Electrically Stimulated Signals from a Long-Term Regenerative Peripheral Nerve Interface

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***Abstract*— Despite modern technological advances, the most widely available prostheses provide little functional recovery beyond basic grasping. Although sophisticated upper extremity prostheses are available, optimal prosthetic interfaces which give patients high-fidelity control of these artificial limbs are limited. We have developed a novel Regenerative Peripheral Nerve Interface (RPNI), which consists of a unit of free muscle that has been neurotized by a transected peripheral nerve. In conjunction with a biocompatible electrode on the muscle surface, the RPNI facilitates signal transduction from a residual peripheral nerve to a neuroprosthetic limb. The purpose of this study was to explore signal quality and reliability in an RPNI following an extended period of implantation. Following a 14-month maturation period, electromyographic signal generation was evaluated via electrical stimulation of the innervating nerve. The long-term RPNI was viable and healthy, as demonstrated by evoked compound muscle action potentials as well as histological tissue analysis. Signals exceeding 4 mV were successfully acquired and amplitudes were consistent across multiple repetitions of applied stimuli. There were no evident signs of muscle denervation, significant scar tissue, or muscle necrosis. This study provides further evidence that after a maturation period exceeding 1 year, reliable and consistent signals can still be acquired from an RPNI.**

I. Introduction

Current technology permits, at most, three degrees of freedom in a hand prosthesis: grasp, flexion, and rotation. This only marginally replicates the numerous degrees of freedom and tactile dexterity unique to the human arm and hand. A modest range of prostheses exists, from passive cosmetic limbs to functional body-powered or myoelectric devices. The current state of the art human interface for prosthetic control is targeted muscle reinnervation (TMR), which relies on skin-surface electromyography (EMG) for signal acquisition [1]. However, this interface exhibits signal instability and requires daily computer calibration for pattern recognition [2]. To improve signal fidelity, recent work has focused on the development of peripheral nerve interfaces to improve signal fidelity. Extraneural cuff electrodes have recently been used in humans to explore electrical stimulation for sensory feedback [3]. These interfaces could be used for prosthetic control, but signal amplitudes are extremely small and unlikely to be sufficient in noisy environments outside the research laboratory. To increase signal amplitudes, multiple groups are exploring the use of intrafascicular electrodes utilizing small electrode sites implanted into the nerve [4]. These devices are capable of providing larger signals from the nerves due to the close proximity of the microelectrode sites to the individual axons. However, both extraneural and intraneural interfaces are limited by micro-shearing forces and foreign body reactions that lead to scarring and signal degradation. Further, these interfaces do not address the biology of the regenerating nerve. Following amputation and nerve division, nerves will continue to regenerate until they reconnect to a target muscle or end organ. Using this biological process to their advantage, other groups are utilizing regenerative sieve and microelectrode interfaces to force axonal regeneration in close proximity to electrode sites at the interface. However, these interfaces all fail to inhibit the formation of neuromas, which cause both pain and signal interference.

Our laboratory has developed a regenerative peripheral nerve interface (RPNI) in a rat hind limb amputation model [5]. This RPNI consists of severing a branch of the sciatic nerve without amputating the entire limb. The nerve ending is then implanted into a muscle graft with an applied electrode and wrapped in an acellular matrix. We have demonstrated both the viability and durability of this interface, along with evidence of neuromuscular amplification of electrophysiological signals over implant durations exceeding 20 months. We have found that the muscle tissue dampens micromotion, improves signal-to-noise ratio, and serves as a target for the regenerating nerve, thereby preventing neuroma formation [6].

The purpose of this study was to explore signal quality and reliability in an RPNI following an extended period of implantation. While we have previously demonstrated that RPNIs remain viable for durations of approximately two years, we have not demonstrated the extent to which these interfaces can be used to create a variety of output signals. Multiple signal levels could potentially be utilized to control prosthesis movement speed or the degree of grip forced produced. Within this study, we examined RPNI signal characteristics following a maturation period exceeding one year in duration. The RPNI demonstrated successful viability, reinnervation, and signal generation.

