulation and optical recording techniques provide the potential for powerful tools that may someday remove the need for invasive wires during electrophysiological recordings.

1. Introduction

Traditional neurophysiology experiments use electrical current through wire or glass electrodes to alter membrane potential and activate neural tissue. However, electrical stimulation produces artifacts on electrical recordings, and suffers from degradation in the electrode/neural interface over long time periods (hours to months depending on the preparation). While techniques to remove electrical stimulation artifact have been developed using both hardware and software methods [1-3], they require a significant amount of additional amplifier circuitry, and remain problematic if stimulation voltage levels saturate the amplifier input range. Similarly, changes in neural membrane potential are typically recorded by sensing the electrical potential with conductive electrodes made from wire or glass. Again, electrical measurements of neural activity are subject to degradation in the electrode/tissue interface [4], and require that metal electrodes be placed as close as possible to the activated tissue, also potentially damaging cells through physical contact and immune system attack.

Several optical methods have been utilized for stimulation and recording, yet they remain limited in their application. Potentiometric dyes have significantly improved their ability to report membrane potential [5]; however, they require invasive procedures, and suffer from toxicity effects over time periods as short as a few minutes. Optical stimulation and recording from genetically modified cells utilize photon activated components [6] and photon emitting or fluorescent reporters of cell function [7] which are added to the cell's genetic material, and provide another optical neurophysiological technique. However, these methods have limited use for research, diagnostic, and therapeutic procedures in humans and animals in which genetic alteration is difficult or impossible. Finally, optically released caged compounds have also provided an attractive means for selectively delivering chemical agents to cells; many of

*Corresponding author: director@vetmed.wsu.edu

OCIS codes: (170.3890) Medical optics instrumentation; (140.3070) Infrared and far-infrared lasers

which are used for stimulation [8]. However, the use of caged compounds is limited to those experiments where they can be infused into the tissue.

Intrinsic optical methods for direct neural stimulation and recording have many purported advantages over electrical means [9-11]. First, intrinsic optical stimulation does not require application of exogenous agents, nor does it elicit large electrical artifact within the neural tissue, which tends to mask early neural events associated with the stimulation. Second, by using focusing optics, it is theoretically possible to direct the stimulation in a two or three dimensional field of view, or provide high resolution patterned stimulation, with a resolution limited only by tissue scattering and diffraction and the numerical aperture of the optics. Third, optical stimulation methods do not suffer from neural interface issues, such as electrochemical reactions, because they do not require tissue/metal contact, and have the potential to be less damaging.

However, direct optical stimulation using infrared photons has several limitations that requires significant investigation and technical improvements before it can be of general use. First, the exact mechanism by which membranes become depolarized is still largely unknown. While a brief heating may temporarily disrupt the membrane, or alter membrane channels allowing ions to flow [12], other possibilities also need further exploration including: direct photic activation of membrane proteins through absorption, and the potential for opto-acoustic effects elicited by the infrared light pulse. Second, infrared light delivery is currently achieved through an optical fiber, without additional optical elements, such that the exact beam profile, location, quality and intensity is difficult to quantify without significant additional diagnostic equipment. As such, probe placement requires trial and error procedures, without full knowledge about the exact stimulus location. Additionally, with the current fiber delivery systems, approximately 1mm of free space is required between the stimulation fiber and target without intervening tissue and fluid, otherwise the stimulus light diffuses, and is no longer effective. This makes it difficult to use the device for implanted applications. Third, the threshold between nerve stimulation and damage is narrow, with cellular damage occurring at only two or three times initial stimulation intensities [13]. This damage threshold limits the amount of optical power that can be applied to the nerve and limits the number of recruited axons. However, putting these limitations aside, if the stimulus light can be focused specifically to the desired location, optical stimulation procedures could revolutionize methods for neural stimulation, especially when only a few nerves need to be stimulated at a time.

Optical methods for recording fast neural events allow imaging technologies to create spatial maps of activity patterns, and, in theory, also do not require contact with the tissue [14]. By illuminating the tissue with light, a variety of tissue optical properties change during activation including: changes in scattering due to intracellular swelling as water follows sodium into a depolarized cell, concomitant changes in the refractive index, and biophysical changes in the structure of membrane proteins and phospholipids that alter their absorbance, fluorescence and birefringence properties during activation [15,16]. Additional optical changes occur on a slower time scale, including changes in blood flow, volume and oxygenation for in-vivo preparations [17]. In isolated nerve preparations, the optical changes can directly follow membrane potential [18-21]. Unfortunately, optically recording the consequences of fast neural signals has several limitations. The procedure is technically challenging because the optical changes are small (one part in 10^4 to 10^6), often smaller than the noise of recording devices [15], requiring hundreds to thousands of averages. Additionally, light scattering is high in neural tissue thicker than 100um, making it difficult to spatially resolve activity without physical contact. As with optical stimulation, optical recording techniques require methods to focus illumination light to the tissue region of interest, then detect changes in the light that are specific to neural activity.

