Chronic Sensory-Motor Activity in Behaving Animals using Regenerative Multi-electrode Interfaces

VH. Desai, S. Anand, M. Tran, A. Kanneganti, S. Vasudevan, JL. Seifert, J. Cheng, EW. Keefer, M.I. Romero-Ortega, *Member, IEEE.*

***Abstract*— Regenerative peripheral nerve interfaces have been proposed as viable alternatives for the natural control and feel of robotic prosthetic limbs. We have developed a Regenerative Multi-electrode Interface (REMI) that guides re-growing axons through an electrode array deployed in the lumen of a nerve guide. While acute studies have shown the use of the REMI in the rat sciatic nerve, the quality of chronic signal recording has not been reported. Here we show that implantation of this interface in the sciatic nerve is stable with high quality recordings up to 120 days and failures mainly attributable to abiotic factors related to pedestal detachment and wire breakage. We further tested the interfacing of REMI with fascicles of the sciatic nerve that primarily innervate muscles (tibial) and skin (sural). When implanted into the tibial nerve, bursting activity was observed synchronous to stepping. However, implantation of REMI into the sural nerve failed due to its small size. While fascicles smaller than 300 μm are a challenge for regenerative interfacing, we show that a modified REMI can be used in an insertion mode to record sensory signals from skin. In summary, the REMI represents an effective tool for recording firing patterns of specific axon types during voluntary movement, which may be used to improve the motor control and sensory feedback in closed loop control systems for robotic prosthesis.**

I. INTRODUCTION

Control of advanced multi-fingered prosthetic hands can be achieved through targeted muscle and skin re-innervation, which relies on surface electromyograph (EMG) recordings of the chest muscles for movement, and indirect tactor stimulation at the stump for sensory feedback [1]. However, the restricted space for EMG electrode placement, few number of independent control channels obtained, and higher cognitive burden on the users, limits this approach [2]. Peripheral nerve interfaces including extra-neural, inter-fascicular, penetrative, and regenerative type approaches that can directly record from motor axons and stimulate sensory afferents in the residual nerve stumps have been proposed to overcome such limitations [3]. While these techniques have shown to be effective for stimulation, long-term multichannel recording of single units from peripheral nerves remains a formidable challenge because of the low amplitude of nerve signals, signal contamination from myoelectric sources, foreign body response as well as abiotic factors of lead wire and connector damage, and material degradation [4][5].

We developed a Regenerative Multi-electrode interface (REMI) which guides the re-growing axons, whether acutely injured or after months of chronic amputation, through an electrode array deployed in the lumen of a nerve guide [6]. In contrast to the closed structure of sieve or micro-channel based interfaces, where axons regenerate through holes of micrometer range diameter, the REMI has an open-space design. REMI can record neural signals starting day 7 post implantation and we recently showed that the molecular mechanisms of regeneration were unaltered by the presence of the multi-electrode array in the nerve conduit [7]. Here, we evaluate its long-term reliability and performance by characterizing the progression of signal quality over time in the rat sciatic nerve. Axon type identification from regenerative interfacing recordings, as well as modality specific stimulation is complicated by the mixed nature of such axons and random interfacing with REMI electrodes. Given the fact that some nerve fascicles are naturally segregated to have predominantly muscle and skin targets (i.e., tibial and sural, respectively), we investigated the feasibility of using the REMI to interface the tibial and sural nerve fascicles to evaluate motor and sensory related neural activity.

II. MATERIALS AND METHODS

Thirty-one adult Lewis rats (150-250g) divided in three groups according to implantation sites were used in this study: sciatic nerve (n=21), tibial (n=4) and sural fascicles (n=6). All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Texas at Arlington.

A. Regenerative Multi Electrode Interface (REMI)

REMI fabrication and surgical implantation have been described before [7]. Briefly, a custom-made Floating Micro-electrode Array (FMA) with 18-pin Parylene-C insulated Platinum/Iridium electrodes (150–250 kΩ for recording; 5-10 kΩ for ground and reference; Microprobes Inc., MD, USA) was secured within the lumen of a polyurethane tube (Braintree Scientific Inc., 5-7 mm length and 1.75 mm inner diameter) and sutured across the two ends of a transected peripheral nerve. The REMIs in the sciatic nerve had varying heights of 0.7-1 mm and 400μm inter-electrode spacing. Electrodes were wired to an 18-pin connector (Omnetics, USA), housed in a titanium pedestal and secured to the pelvis using bone cement. Dual REMI implants were used in the tibial and sural nerves with shorter