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II. METHODS

A. Surgical Preparation of an RPNI

All animal care, housing, and experimental surgeries were approved by the University of Michigan Committee on Use and Care of Animals (UCUCA). Our rat hind limb model utilizes adult, male, specific pathogen-free Fisher 344 (F344) rats (Harlan Laboratories, Inc., Haslett, Mich.), weighing 300-350 grams at the time of surgery. The rat was first dosed with the analgesic buprenorphine hydrochloric acid (0.05 mg/kg) (Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) followed by anesthetic induction with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) (Lundbeck Inc., Deerfield, IL), supplemented as needed. The left hind limb was shaved and cleaned. An incision was made in lateral left thigh of the hind limb. The biceps femoris muscle was split longitudinally to identify the peroneal nerve near its bifurcation point off the sciatic nerve in the mid-thigh. The peroneal nerve was then carefully dissected, freed from the surrounding tissue, and then sharply divided. Another incision was then made in the lower hind limb to expose the extensor digitorum longus (EDL) muscle. The EDL was freed from the surrounding tissue and transferred to the location of the divided peroneal nerve in the thigh. The muscle graft was secured to the femur using 7-0 prolene sutures. A small incision was made in the surface of the EDL. The divided peroneal nerve was then implanted into the muscle pocket and secured with 9-0 nylon sutures. A piece of single-layer acellular extracellular matrix (Cook Biotech, Inc., West Lafayette, IN) was cut to size (about 1 cm x 2 cm), hydrated, dipped in 70% alcohol, rinsed, and then wrapped around the construct.

B. Surgical Procedure at Testing

Following a 14-month maturation period, the subject underwent endpoint electrophysiological testing under continuous inhalational isoflurane for anesthesia and buprenorphine for analgesia. The RPNI was exposed, and the peroneal nerve branch to the RPNI was isolated. The tibial branch of the sciatic nerve was transected to prevent extraneous activation of lower limb musculature that could cause signal interference. A bipolar stainless steel hook electrode was then placed around the peroneal nerve. Two platinum needle electrodes were inserted into the middle of the RPNI muscle belly (~5 mm longitudinal separation), and