In spite of the limitations, of optical stimulation and recording techniques, the combination of the two techniques could provide a powerful tool in the future if some of the technical challenges for light delivery and detection can be overcome. We investigated the application of pulsed, infrared laser light as a method to stimulate nerves, while simultaneously recording optical responses from the tissue. These techniques may promote neurophysiological procedures that use only photons.

2. Methods

We extracted nerves from the walking legs of lobsters, *Homarus americanus* (Sea View Lobster, Kittery, ME, USA), using the Furusawa pulling out method [22], tied each end with silk suture, and placed them in a nerve chamber. Detailed procedures are outlined in our earlier papers [11,15,21]. The nerve chamber included 8 wells, each 2mm wide, 5mm long and 4mm deep (Fig 1D). Electrodes, constructed from 28 gauge bare silver wire approximately 5mm in length, were seated within each nerve chamber well. The nerve bundle rested in a channel that ran through each well. Petroleum jelly was used to electrically isolate each well, then the chamber was filled with a lobster saline bath solution (525 mM NaCl, 13.3 mM KCl, 12.4 mM CaCl₂, 24.8 mM MgCl₂ and 5 mM dextrose). Electrodes within the chamber were used to stimulate and record the electrical responses.

Nerves were stimulated using both electrical and optical methods. Electrical stimuli (0.2ms pulse width) were applied via electrode pairs (Fig 1C; ESTIM1 and ESTIM2) on either side of the chamber, attached to a stimulus isolator (Model A365, World Precision Instruments, Boston, MA). We increased the stimulus intensity in increments of 10uA until action potentials were recorded. Stimuli were applied at 1-2s random inter-stimulus intervals.

For optical stimulation, both whole nerves and fascicular sections were evaluated (Fig. 1A). A section of nerve at either end of the chamber was isolated and the surrounding ringer above the nerve was removed (Fig 1C,D; OSTIM1 and OSTIM2). We found it critical that some fluid remain in contact with the nerve, presumably to dissipate some of the optical stimulation energy that might otherwise damage the nerve through heating. A Renair (Acculight Corp., Bothell, WA) diode laser coupled to a 0.6mm core optical fiber was situated above the nerve surface (approximately 0.75mm), perpendicular to the longitudinal axis (Fig. 1B). The fiber was a low OH multimode silica fiber, 0.6mm in diameter and had a numerical aperture of 0.22 (P600-2-VIS-NIR, Ocean Optics, Dunedin, FL). In order to adequately transmit infrared light, the optical stimulation fiber material must not significantly absorb the 1850nm wavelength. Additionally, since we did not use additional optical elements, the numerical aperture determined the beam profile emitted from the fiber.

Single stimulus light pulses were applied to the nerve at varying widths ranging from 0.2 to 2.0ms using a wavelength of 1856 nm and 100% power setting. Since we did not have access to equipment to measure the actual radiant exposure of the stimulator, we could not assess the amount of light delivered within each pulse. However from the Renair users manual, and from measurements made in previous studies by others [9,10,12,13], we estimate that these pulse widths corresponded to a radiant exposure ranging approximately from 0.1 to 1.0J/cm² at the fiber tip. The actual radiant exposure required to stimulate the nerve depended on many factors including the quality of the fiber tip, the distance of the fiber to the nerve, the amount of material surrounding the nerve (amount of water, glial sheath thickness, other connective tissue). Without an easy to implement method to determine the actual amount of light delivered directly to the neural membrane, the radiant exposure stimulation level (as set by pulse width our experiments) was determined empirically for each experiment by starting with the shortest pulse width of 0.1ms, and increasing the width until action potentials appeared. The shorter

widths activated one or a few axons with less damage to the nerve. The longer pulse widths were required to activate enough axons to produce a change in the optical recording signal.

