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Motor Neuron Activation in Peripheral Nerves Using Infrared Neural Stimulation

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Abstract

Objective—Localized activation of peripheral axons may improve selectivity of peripheral nerve interfaces. Infrared neural stimulation (INS) employs localized delivery to activate neural tissue. This study investigated INS to determine whether localized delivery limited functionality in larger mammalian nerves.

Approach—The rabbit sciatic nerve was stimulated extraneurally with 1875 nm-wavelength infrared light, electrical stimulation, or a combination of both. Infrared-sensitive regions (ISR) of the nerve surface and electromyogram (EMG) recruitment of the Medial Gastrocnemius, Lateral Gastrocnemius, Soleus, and Tibialis Anterior were the primary output measures. Stimulation applied included infrared-only, electrical-only, and combined infrared and electrical.

Main results—81% of nerves tested were sensitive to INS, with 1.7 ± 0.5 ISR detected per nerve. INS was selective to a single muscle within 81% of identified ISR. Activation energy threshold did not change significantly with stimulus power, but motor activation decreased significantly when radiant power was decreased. Maximum INS levels typically recruited up to 2–9% of any muscle. Combined infrared and electrical stimulation differed significantly from electrical recruitment in 7% of cases.

Significance—The observed selectivity of INS indicates it may be useful in augmenting rehabilitation, but significant challenges remain in increasing sensitivity and response magnitude to improve the functionality of INS.

Keywords

electromyogram; motor recruitment; selectivity; electrical stimulation; peripheral nerve; sciatic; laser stimulation; optical stimulation; extraneural

1. Introduction

Peripheral nerve interfaces are useful in restoring function to paralyzed limbs by stimulating the axons of lower motor neurons (Polasek et al., 2009; Popovic et al., 2007; Weber et al., 2005). Improving control over the axons which are activated is a key interface design parameter, as improved selectivity of activation can increase functionality (Badia et al., 2011; Butson et al., 2011; Fisher et al., 2009; Maks et al., 2009; Nielsen et al., 2011; Richter et al., 2011b; Schiefer et al., 2010). Efforts to improve selectivity in electrically-based interfaces have involved bypassing the resistive outer layers of the peripheral nerve by

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introducing electrodes to the endoneurial space (Badia et al., 2011; Dhillon and Horch, 2005; Dowden et al., 2009; Fitzgerald et al., 2012; Rutten et al., 1991; Sergi et al., 2006). This increase in selectivity comes at the cost of increased invasiveness to the nerve. While shown effective in interfacing with small populations of axons (Badia et al., 2011; Dowden et al., 2009), reports of chronic response reported decreased innervation and increased muscle atrophy (Fitzgerald et al., 2012). An ideal interface would be capable of selectively, but non-invasively, activating axons within the peripheral nerve.

Optical neural interfaces do not require an electrochemical interface with the body, and optical energy can be shaped and delivered in ways unique from electrical stimulation. Stimulation using infrared light can be achieved without modifying targeted neurons or introducing exogenous chemicals (Izzo et al., 2006; Wells et al., 2005b). Infrared neural stimulation (INS) can evoke motor response when applied to the surface of a peripheral nerve (Duke et al., 2009; Richter et al., 2011a; Wells et al., 2007c, 2005b). Functional mapping of INS-evoked motor response on the rat sciatic nerve indicates localized, low-level activation of muscle groups is possible (Wells et al., 2007b). Sensitivity of the rat sciatic nerve to extraneural INS has been reported as either generally uniform (Wells et al., 2007b), or localized to small regions occurring on only a fraction of rat sciatic nerves (Duke et al., 2012a). Activation thresholds of INS applied to peripheral nerves occur below acute radiant exposure damage thresholds (Wells et al., 2007c), but thermal loading limits stimulation at a single site to 4 Hz (Wells et al., 2007a). Electrical stimulation combined with infrared delivery has been used to lower INS thresholds for individual muscle twitches (Duke et al., 2012a, 2009). These works indicate INS may offer selective, extraneural activation in small mammalian nerves, which may be further improved by controlling energy delivery to very small regions of the nerve. Important steps in developing INS include translation to larger nerves, quantifying motor recruitment above stimulation thresholds, and measuring nerve sensitivity to extraneural INS.