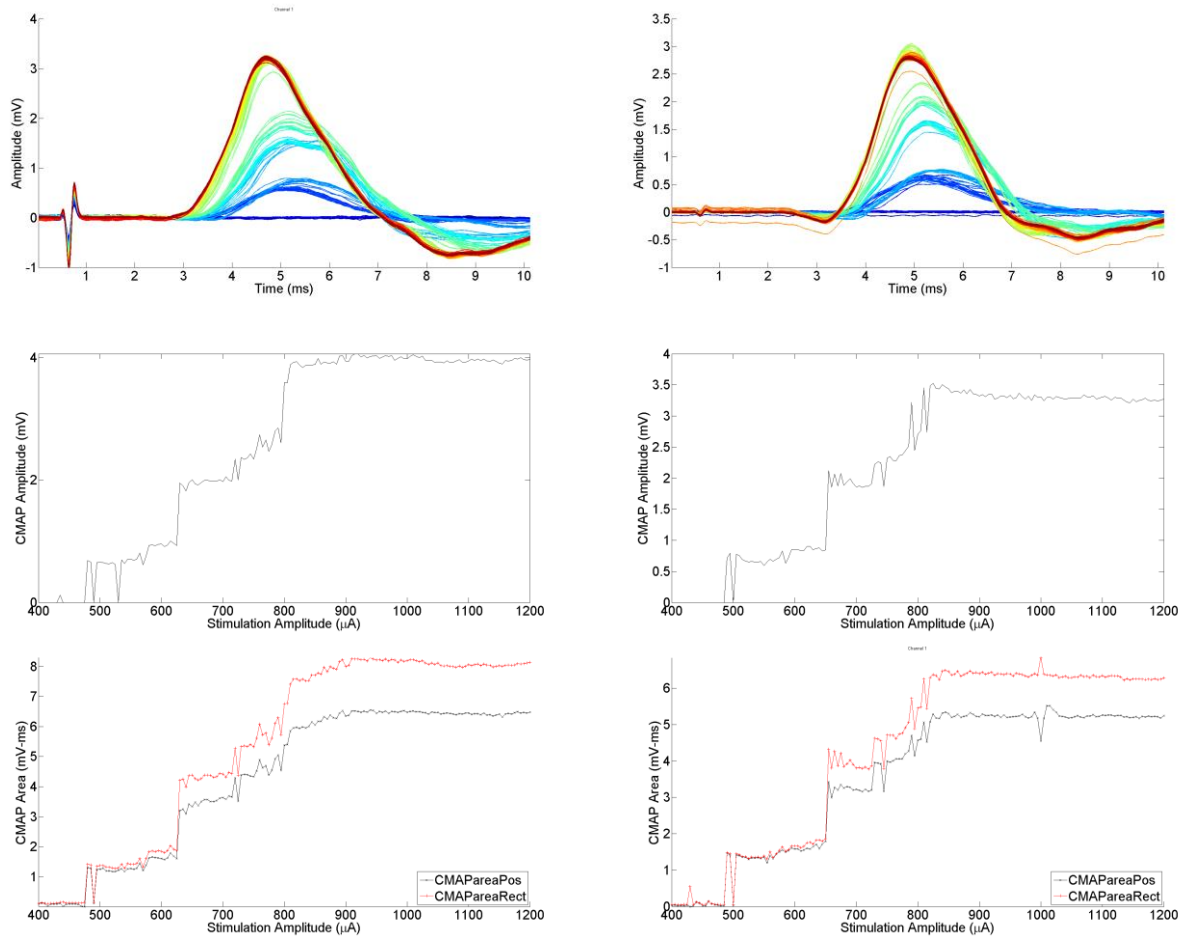

Figure 1: Example Comparison of Monopolar and Bipolar RPNI EMG Signals. Left: Monopolar. Right: Bipolar. Top Row: Pile plots of signals acquired from RPNI in both recording configurations. Color represents the stimulation intensity with Blue as the lowest (400 μA) and Red as the largest (1200 μA). Middle Row: Peak-to-Peak Voltage amplitude of the recorded CMAP response as a function of amplitude. Monopolar signals were slightly larger and more consistent than the bipolar recording. Bottom Row: Both the positive area and overall rectified area of the CMAP response as a function of amplitude are displayed.

a third needle was inserted in the web space between the second and third toes of the ipsilateral foot as a ground.

C. Electrophysiological Data Collection

Electrophysiological RPNI signals were acquired from the electrodes using a multichannel acquisition system (TDT RZ2, Tucker-Davis Technologies, Alachua, FL, USA). EMG signals were fed through an anti-aliasing filter from 2 Hz – 7.5 kHz and acquired using a TDT PZ3 differential instrumentation preamplifier. For bipolar acquisition, the distal needle electrode served as the primary input, the proximal electrode served as the reference, and the needle in the toe served as the ground. For monopolar data acquisition, the reference and ground inputs were shorted. RPNI signals were then sampled at 50 kHz for offline analysis in MATLAB. Electrical stimulation pulses were generated using the same equipment and fed through a current amplifier/stimulus isolator to deliver constant current stimuli to the innervating nerve to activate the RPNI. Driven compound muscle action potentials (CMAPs) were analyzed and characterized as a function of the stimulus pulse amplitude. All stimulation pulses were cathodic first, biphasic, 100 µs per phase delivered at a rate of 1 pulse per second. Stimulation pulses ranged from 5 to 1500 microamperes (µA). After determining the range at which stimulation induced a response over the noise threshold, signal reliability was examined by stimulating multiple amplitudes within the response range with 50-100 pulses per amplitude. The test protocol allowed measurement of multiple electrophysiological characteristics as described previously [7]. To obtain the maximal CMAP peak-to-peak amplitudes, the nerve was stimulated with increasing current until the maximal CMAP voltage response was reached. The area under the CMAP curve was calculated using a trapezoidal integral, where the depolarization area was defined as the CMAP area of the positive waveform above baseline, whereas the rectified area included the additive area below the baseline.