With our present setup, it was not possible to stimulate the nerve optically and electrically in the same location for two reasons. First, during optical recordings, the polarizer block was positioned directly over the chamber containing the stimulation wires, blocking access by the optical stimulation fiber. Second, if the infrared stimulus light directly illuminated the silver wire, we observed a large electrical artifact (1mV or larger), perhaps due to a thermally initiated electrochemical potential between the silver and the saline solution.

In order to optically record neural activity, an LED emitting 665 nm light (2800 mcd, 5 mm dia., Panasonic, Model LN261CAL) passed through a polarizer (POL1, VIS 4 K, Linos Photonics, Milford, MA) that was oriented 45 degrees with respect to the long axis of the nerve and illuminated the nerve through a glass optical window in the chamber (Fig. 1C). A second polarizer was oriented 90 degrees with respect to the first polarizer (POL2) and a photodiode (PD, UDT-555UV/LN, UDT Sensors, Hawthorne, CA) detected polarized light changes (birefringence). Since the LED contained a built in lens for focusing the source illumination light, no other lenses were required. In order to collect as much of the light emitted from the nerve as possible, a large (1cm diameter) photodiode was placed as close as possible to the nerve, with only the top polarizer between the nerve and the detector (POL2, Fig 1C).

All signals were amplified with a laboratory built amplifier system using a commercially available amplifier circuit (MAX4478, Maxim Semiconductor, Sunnyvale, CA), filtered between 0.1 Hz and 3.2 KHz, digitized at 20 KHz and archived for post-hoc analysis. For acquisition of single action potentials, we used a low noise amplifier circuit (AD797, Analog Devices, Inc, Norwood, MA).

3. Results

Electrical Stimulation

Electrical stimulation at 200uA produced single action potentials from the largest nerves, with fascicular sections requiring as little as 40uA. Increasing stimulation current recruited action potentials from more axons, producing population action potentials from both large and small axons. By using propagation velocity as an index of axon diameter, we observed that the largest axons were recruited with the lowest electrical stimulation currents as would be expected due to their lower axial and membrane resistance. Increased electrical stimulation current recruited smaller axons, corresponding inversely to the stimulus current (data not shown) [21]. In order to activate enough axons to generate significant optical signals, electrical stimulus intensities of 2mA were required.

Electrical responses were recorded on both sides of the chamber (ER1 and ER2), and the temporal spread in action potentials were observed based on differences in propagation velocity and axon diameter (Fig. 2). When stimuli were presented on the right side of the chamber, the electrical responses appeared inverted because action potentials arrive first on the electrodes with opposite polarity. Optical birefringence responses to electrical stimulation, as measured by the change in light polarization, were similar to those previously reported [11,15,21]. Since electrical stimulation used between 3 and 30 volts (depending on the impedance of the tissue), and since the photodiode amplifiers were highly sensitive, a small amount of electromagnetically induced signal could be observed on the photodiode output during the electrical stimulus pulse. The optical changes exhibited a slow recovery time and therefore, the birefringence signals did not exhibit the same temporal structure of the population action potentials seen in the electrical responses (Fig. 2).

Optical Stimulation

Onset, peak and duration for electrical and birefringent responses were comparable using optical stimulation with 2ms pulse widths (Fig. 2). Since high optical power and a 2ms pulse width was needed to optically activate a sufficient number of axons to generate a detectable birefringence signal, only a two to four trials were possible before nerve damage occurred. Additionally, the electrical and birefringence responses were an order of magnitude smaller than the electrically stimulated nerves because fewer axons were recruited in the response. Placement of the laser was essential for optimal optical stimulation, with a 50μm error in height or position leading to lack of detectable action potentials. Since the optical stimulation fiber was placed approximately 15mm distal from the center of the electrical stimulation wire pairs, we expected a 5 to 15ms delay in the responses to optical stimulation when compared to electrical stimulation (population axonal propagation velocities that ranged from 1.0 to 3.0m/s [21]). We measured an average delay of 13.6 ± 2.1 ms from the stimulus time to the earliest responses which corresponded to a propagation velocity of approximately 1.1m/s.

In order to assess our ability to optically stimulate individual axons, we positioned the optical stimulation fiber over the nerve on the left side of the chamber, and gradually increased the pulse width. Action potentials were noted with a minimal optical stimulation pulse width of 400μs (Fig. 3). Low level optical stimulation, that recruited only one or two axons, could be maintained for many hundreds of trials, similar to electrical stimulation (data not shown), limited only by the normal lifetime of the extracted nerve. Incremental increases in the pulse width correlated with an increase in the number of action potentials, suggesting increased axonal recruitment. Pulse widths above 1ms quickly damaged the nerve and action potentials could no longer be produced. Pulse widths of this duration induced a change in the color of the underlying nerve, making it more opaque, especially when the nerve was suspended in air during optical stimulation.