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VH. Desai, S. Anand, M.Tran, A. Kanneganti are with the University of Texas at Arlington, Arlington TX. S. Vasudevan is with UT Arlington and the UT Southwestern Medical Center, Dallas, TX. J. Cheng is with UT Southwestern Medical Center and EW. Keefer is with Nerves Incorporated, Dallas, TX. JL. Seifert and MI. Romero-Ortega are with the University of Texas at Dallas and UT Southwestern Medical Center, Dallas, TX. (email: Mario.Romero-Ortega@utdallas.edu)

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(0.5 - 0.8 mm) electrodes and wired to a Zero Insertion Force (ZIF) circuit board connector (Plexon Inc., TX, USA) (Fig. 2A). The connector was housed in an Acrylonitrile Butadiene Styrene (ABS) pedestal attached to a polypropylene mesh (Ethicon) and sutured to the pelvis musculature. Sural REMIs consisted of High Density FMA (HD-FMA) with 16 recording electrodes of alternating heights (0.6 - 0.7 mm) and 250 µm inter-electrode space, arranged in two rows on a 2.95 x 1.6 mm ceramic base (Fig. 3A).

B. Neural Signal Recording and Analysis

Electrophysiological signals from the REMI were obtained from wake behaving animals inside a Faraday cage using an Omniplex Data Acquisition System (Plexon Inc., USA) at 40 kHz sampling frequency in a bandwidth of 100-8000 Hz (Bessel 4-pole analog filter). Signals were differentially amplified with a reference or ground signal generated by the preamplifier. Each recording session, at least 5 min long, was videotaped (80 frames/sec) and synchronized with neural data using Cineplex Behavioral Research System (Plexon). Recordings were performed weekly after implantation, until failure or up to 120 days. Signals were high-pass filtered offline at 800 Hz to attenuate EMG contamination and power line interference. Waveforms that crossed a fixed threshold of (-4.5) standard deviation from the mean distribution of signal were extracted in windows of 1400 µsec. Large amplitude movement artifacts were manually removed and remaining waveforms were classified into Single Unit (SU) spikes using Principal Component Analysis (PCA) based semi-automated spike sorting techniques in Offline Sorter (Plexon). The entire process was done by a single investigator to minimize variability [8]. Further, to distinguish SU from Multi Unit (MU) activity, putative spike waveforms were subjected to the uniform criteria of having a) repetitive consistent biphasic or tri-phasic shape and b) less than 1% waveforms within an Inter Spike Interval (ISI) of less than 3 msec. c) Signal-to-Noise Ratio $\geq$ 3 [9]. P-P amplitude was defined as the difference between the maximum and minimum voltage of the mean waveform of all spikes in a unit. Since, the spectral properties of residual waveforms and background activity in *in vivo* neuronal recordings are similar [10], noise was defined as the collection of residual waveforms obtained after subtracting the mean waveform from each individual waveform within the unit [11] and SNR as described in (1)

$$\text{SNR} = \frac{\text{Vp-p}}{2 * \text{Standard Deviation (SD) of noise}} \quad (1)$$

III. RESULTS

A. Stability in Quality of Spontaneous Sciatic Nerve Activity

Nerve regeneration was observed in all 21 animals and gross examination of the explanted tissue revealed an average of 14 out of 18 FMA electrodes to be embedded within the regenerated tissue. Average array yield (percentage of electrodes recording SU from the number of embedded electrodes) was found to be 18.23 $\pm$ 2.01% (Mean $\pm$ SEM). As reported previously, spontaneous action potentials were recorded from the regenerating sciatic nerve since 7 days post implantation [6][7]. The average number of SUs acquired per implant fluctuated from 5.3 $\pm$ 1.3 at day 21 to 2.9 $\pm$ 0.6 at day 28, though this decrease was not statistically significant, and remained relatively stable from 28 to 120 days (Fig 1B).