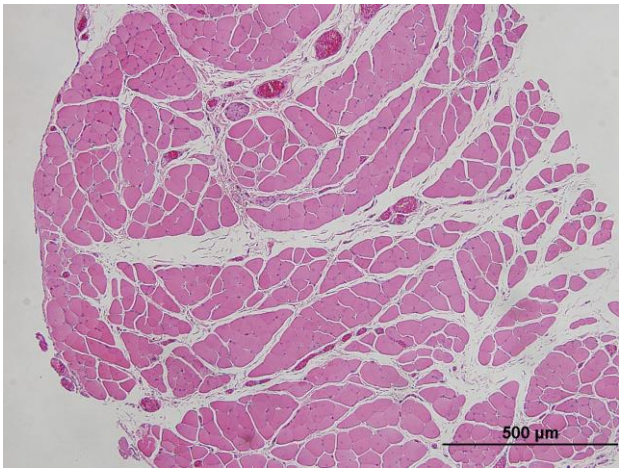

Figure 2: Histological Image of RPNI. Above tissue slice was stained with H&E. Image demonstrates that the muscle fibers are healthy with no significant signs of scar tissue.

III. RESULTS / DISCUSSION

The long-term 14-month RPNI examined in this study was viable and healthy, as demonstrated by the evoked compound muscle action potentials. High amplitude signals were successfully acquired, shown in Figure 1. There were no evident signs of muscle denervation, which would have resulted in fibrillations following activation of the RPNI. No

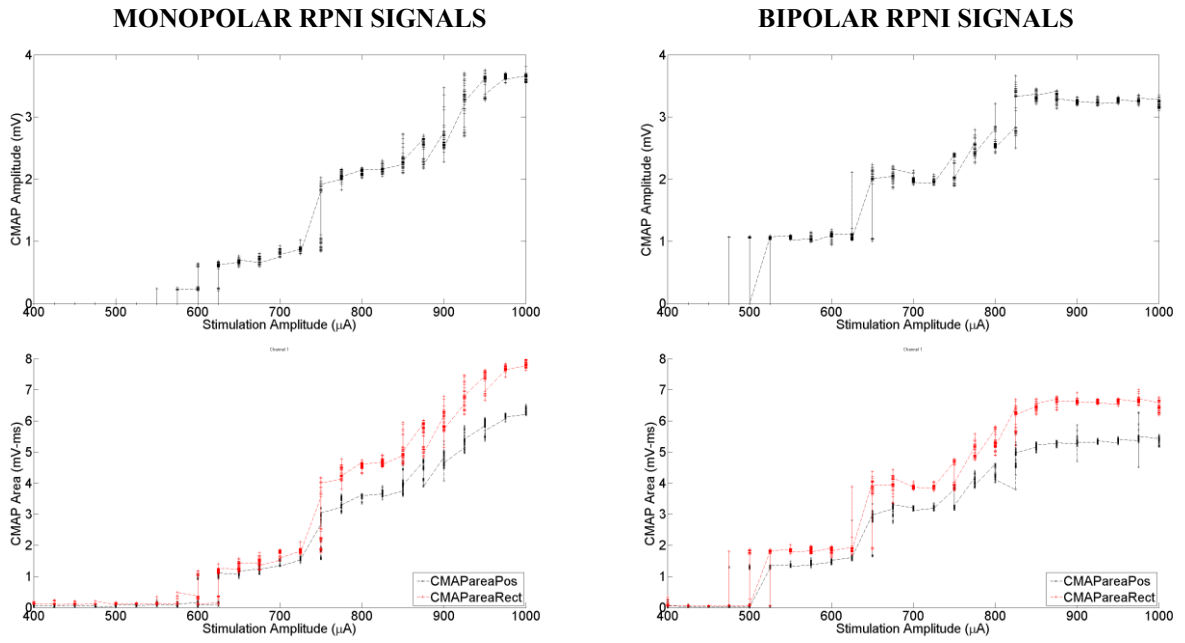

Figure 3: Plots of Reliability and Consistency of CMAP Amplitude and Area. Left: Monopolar Recordings. Right: Bipolar Recordings. Stimulation amplitudes were applied from 400 – 1000 µA in 25 µA increments. Each intensity was applied to the nerve 50 times before increasing to the next amplitude. All recorded responses are plotted on each of the plots. Vertical lines at each amplitude are indicative of the range of responses recorded. Top Row: Amplitude as a function of applied stimulation intensity. Bottom Row: Positive depolarization as well as rectified area are plotted as a function of applied current.