The optically stimulated axons seen in Figure 3 had medium to small (20 to 100μm) diameters since they exhibited propagation velocities of 1.0 to 1.5m/s, suggesting that optical stimulation activated smaller diameter axons, or due to the axon position relative to the stimulation probe, especially if the smaller axons were around the periphery of the nerve bundle.

4. Discussion

These results represent a significant step toward using optical techniques to directly stimulate and record neural activity without the use of dyes or other agents. The advantages of optical techniques lie in the ability to employ numerous strategies for focusing and controlling the light in order to activate a subset of axons, which cannot be done with electrical methods. However, several limitations must be overcome to make optical stimulation techniques practical, including minimizing the effects of photon scattering by tissue, and increasing the power and specificity in light delivery. Optical detection techniques require increased sensitivity and lower noise in photo-detectors, and optical sectioning methods, such as optical coherence tomography, to increase the spatial resolution and exclude non-specific light.

By themselves, the results from the two techniques presented here are consistent with previous reports of optical stimulation and recording. However, in combination, these techniques could someday provide better non-invasive methods for stimulating and recording from neural tissue. Our results show that simultaneous optical stimulation and recording can provide similar information as electrical methods, especially when only a few axons require activation.

In order to improve the application and minimize diffusion of optical stimuli for general use, an optical element is needed to allow the stimulus probe to make contact with the tissue, and focus the light to a particular point within the tissue. In particular, liquid or tissue between the

laser and nerve interfered with stimulation and did not generate action potentials, presumably because the light was diffused by the liquid or tissue. We devised a focusing element using a simple 1mm ball lens, with the stimulation optical fiber placed nearly in contact with the lens, but were unsuccessful at stimulating nerves in this configuration. Since we lacked equipment to adequately image the stimulus light beam profile, we could not determine the reason for our failure. Also, when nerves were completely suspended in air, without fluid underneath or around the nerve, neural activation rapidly degraded after one or two pulses.

Since the laser light may induce activation through thermal changes, rapid tissue heating may accelerate damage to the axons [9]. Although post-stimulus histology was not performed, we used a dissecting microscope throughout the experiments and noted gross changes in nerve color and opacity at optical pulse widths longer than 1ms when the nerve bundle was suspended in air during stimulation, but to a lesser extent when the nerve had some contact with the bath solution. Earlier work by others reported that rat sciatic nerves were histologically undamaged using similar stimulation wavelengths with short pulse widths, even at two weeks following the stimulation [13]. Unmyelinated nerves, as found in the lobster leg, may be more susceptible to thermal damage due to the increased water content and higher wavelength absorption. However, additional studies are required to compare optical stimulation of the two nerve types.

Optical detection of action potentials using birefringence methods mirrored electrical changes in sensitivity and onset. The delayed peak and duration of the optical signal when compared to the electrical recording is consistent with the hypothesis that optical signals measure neural swelling [16]. Since the location of the optical stimulator was further from the optical recording window than the electrical stimulus wires (Fig. 1), the time differences in electrical and optical depolarization onset with optical stimulation seen in Figure 2 are due to differences in the stimulus location relative to the recording site. The average optical stimulus location delay of the earliest signals in the response was 13.6ms (1.1m/s), which is slow compared to the fastest action potentials (~3.0m/s), and could be explained by a longer time required to activate the nerve optically over electrically.

Figure 2 also shows that the electrical and optical responses to optical stimulation were one order of magnitude smaller than the responses to electrical stimulation. Our optimal optical stimulation paradigm recruited many fewer axons than electrical stimulation, thus the size of both the electrical population action potentials and the optical birefringence changes were smaller. Higher optical stimulation intensities rapidly damaged the nerve, thus we could not achieve larger responses with optical stimulation. Indeed, the ability to be more specific in stimulating a subset of the axons within a bundle represents one advantage of optical stimulation over electrical stimulation; however, when large numbers of axons need to be recruited, optical stimulation was not as effective because the higher energy required damaged the nerve.