Our goal of investigating INS is to explore the design parameters relevant to translating INS towards a selective and functional peripheral nerve interface. Previous studies have used the rat sciatic nerve to study INS (Duke et al., 2009; Wells et al., 2007b). The rabbit sciatic nerve used in this study is roughly twice the diameter of the rat sciatic nerve, (Hsu et al., 2011; Tyler and Durand, 2003), and was chosen to gain understanding about how INS scales with nerve size. The first aim of this study is to quantify sensitivity and response of a multi-fascicular mammalian peripheral nerve model to single-channel INS, and characterize motor recruitment below reported acute damage thresholds. We hypothesized that at least 3 infrared-sensitive regions (ISR) could be identified on each nerve, as this is the minimum number of ISR required to interleave 4 Hz stimulation across multiple nerve sites for tonic, 12 Hz stimulation. We hypothesized that motor recruitment would follow a monotonically increasing response to increasing pulse energy below the acute damage threshold, that at least 50% of ISR provide selective activation of a single muscle, and that a single channel of INS stimulation would recruit at least 10% of a muscle. The second aim of this study was to investigate whether co-located and entrained infrared stimulation would augment extraneural electrical stimulation to improve selectivity of activation. Combined electrical and infrared stimulation was compared to electrical stimulation for full-muscle recruitment. We hypothesized that combined stimuli would alter recruitment of enough axons to provide significant differences in full-muscle recruitment over electrical stimulation alone.

2. Methods

2.1 Surgical preparation and EMG recording

The following procedures were approved by the Case Western Reserve University Institutional Animal Care and Use Committee (IACUC). New Zealand white rabbits

(*Oryctolagus cuniculus*) (Male, 2.8–4.5 kg) were initially anesthetized using Ketamine and Xylazine. Anesthesia was maintained using Isoflurane at 2–4% supplied through an endotracheal tube with oxygen. Reflex checks and vital signs were used to determine anesthesia level. Heart rate, temperature, oxygen saturation of the blood, and exhaled carbon dioxide were measured to maintain stasis. Blunt dissection techniques were used to separate the semitendinosus and biceps femoris and expose the sciatic nerve proximal to the popliteal fossa.

Electromyogram (EMG) signals were recorded to quantify motor response using bipolar intramuscular wire pairs placed in the Lateral and Medial Gastrocnemius muscles (LG and MG, respectively), Soleus (Sol), and Tibialis Anterior (TA) (Figure 1A). Two independent recording channels were implanted in each MG and LG to better record response in these physically larger muscles. Intramuscular electrodes were made by removing 3–5 mm of insulation from 100 μm 316LVM stainless steel wire (Fort Wayne Metals, Fort Wayne, IN), and implanted using a hypodermic needle tip. An amplification factor of 330, a 10–500 Hz band-pass filter, and a 60 Hz mains-notch filter were applied to the recorded EMG signals (1902 Amplifier, Cambridge Electronic Design, Cambridge, England, UK), and sampled at 5000 Hz to accurately capture EMG shape and timing (USB-6259 BNC, National Instruments, Austin, TX). Electrical and infrared stimulus triggering was controlled using custom LabVIEW software (LabVIEW 2010, National Instruments, Austin, TX). Stimulator triggers were recorded with EMG signals. EMG recordings started approximately 40 ms before and continued approximately 50 ms after the stimulus trigger.

2.2 Infrared Deposition

Infrared energy was generated at 1875 nm using a continuous-wave diode laser (Capella 1870, Lockheed-Martin Aculight, Bothell, WA). Pulse energy was controlled by adjusting pulse power and duration. Infrared light was delivered through the polished tip of a 400 μm optical fiber (Ocean Optics, Dunedin, FL). Prior to each experiment, spot size was measured using the knife edge technique (Khosrofian and Garetz, 1983) and a power meter (PS19Q, Coherent Inc. Santa Clara, CA). The fiber tip was positioned 200–1000 μm from the nerve surface using a 3-axis, 10 μm -resolution micromanipulator (M325, World Precision Instruments, Sarasota, FL). Infrared pulses were applied at 1–2 Hz to minimize pulse-to-pulse thermal interactions (Wells et al., 2007a).

2.3 Optical stimulation

Radiant exposure to INS was limited to 2.6 J/cm² to avoid the acute damage thresholds reported by (Duke et al., 2012a, 2009; Wells et al., 2007a). The nerve surface was scanned with infrared light to identify regions where INS triggered a detectable EMG response. The nerve was scanned side to side in 100–200 μm transverse rows along the accessible length of the nerve. When the edge of the accessible area was reached, the nerve was scanned in longitudinal columns. The tip of the optical fiber was positioned between 250 and 1000 μm from the nerve surface using the micromanipulator. Pulse power and duration was initially set to target energy density of 1.3 J/cm² when the fiber is positioned ~750 μm from the nerve. If no ISR were observed, radiant exposure was increased and the surface was rescanned.