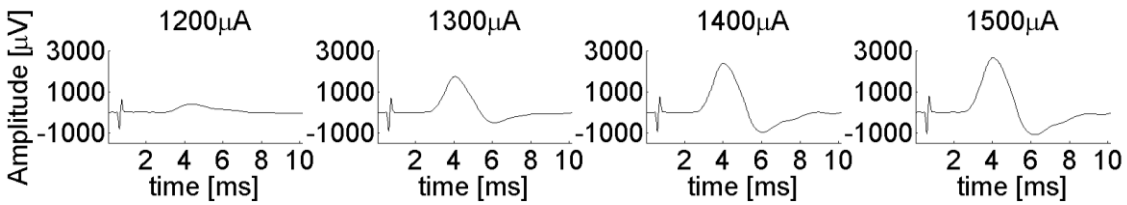

Figure 4: Average CMAP Responses. Depicted above are 4 plots summarizing repeated stimulation at stimulation intensities from 1200 – 1500 μA. Data were recorded in bipolar configuration. 100 total pulses were applied at each amplitude, and the average response was calculated at each amplitude. Average responses follow a similar stimulus response curve as the raw data.

significant scar tissue or muscle necrosis were observed intraoperatively. Throughout the six hours of continuous acquisition, EMG signals generated by the RPNI maintained similar high amplitudes to when the experiment began. Histological analysis was performed on the RPNI muscles harvested at the end of the testing period. An example image stained with hematoxylin and eosin (H&E) is depicted in Figure 2. Overall, the muscle appeared healthy with no significant signs of scar tissue or muscle degeneration.

Response threshold of the RPNI in this study was ~500 μA, though some variation was observed, likely due to exact hook electrode placement and the amount of saline / blood present nearby the stimulation site. Figure 1 depicts a typical electrophysiological evaluation from the RPNIs studied. Both monopolar and bipolar recording configurations were utilized to examine if any large differences were apparent in recording quality. As can be observed, response amplitudes were similar in both configurations, however maximum peak-to-peak voltages and areas were slightly less in the bipolar configuration (Figure 1). This is expected and likely due to some subtraction of common signals present at both closely spaced electrodes.

Ultimately, neuroprosthetic users will require consistent responses when they choose to volitionally activate the RPNI. To mimic these repeated activations, multiple repetitions of each stimulation intensity were applied to the nerve. Figure 3 depicts the overall responses to these repeated stimuli. In both the monopolar and bipolar configurations, some variability is observed at low stimulus intensities, likely due to only partial activation of the nerve that does not result in a suprathreshold response to each stimulation pulse. When beyond the threshold, there is little variation in the amplitude and area responses at each intensity. Similarly to the overall CMAP response amplitudes and areas, the overall repetitive waveform can also be observed. In Figure 4, four example amplitudes were selected from another suprathreshold dataset. As can be seen, the overall average CMAP waveform response increases with larger applied stimuli. These overall response characteristics of the RPNI to both single and repetitive stimuli strongly suggest its potential in transducing graded output signals for control of high-fidelity prostheses.

IV. CONCLUSION

Previous studies from our team have demonstrated the viability of RPNIs for durations exceeding 20 months. We have also validated that signals remain reliable and consistent for over 6 months with both stainless steel and conductive polymer electrodes implanted in these interfaces [5]. While the results observed in this study do correspond with previous studies of tissue viability, only a single subject has been evaluated to this long extent. This study provides further evidence that after a maturation period exceeding 1 year, reliable and consistent signals can still be acquired from an RPNI. Furthermore, repeated activation of the RPNI results in consistent amplitude responses over more than 50 repeated stimuli. Future studies will explore the reliability of RPNIs monitored at regular intervals in this fashion as well as validation of volitionally generated control signals for the ultimate purpose of controlling prosthetic limbs.

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