Better efficiency at delivering stimulation light to more specific locations within the tissue using shorter pulse widths may greatly improve optical stimulation. Additionally, high speed optical scanning techniques could be used to deliver shorter pulses to multiple regions in the tissue, and thus recruit more axons without the need to increase total optical power. While one of the advantages of optical stimulation is minimal or no stimulus artifact on the electrical recording, if optical detection of neural activity is required at the same location as the stimulus, an optical stimulus artifact is possible if the detector is sensitive to infrared wavelengths. Thus, the detector must be gated or filtered to exclude the stimulus light.

Data from these experiments suggest that the infrared laser is capable of depolarizing the lobster leg axons and can generate action potentials. Since we could simultaneously stimulate and record neural activity with optical methods, this study paves the way for neurophysiology

without the need for wires or electrical conduction. Both the optical stimulation and recording paradigms require further development in order to probe deeper into tissue or direct the stimulus to specific nerve types. Pilot studies are currently underway to evaluate the concurrent application of optical stimulation and recording of mixed sensorimotor sciatic nerve and cortical activity using an in-vivo rat model.

6. Acknowledgments

This work was made possible through an NIH SBIR to the Aculight Corporation (5R44NS051926-03), NIH Grant MH60263, and the Murdock Foundation. JLS is supported by a fellowship from the Poncin Foundation.

5. References

1. Babb TL, Mariani E, Strain GM, Lieb JP, Soper HV, Crandall PH. A sample and hold amplifier system for stimulus artifact suppression. *Electroencephalogr Clin Neurophysiol* 1978;44(4):528–531. [PubMed: 76562]
2. Klop WM, Hartlooper A, Briare JJ, Frijns JH. A new method for dealing with the stimulus artefact in electrically evoked compound action potential measurements. *Acta Otolaryngol* 2004;124(2):137–143. [PubMed: 15072415]
3. O'Keefe DT, Lyons GM, Donnelly AE, Byrne CA. Stimulus artifact removal using a software-based two-stage peak detection algorithm. *J Neurosci Methods* 2001;109(2):137–45. [PubMed: 11513948]
4. Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. *J Neurosci Methods* 2005;148(1):1–18. [PubMed: 16198003]
5. Zecevic D, Djuricic M, Cohen LB, Antic S, Wachowiak M, Falk CX, Zochowski MR. Imaging nervous system activity with voltage-sensitive dyes. *Curr Protoc Neurosci*. 2003Chapter 6:Unit 6.17
6. Airan RD, Hu ES, Vijaykumar R, Roy M, Meltzer LA, Deisseroth K. Integration of light-controlled neuronal firing and fast circuit imaging. *Curr Opin Neurobiol* 2007;17(5):587–592. [PubMed: 18093822]
7. Kain SR, Ganguly S. Overview of genetic reporter systems. *Curr Protoc Mol Biol*. 2001Chapter 9:Unit9.6
8. Mayer G, Heckel A. Biologically active molecules with a 'light switch'. *Angew Chem Int Ed Engl* 2006;45(30):4900–4921. [PubMed: 16826610]
9. 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(6):0640031–12.
10. Richter CP, Bayon R, Izzo AD, Otting M, Izzo AD, Richter CP, Jansen ED, Walsh JT Jr. Laser Stimulation of the Auditory Nerve. *Lasers in Surgery and Medicine* 2006;38:745–753. [PubMed: 16871623]
11. Foust AJ, Beiu RM, Rector DM. Optimized Birefringence Changes During Isolated Nerve Activation. *Applied Optics* 2005;44(11):2008–2012. [PubMed: 15835348]
12. Wells J, Kao C, Konrad P, Milner T, Kim J, Mahadevan-Jansen A, Jansen ED. Biophysical mechanisms of transient optical stimulation of peripheral nerve. *Biophys J* 2007;93(7):2567–2580. [PubMed: 17526565]
13. Wells JD, Thomsen S, Whitaker P, Jansen ED, Kao CC, Konrad PE, Mahadevan-Jansen A. Optically Mediated Nerve Stimulation: Identification of Injury Thresholds. *Lasers in Surgery and Medicine* 2007;39:513–526. [PubMed: 17659590]
14. Rector DM, Carter KM, Volegov PL, George JS. Spatio-temporal mapping of rat whisker barrels with fast scattered light signals. *Neuroimage* 2005;26(2):619–27. [PubMed: 15907319]
15. Foust AJ, Schei JL, Rojas MJ, Rector DM. In vitro and in vivo noise analysis for optical neural recording. *J Biomed Optics* 2008;13(04):044038.
16. Foust AJ, Rector DM. Optically Teasing Apart Neural Swelling and Depolarization. *Neuroscience* 2007;145:887–889. [PubMed: 17303339]
17. Villringer A, Chance B. Non-invasive optical spectroscopy and imaging of human brain function. *Trends Neurosci* 1997;20(10):435–42. [PubMed: 9347608]