When an optically sensitive region was detected, the fiber was positioned to center the infrared beam over the sensitive region based on evoked EMG amplitude. The red pilot light of the laser was used to visualize the location of the ISR on the nerve surface. The ISR position was recorded on a diagram of the nerve relative to the common peroneal and sural branches (Figure 1A). When time permitted, response was measured as a function of position on the nerve. The ISR was scanned in the longitudinal and transverse directions in

50–100 μm steps with 0.7–2.6 J/cm^2 pulses. The largest above-threshold region across all pulse durations tested was taken as the spatial measurement of the ISR.

Early data collection revealed periodic decreases in recruitment response that would return a few minutes later. The points where response dropped did not correspond to particular pulse energies/durations, so nerve hydration was investigated as a significant factor. Recruitment response was measured over time with fixed stimulus and periodic application of 0.25–0.5 ml lactated Ringer's solution to the nerve surface. At least 30 seconds were allowed to pass between applications of 8 infrared pulses, delivered at 2 Hz. The response rate to stimulus was defined as percentage of applied optical pulses resulting in detectable EMG, and the threshold for response rate set to 30% to exclude random twitches and noise events. When the recruitment response rate dropped below the threshold during data collection, data points were excluded until the response rate recovered. Because the response rate was defined as the ratio of detected EMG signals to applied pulses, changes in recruitment amplitude would still be captured for detectable EMG responses.

Motor recruitment curves at ISR were recorded in response to infrared pulse durations from 0.2–3.4 ms in 0.1 ms steps. Maximum pulse duration was limited based on infrared power to remain below the calculated damage threshold. Order in which pulse duration was applied was randomized, and at least 8 pulses were applied at each pulse duration level, at a rate of 2 Hz. The activation threshold of a recruitment curve was defined as the lowest pulse duration or energy at which the response rate exceeded 30%.

2.4 Electrode fabrication

To emulate geometries previously identified effective in combining infrared and electrical stimulation (Duke et al., 2012a, 2009), a nerve cuff with three electrodes was developed (Figure 1B). Polydimethylsiloxane (PDMS) was mixed and cured in 3D-printed molds to form flat-interface nerve electrodes (FINE) (Tyler and Durand, 2003). The nerve channel cross-section was 0.8 mm by 4.5 mm. The infrared delivery window was created by cutting a 1.3 mm by 4.5 mm opening spanning the width of the nerve channel into the top PDMS layer, allowing direct IR delivery to the nerve. Electrical stimulation was applied through electrodes stabilized in the PDMS cuff. Square, 0.64 mm^2 platinum electrodes were located on either side of this window on the top half of the nerve channel, longitudinal to the nerve and 1.3 mm apart. A third electrode was placed on the bottom half of the nerve channel, directly below the opening.

2.5 Electro-Infrared Stimulation

The PDMS nerve cuff positioned as close as possible to an identified ISR to deliver electrical and combined electrical and infrared stimulus. After device implantation, optical fiber position and radiant exposure was adjusted to re-establish infrared-evoked response, if possible. Electrical stimulation was supplied using a custom current-controlled stimulator (FSPG, Crisptronics LLC, Cleveland, OH). Stimulus pulses were biphasic with a rectangular stimulus phase and passive, current-limited charge-balancing phase (Figure 1C). Muscle recruitment was measured as a function of electrical pulse amplitude, in response to pure electrical or combined electrical and infrared stimulus. Nine different electrode configurations were used to generate recruitment curves on each nerve. Each configuration employed one of the three electrodes as the cathode, with anodic return through either one of the other two contacts or a distant percutaneous electrode. Infrared stimulus was set to ~150% of the previously observed infrared threshold. This ensured some safety margin below damage thresholds, while maximizing nerve region affected by INS. Electrical and optical stimulation was entrained so the stimulation phases of each ended at the same time (Figure 1C), matching previous reports of combined stimulus (Duke et al., 2009). Electrical

and optical stimuli were coordinated using custom LabVIEW software (LabVIEW 2010, National Instruments, Austin, TX). Electrical amplitude was randomized, with at least 6 repeat measures for each amplitude throughout the trial. Electrical-only and combined stimulus pulses were applied in pairs at each electrical amplitude in randomized order. All stimuli were applied at 1–2 Hz to reduce inter-pulse interaction and fatigue.