Overall, a total of 161 SUs with average P-P amplitude of 100.83 $\pm$ 5.40 µV were recorded over the entire duration of this study. SUs as high as 850 µV and SNR 24:1 were also recorded occasionally, however excluded from statistical tests. A significant increase was observed from 81.92 $\pm$ 8.47 µV at day 14 to 125.6 $\pm$ 16.96 µV at 28 days post implantation ($p=0.0003<0.05$; Fig 1C), perhaps associated with maturation of axons. Despite the initial damage caused by nerve transection associated with REMI implantation and subsequent regeneration through the conduit, the average SNR of SUs was high at 5.1:1 $\pm$ 0.1:1 and remained consistent throughout the study, with no significant changes at any of the eight time points evaluated (7- 120 days; Fig. 1D). Representative examples of SU waveforms across all time points are shown in Fig 1E.

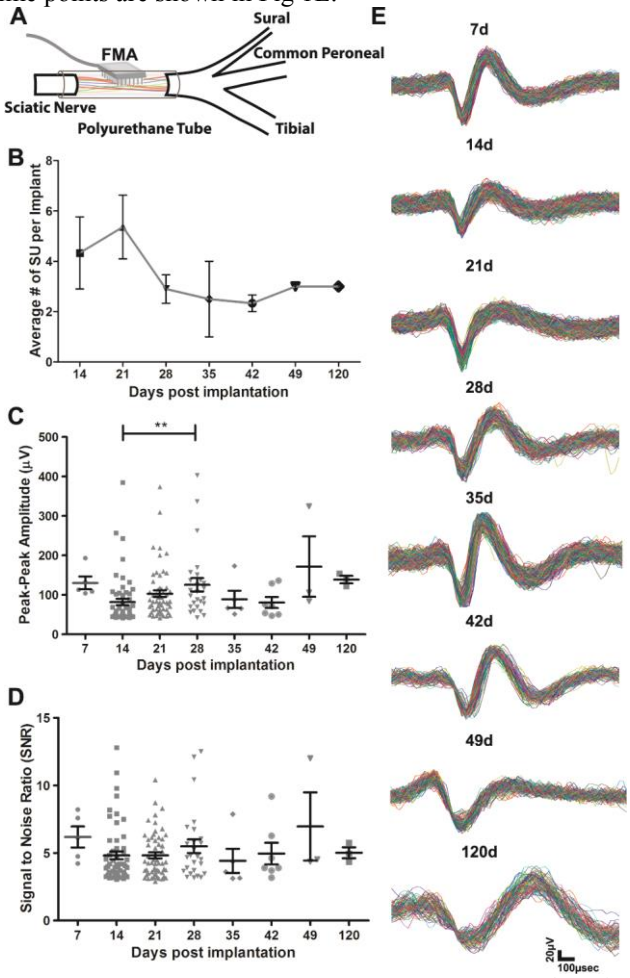

Figure 1. Signal quality from sciatic implanted REMIs. A) Schematic of the REMI implant. Quality metrics over time: Average (B) Number of SU per implant (C) P-P amplitude, and (D) SNR of SUs. Individual data points presented (C-D) along with Mean and the Standard Error of Mean (B-D). Statistical analysis by non-parametric Kruskal-Wallis test, and Dunn's multiple comparisons post-test; **indicates significance $p<0.01$). (E) Representative examples of SU waveforms from 7 to 120 days

Failure Mechanism: Majority of the REMIs implanted in sciatic nerve (n=13 out of 17; 76%) failed due to abiotic factors such as loss of connector due to pedestal detachment or wire breakage. We reasoned that our weekly recording sessions contributed to the pedestal failure and to confirm this possibility we minimized the handling of the pedestal in two animals. In one we delayed the testing until 49 days post implantation, and in the other we placed the connector sub-dermally until it was resurfaced at 110 days, and signals acquired at 120 days post implantation. In these two animals successful recordings were obtained, with the number of SUs, P-P amplitude, and SNR levels comparable to those observed in the first weeks of implantation, suggesting that frequent handling of the connector/pedestal contributes to their failure, and that the nervous tissue/electrode interface in the REMI is rather stable.