18. Cohen LB, Keynes RD, Hille B. Light scattering and birefringence changes during nerve activity. *Nature* 1968;218:438–441. [PubMed: 5649693]
19. Tasaki I, Watanabe A, Sandlin R, Carnay L. Changes in fluorescence, turbidity, and birefringence associated with nerve excitation. *Proc. Natl. Acad. Sci* 1968;61:883–888. [PubMed: 4301149]
20. Stepnoski RA, LaPorta A, Raccuia-Behling F, Blonder GE, Slusher RE, Kleinfeld D. Noninvasive detection of changes in membrane potential in cultured neurons by light scattering. *Proc. Natl. Acad. Sci* 1991;88:9382–9386. [PubMed: 1946349]
21. Carter KM, George JS, Rector DM. Simultaneous birefringence and scattered light measurements reveal anatomical features in isolated crustacean nerve. *J. Neurosci. Methods* 2004;135:9–16. [PubMed: 15020084]
22. Furusawa K. The depolarization of crustacean nerve by stimulation or oxygen want. *J Physiol* 1929;67:325–342. [PubMed: 16994035]

Figure 1.

Walking lobster leg claws with separated nerve fascicles were extracted utilizing the Furusawa method (A). A single fascicle placed in the nerve chamber and immersed in ring is depicted in B. The 0.6mm optical fiber for laser stimulation, coming from the top left corner, was attached to a micro-manipulator and positioned over the nerve. In the middle of the chamber, the nerve was suspended over the optical window and illuminated by a 665nm LED for detection of the optical response. Panel C illustrates a side view of the optical recording setup with the nerve represented by the white line in the middle of the chamber. An LED emitting 665nm light illuminated the nerve. Crossed polarizers (POL1 and POL2) were placed at 45 degrees with respect to the long axis of the nerve and sandwiched above and below the middle section to detect changes in birefringent light with the photodiode (PD). Panel D shows a top view of the chamber which has four wells on each side of the chamber which contained silver wire and were isolated with petroleum jelly. Two pairs of electrical stimulation wires (ESTIM1 and ESTIM2) were on either side of the center section along with two pairs of electrical recording wires (ER1 and ER2). Since the polarizers blocked the central portion of the chamber, optical stimulation positions (OSTIM1 and OSTIM2) needed to be on either end of the nerve. The optical window in the middle of the chamber was used to illuminate the nerve with 665nm LED light. A ruler at the bottom of panel D shows approximate distances for each section from the center of the chamber.

Figure 2.

Electrical and optical (birefringent) responses from electrical and optical stimulation are shown for four lobster walking leg nerves. Both electrical (2mA current) and optical (2ms pulse width) stimulation were performed on the left side and right side of the nerve causing population action potentials to propagate in the left to right or right to left direction, respectively. Electrical responses were recorded on both the left side (ER1, electrical response black line) and the right side (ER2, electrical response gray line) of the chamber. Electrical stimulation traces represent an average of 20 stimuli while the optical stimulation traces are an average of 4 stimuli. Vertical lines indicate time of stimulation. Note the lack of noticeable stimulus artifact in the optical stimulation traces.

Figure 3.

Electrical responses to mild optical stimulation (left side stimulation) in a nerve fascicle shows action potentials at a minimal pulse width of 400us. With increasing optical pulse widths, more axons were activated. Black lines are data collected by the left electrode position (ER1, Fig. 1D), which was closer to the optical stimulation fiber, while the delayed gray traces show recordings from the electrodes on the right side (ER2, Fig. 1D), farther from the stimulator. The distance between the recording electrodes was approximately 21mm, thus action potentials propagated at a rate between 1.0 and 1.5m/s. At 1ms stimulation pulse width, action potentials are no longer noted, likely due to thermal injury of the fascicle. The vertical line indicates the time of stimulation. These data were collected with a very low noise amplifier and a much

higher gain than the data in Figure 2. At this gain, a small stimulus artifact is visible due to electrical crosstalk between the laser trigger and the electrodes. This artifact could be removed by shielding the laser drive cable.