2.6 Data Analysis

EMG response was the primary outcome measure used in analyzing response to applied stimuli. Stimulus triggers were used as reference time points for identifying evoked responses. Early EMG responses were analyzed manually to determine that EMG response typically began within 5 ms and was complete within 10–13 ms of the trigger. An automated process was developed to measure evoked EMG response. The DC offset of all EMG signals was removed, and the RMS value of the signal during the 40 ms prior to the trigger was computed to estimate baseline noise. Threshold was set at 6 times this RMS, and the algorithm detected threshold crossings in the 15 ms after each stimulus trigger. Contiguous signal deflections that maintained the same polarity for longer than 0.5 ms but less than 4 ms were classified as part of the EMG signal and used to determine EMG start and stop times. Muscle recruitment was measured as the rectified EMG signal integrated from start to stop time.

Delay between stimulus and EMG initiation was compared for infrared and electrical stimuli applied to the same nerve region. Infrared light was delivered through the opening in the nerve cuff, and electrical stimulation applied through each of the contacts 0.6 mm proximal and distal to the opening. Recruitment curves were generated with each energy modality, varying pulse amplitude for electrical stimulation and pulse duration for infrared stimulation. Delay between stimulus and the start of evoked EMG was measured manually by plotting EMG signals relative to the stimulus triggers. The electrically-evoked EMG delay was measured from the end of the 50–100 μ s cathodic stimulus phase to the initial EMG deflection. The optically-evoked EMG delay was measured from the end of a minimum-duration infrared pulse triggering an EMG response to the initial EMG deflection.

All statistical testing was performed with a significance level of $\alpha = 0.05$. Wilcoxon signed rank tests were used when comparing measured results against a hypothesized mean. Wilcoxon rank sum tests were used to compare pairs of distributions. Significance and confidence intervals for selectivity were computed using a proportions test, with each sensitive region classified as selective or not. Differences in recruitment curves between electrical and hybrid stimulation were tested using a two-sample Kolmogorov-Smirnov test sensitive to differences in shape and location of the cumulative distribution of two samples (Chen et al., 2009). Recruitment pairs identified as statistically significant were examined visually to determine whether consistent changes in activation were present in response to combined stimulus. Changes in recruitment threshold or slope were considered functionally relevant, but statistically significant differences in recruitment at a few stimulation amplitudes was not. Plotting, data analysis, and statistical computations were performed using MATLAB 2012a (Mathworks, Natick, MA), and Minitab 16 (Minitab Inc. State College, PA).

3. Results

3.1 Nerve Sensitivity to INS

30–50 mm sections of 2.5–4.5 mm-wide sciatic nerves (N=32, 25 rabbits) were scanned to identify infrared-sensitive regions (ISR). An ISR was classified as unstable if motor response was observed, but did not remain long enough to record recruitment data. Adding a stabilizing stage below the nerve helped decrease movement of the nerve and increase

response stability. 26/32 (81%) scanned nerves were responsive to infrared pulses. 1.7 ± 0.5 (mean $\pm$ 95% confidence interval) ISR per nerve were observed across all 32 nerves. 2.0 ± 0.5 (mean $\pm$ 95% confidence interval) ISR per nerve were observed among the 26 infrared-sensitive nerves. A typical result is illustrated in (Figure 2A), and a composite result across all nerves is illustrated in (Figure 2B). The maximum number of ISR detected on a single nerve was 6. Scans of the sural sensory branch did not yield any motor response, as expected. Lower ISR frequency proximal to the common peroneal branch or distal to sural branch (Figure 2B) was due to nerve-to-nerve variation in branch point locations and physical limitations of scanning above and below these points, rather than intrinsic lower sensitivity. EMG responses in 11 ISR were measured as a function of position and pulse energy to measure ISR size. Average transverse and longitudinal measurements were $360 \pm 90 \mu\text{m}$ and $470 \pm 140 \mu\text{m}$ (mean $\pm$ 95% confidence interval), respectively.

3.2 Motor Recruitment with Infrared Stimulus

Recruitment from fixed stimulus was measured over time to investigate effects of hydration on motor response. 12 trials were performed across 6 animals. LRS was added prior to each trial, and at least 3 minutes elapsed before beginning a trial. For 8/12 trials, LRS was applied twice mid-trial (Figure 3A, B), whereas in 4/12 trials no LRS was added once the trial began. 4/8 of the intra-trial hydration cases showed a significant decrease in response immediately following LRS application (Figure 3A). The other 4/8 cases showed no significant change in recruitment after saline application (Figure 3B). Of the 4 trials where the nerve was not hydrated mid-trial, only 1 trial showed brief reduction of response that returned within a few minutes.

Recruitment as a function of pulse duration/energy was recorded in 42 sensitive regions (21 nerves, 18 rabbits). Stimulation was applied with pulse durations between 0.2 and 3.4 ms, in 0.1 ms increments. Recruitment curves were analyzed across recorded EMG channels to measure selectivity. An ISR was classified as selective if a single muscle could be recruited before another reached threshold (Figure 3C), and classified as non-selective if multiple muscles reached the threshold response rate simultaneously (Figure 3D). Single-muscle selectivity was observed in 34/42 sensitive regions (95% confidence interval: 67–91%, single-parameter proportions test). When the definition of selectivity was relaxed to allow co-activation of the functionally-similar MG and LG, 39/42 cases exhibited selectivity (95% confidence interval: 81–99%). Multiple muscles could be activated within most ISR, with increased pulse energy. In only 12/42 (29%, 95% confidence interval: 16–45%) ISR activation was limited to a single muscle below the calculated damage threshold.

Mean recruitment was observed to follow a generally monotonic relationship with increase pulse duration. Across 209 recruitment curves (21 nerves, 17 rabbits) Spearman's ρ was 0.68 ± 0.04 (mean $\pm$ 95% confidence interval), and was significantly greater than zero for 83% of the recruitment curves.

To examine whether short, high power infrared delivery was more effective than longer, lower power delivery, recruitment curves were generated using infrared pulses delivering radiant power at 4.4, 3.4, and 2.3 W. Data was collected at 12 sensitive regions across 7 nerves/rabbits at 4.4 and 3.4 W, but time only permitted collection at 2.3W in 4 trials. Pulse duration thresholds increased significantly with decreased radiant power (Figure 4A), but total energy thresholds did not (Figure 4B). Maximum recruitment decreased significantly with decreased radiant power when compared to recruitment at 4.4 W (Figure 4C). Because in many cases lower power stimulation failed to activate all muscles that were stimulated using full power, Sol and TA lacked sufficient samples at 2.3 W to compute confidence intervals or significance. ANOVA showed radiant power ($p=0.04$), but not muscle ($p=0.93$), to be a significant factor in recruitment level.

Maximum optical and electrical recruitment was compared across 57 recruitment curves (12 nerves, 10 rabbits). INS recruitment was lower magnitude than electrical recruitment (Figure 5A). Mean INS recruitment across muscles was 2–9% of total muscle recruitment, and was significantly below 10% for all muscles (Figure 5B). Cases wherein muscles were not activated to any detectable level were excluded.

Delay from infrared or electrical stimulation to EMG response start time was recorded with electrical stimulus applied 0.6 mm proximal or distal to a region stimulated by infrared (3 nerves, 3 rabbits). The minimum pulse duration activation threshold was determined for each muscle activated by infrared, and ranged 0.8–2.0 ms. Across all stimulated muscles within each trial, infrared activation delays were significantly longer than electrical delays from the proximal or distal electrode. Results from one trial are shown (Figure 6). Mean infrared response latencies ranged 0.96–2.5 ms longer than mean electrical response latency.

3.3 Electro-Infrared Stimulation

Combined extraneural electrical and infrared stimulation was tested in 12 nerves in 7 rabbits using the windowed nerve cuff (Figure 1B). In 7/12 nerves EMG response to infrared stimulus was observed before beginning the trial. Analysis of paired electrical and combined stimulus recruitment curves with a 2-sample Kolmogorov-Smirnov test identified 873/930 (93%) recruitment pairs as not statistically different. Of the 67 (7.2%) significant pairs, 31 (46%) were generated on the same nerve during a single experiment and exhibited strong inhibition of Sol (Figure 7A), mixed inhibition of MG and LG, and decreased activation threshold for TA. All EMG responses were small when this trial was performed relative to earlier in the same experiment, indicating that the entire muscle was not activated by electrical or combined stimulation. This may be due to fatigue, or other confounding factor affecting the nerve state. 13/36 of the remaining statistically significant pairs showed electro-infrared activation at pulse amplitudes below the electrical-only activation threshold (Figure 7B), but recruitment differences between the two modalities disappeared above the electrical threshold. Of the remaining 23 statistically significant cases, combined stimulus shifted recruitment to slightly higher thresholds or decreased slope in 11 cases; showed an increase in recruitment slope in 3 cases; exhibited high variability in either or both recruitment curves in 9 cases; and showed no identified trend in 2 cases. Of the 9 different electrode configurations, 21/36 (58%) of the significant recruitment pairs were generated when the contact directly below the infrared delivery window was involved as cathode or anode. Of the other electrode arrangements, only the distal anode with proximal cathode appeared more often than chance, with 7/36 (19%) cases involving this arrangement.

4. Discussion

4.1 Nerve Sensitivity to INS

Nerve sensitivity to extraneural INS will impact the design of peripheral interfaces, as low sensitivity will require increased flexibility in infrared delivery. Measured nerve sensitivity to INS was low, with typically only 1–2 sensitive regions measured per nerve (Figure 2A). Additionally, response to infrared stimulus was observed in only 81% of nerves. This result contrasts with the more uniform sensitivity reported by Wells *et al.* (2007b) in rat sciatic nerve, but are in accordance with reports from Duke *et al.* (2012a) that sensitivity is localized to small regions and only occurred in 76% of rat sciatic nerves (Duke *et al.*, 2012a; Wells *et al.*, 2007b). Observed differences in sensitivity may be due to differences in nerve size between rat and rabbit, stimulating laser characteristics, or response measurement. The rabbit sciatic nerve is roughly twice the diameter of the rat sciatic nerve (Hsu *et al.*, 2011; Tyler and Durand, 2003). Since penetration depths of the wavelengths used are expected to be 300–600 μm (Wells *et al.*, 2007b), a smaller proportion of the rabbit nerve is exposed to

infrared than in the rat. This may be a significant limitation in scaling INS to larger nerve targets. Results of this study also indicate that shorter and higher-power pulses are more effective for stimulation (Figure 4). The pulsed laser source used by Wells *et al.* (2007b) delivers higher power pulses than could be achieved with the continuous-wave source used in this study, and may have impacted results (Wells *et al.*, 2007b). Finally, Wells *et al.* (2007b) monitored a different set of muscles than those observed in this study or the that by Duke *et al.* (2012a) (Duke *et al.*, 2012a). If non-recorded muscles were activated in the current study, it was not enough to cause visible movement leg movement. Because the focus of this study was evaluating functional output, undetected activation of small muscles should not alter conclusions about functional utility targeting large muscles used for foot positioning and control.

Measurements of the spatial extent of response to infrared light indicate that ISR are small, typically less than 400–500 μm in the longitudinal or transverse directions, similar to that reported for the rat sciatic nerve (Duke *et al.*, 2012a). The primary caveat to both studies' measurements is that infrared energy was applied using a 400 μm diameter fiber, which sets a lower limit on reliable feature size measurement. These results do provide an upper bound on optically sensitive region size, though, and indicate sensitive regions are relatively small.

4.2 Infrared-Evoked Muscle Recruitment

Previous INS work indicated that rehydration of the nerve was necessary for maintaining nerve viability (Wells *et al.*, 2007a, 2007b), but the effect on INS response was not reported. Trials involving fixed stimulus applied over regular intervals showed that application of lactated Ringer's solution (LRS) may temporarily reduce or abolish response to INS (Figure 3A, B). This may be due to absorption of infrared light by excess water on the nerve surface, reducing energy delivery to nerve tissue. Scanning the nerve surface with infrared light required an open preparation, and several hours were needed to scan the surface multiple times to identify as many sensitive regions as possible. Fluids were regularly applied to the nerve to maintain health of the nerve, but the timing between fluid application and scan pulse was not precisely controlled. Hydration levels would change during scanning, introducing expected variability in the results. Because multiple scans were performed at various hydration levels, the variability observed does not affect the overall study conclusions. These results indicate that to reduce variability in future studies, nerve hydration should be closely controlled.

While infrared energy delivery at the wavelengths used for INS is expected to be thermally constrained over the pulse durations used (Wells *et al.*, 2007a), axon response may be sensitive to the pulse duration used. Stimulation at lower power levels did not change activation energy thresholds, but significantly altered maximum recruitment (Figure 4C). That activation energy thresholds were maintained when activation was achieved, but maximum recruitment was not, indicates that a smaller population of axons was activated despite delivery of the same energy. Shapiro *et al.* demonstrated that rapid heating of a cell membrane in an ionic solution using infrared light drives a capacitive current across that membrane (Shapiro *et al.*, 2012). If this capacitive current is the driving mechanism of INS, then this small capacitive current may diffuse away from the deposition site, instead of causing activation, during long-duration and low-power delivery. Sustained, subthreshold currents could also have created an activation block by closing sodium inactivation gates (Grill and Mortimer, 1995). This would suggest that intra-axonal dynamics may be an important factor in designing INS delivery.

Previous measures of INS selectivity in peripheral nerve have shown motor activation patterns change in response to changing stimulus location (Wells *et al.*, 2007b, 2005a), but did not report recruitment patterns within the same location and across muscles. In 34/42

(81%) of ISR, a single muscle could be activated before another reached threshold. This result was significantly above 50%, indicating that the localized delivery of INS generally results in the ability to selectively activate a single muscle, when an ISR can be identified. This result, paired with the results indicating that a very small proportion of the muscle can be activated with INS (Figure 5), indicates that selective sub-fascicular activation can be achieved using extraneural INS. Maximum motor response remains a limiting factor to implementing INS, though, because an average of only 2–9% of each muscle was activated with INS (Figure 5). This low level of activation means that for an INS interface designed to provide graded control over activation of the first 20% of a muscle would require 2–10 INS channels, this increases to 6–30 stimulation sites if three channels of 4 Hz stimulation are required to achieve 12 Hz stimulation. If not all stimulation sites stimulate the target muscle, this number would increase further. These requirements far exceed the 1–2 available ISR typically found on the nerves investigated.

4.3 Combined Electrical and Infrared Stimulus

Previous work indicated that infrared stimulation thresholds could be lowered with entrained electrical stimulation (Duke et al., 2012a, 2009). Motor recruitment using INS alone offered limited motor recruitment capabilities (Figure 5), so it was reasoned that including electrical stimulation ramped from below threshold to supramaximal activation should reveal whether INS activates a significant axon population to a subthreshold level. The primary difference from previous studies is that differences in full-muscle recruitment were quantified, instead of just stimulation threshold shifts. Combining electrical and infrared stimulus revealed significant differences from electrical recruitment in only 7% of recruitment comparisons. Of the cases that were significantly different, the largest difference observed was recruitment inhibition observed in a single trial (Figure 7A). This behavior resembled the action potential inhibition observed by Mou *et al.* (Mou et al., 2012) and Duke *et al.* (Duke et al., 2013), rather than an increase in excitability from INS (Figure 6A). Results presented by and Duke *et al.* (Duke et al., 2012b) indicate that applying infrared light at frequencies above 4 Hz, which would be expected to cause sustained tissue temperature increases, may be used for significant force generation (Duke et al., 2012b). This indicates that combined stimulation may be more effective when infrared is used to modulate local nerve temperature and affect electrical excitability, as opposed to low-frequency, entrained stimulation.

4.4 Neural Activation with Infrared Light

At this time, the mechanism by which infrared light stimulates neural activity has not been identified, though it is under active investigation. Wells *et al.* investigated electric field generation, photochemical, and mechanical effects driven by infrared energy, but concluded that instead INS was driven by establishing a spatial or temporal thermal gradient (Wells et al., 2007a). More recently, infrared light applied to excitable cells has been shown to drive release of mitochondrial calcium and changes in the effective capacitance of the cell membrane (Dittami et al., 2011; Shapiro et al., 2012). Temperature sensitive ion channels (Fernandes et al., 2012), opened by local heating by infrared absorption may also affect excitability of the sensory and motor neurons (Anand et al., 2008; Facer et al., 2007). Any of these factors, or some combination, may alter the local electrical state of the affected cell and initiate action potentials.

Comparing infrared-evoked and electrically-evoked EMG responses provided support that infrared light activates motor neurons directly, and that electrical and infrared stimulation activate axons differently. Support that infrared activates motor neurons directly comes from the latency difference between the two methods (Figure 6). Electrical stimulation is expected to directly activate motor neurons instead of a reflex pathway, and is supported by results

showing stimulation-to-EMG latencies below 2 ms in this study. That infrared latencies are only 0.96–2.5 ms longer than electrical latencies does not support motor activation of INS through a monosynaptic reflex pathway. Reports of the Hoffmann's response (H-reflex) latency are 5 ms in the rat sciatic nerve and is reported to be longer in the sciatic nerve of the anesthetized rabbit (Chen and Wolpaw, 1995; Zheng, 1988; Zhou et al., 1997).

The increased latency observed with infrared stimulation could be due either to a slow or delayed activation mechanism, or due to activation of slower-propagating, small-diameter axons and muscle fibers. If the 2.5 ms additional latency is due to a delayed activation mechanism, then action potentials can initiate after the infrared pulse ends. This may support activation by calcium release or thermally-sensitive ion channels, depending on the time constants of each. If the delay is instead due to decreased conduction velocity, then direct activation mechanisms such as a capacitive current that is greatest during the infrared pulse may be more likely. Wells *et al.* presented data showing 2.6 ms delay between threshold energy delivery and ENG measured on the rat sciatic nerve 6 mm from the stimulation site (Wells et al., 2007a). This translates to a conduction velocity of 2.4 m/s, and either a sub-0.5 μm diameter myelinated or $\sim 1.1 \mu\text{m}$ unmyelinated fiber (Ritchie, 1982). It is possible that ENG results reported by Wells *et al.* were recorded from an unmyelinated axon. Because delays are similar to those reported in this study, a simpler explanation for both observations is that the mechanism of activation takes longer than electrical activation. This does not preclude the possibility of a combination of both mechanism delay and increased conduction velocity, though. Additional research into the mechanism of INS may help to improve infrared effectiveness and efficiency.

5. Conclusion

Extraneural INS was investigated as a means to provide selective motor activation without penetrating the epineurium or perineurium. Rabbit sciatic nerve was used in this study to investigate scaling INS to a larger nerve model than had been previously investigated. Results indicate that nerve sensitivity to extraneural INS is localized to small regions of the nerve, and that these regions occur infrequently. The evoked motor response was found to be selective to a single muscle in the majority of regions identified, but the maximum achieved recruitment fell below functional motor activation levels. Combining entrained electrical and infrared stimuli offered limited utility over electrical stimulation alone. Results indicate that nerve sensitivity and the motor response to extraneural INS can provide localized activation of small populations of axons without penetrating perineurium or epineurium.

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Figure 1. Methods for infrared and electrical stimulation of rabbit sciatic nerve

A) Continuous-wave infrared light was coupled onto a 400 μm diameter optical fiber, which was positioned relative to the nerve using a micromanipulator. Intramuscular EMG response was recorded in Medial Gastrocnemius, Lateral Gastrocnemius, Soleus, and Tibialis Anterior. B) Polydimethylsiloxane (PDMS) nerve cuff used to combine optical and electrical stimulation. Optical energy was delivered to the nerve surface through the gap forming the infrared delivery window. C) Electrical and optical stimuli are aligned so cathodic electrical stimulation ends with the IR pulse.

Figure 2. Locations of detected optically sensitive regions

Infrared-sensitive regions (ISR) detected on the rabbit sciatic nerve. Identified regions were classified as unstable if recruitment was observed but could not be maintained. Infrared sensitivity was observed in 26/32 scanned nerves. A) Typical result from one trial exhibiting infrared sensitivity. B) Composite of all identified sensitive regions observed, $n = 32$ nerves/25 rabbits.

Figure 3. Mean and 95% confidence intervals under infrared-only stimulation

A, B) Recruitment over time with constant stimulus was affected by nerve hydration. A) Example of decreased recruitment after application of 0.25–0.5 ml of lactated Ringer's solution (LRS). B) Example where LRS application did not reduce recruitment. C, D) Recruitment curves as a function of pulse duration showing: C) selective recruitment and D) non-selective recruitment.

Figure 4. Comparing response versus infrared pulse power

A) Minimum pulse duration required for activation decreases with increasing pulse power. B) Minimum pulse energy required for activation does not change significantly with pulse power. C) Ratio of recruitment at 3.4 and 2.3 W to recruitment at 4.4 W. * $p < 0.05$, ** $p < 0.01$ A,B) Wilcoxon rank sum test. C) Wilcoxon signed rank test.

Figure 5. Maximum electrical and infrared recruitment compared

A) Example electrical and infrared recruitment curves (mean and 95% confidence interval) for a single muscle B) Percentage of maximum electrical recruitment achieved with infrared stimulation. N = 12 nerves in 10 rabbits. Computed mean with 95% confidence intervals, and p-values for Wilcoxon signed rank test results against mean = 10%.

Figure 6. EMG response latency for electrical and infrared stimuli

Delay between threshold stimulus delivery and EMG response for electrical and infrared stimuli (3 nerves, 3 rabbits). Electrical stimulation was applied 6 mm proximal or 6 mm distal to infrared stimulus. EMG delay was significantly longer for muscles activated by infrared pulses, than activated by electrical stimulus.

Figure 7. Electrical and combined stimulus recruitment curves

Examples of full-muscle recruitment using electrical and combined electrical and infrared stimulation. A) Inhibition of soleus, observed under high muscle fatigue. B) Example of combined stimulus yielding statistically but not functionally significant recruitment difference over electrical stimulation. Inset: Example of optical activation below the electrical activation threshold, electrical amplitude = 0.05 mA. Recruitment curves show mean and 95% confidence intervals across applied pulses.