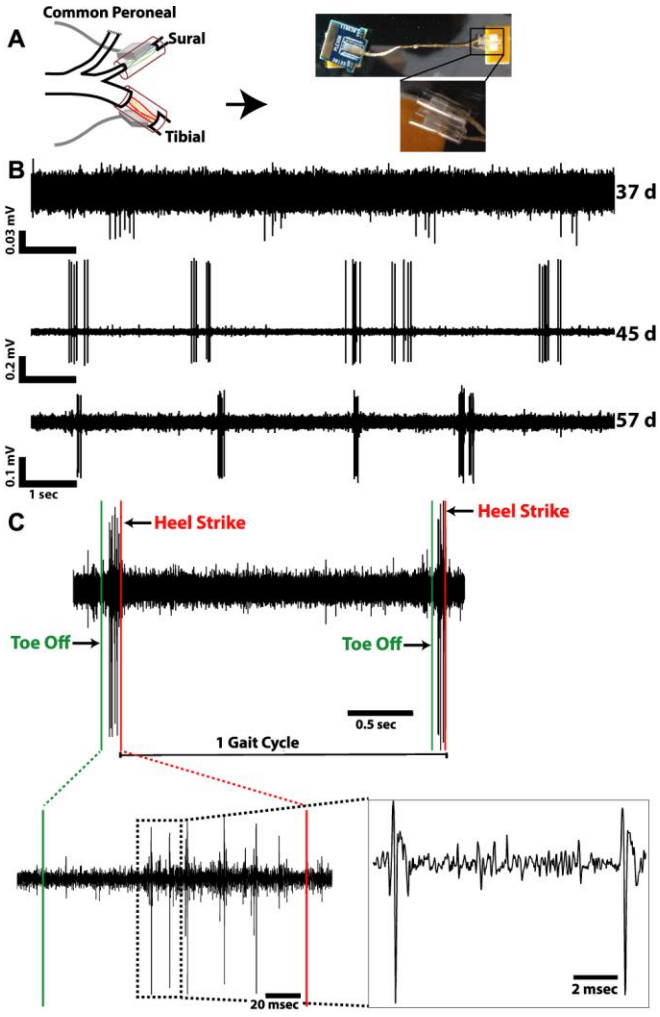

Figure 2. Tibial nerve activity during bipedal locomotion. (A) Schematic of the REMI fascicular implant and a photograph of the Dual REMI connected to a ZIF connector. (B) Bursting neural spikes observed during treadmill walking at 37, 45, and 57 days post implantation. (C) Neural activity during one representative gait cycle at 57 day with inset (left) showing time magnified view of signals from toe off (green) to heel strike (red) and individual action potential spikes within a burst (right).

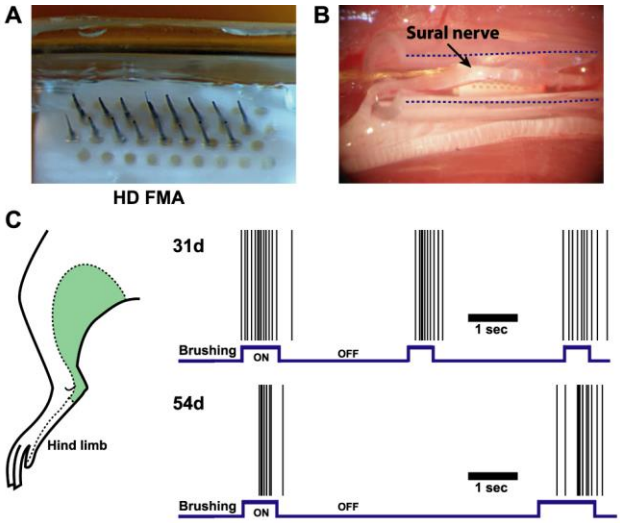

Figure 3. Sensory action potentials from the sural nerve. (A) HD-FMA designed to interface with the sural nerve. (B) Sural nerve placed on the FMA after epineurium removal. (C) Schematic shows receptive field (dotted line) of sural nerve on the lateral side of the hind limb adapted from [14]. Shaded region represents area brushed in this experiment. Raster Plot of spikes evoked by Q-tip brushing at 31 and 54 day post implantation (anesthetized animal).

B. Efferent activity from Tibial nerve during bipedal locomotion

Four animals were trained for bipedal treadmill walking (3cms^{-1}, 30 min/day, 30 days) before REMI implantation in the tibial nerve. Neural activity was successfully recorded from two animals during 30 gait cycles with an average cycle duration of 1.48 $\pm$ 0.65 seconds (Mean $\pm$ SD) at 37, 45, and 57 days post implantation, and correlated with the timing of heel contact and toe off (Fig 2C). Some electrodes showed tonic firing pattern when the animal was standing on its hind limbs, while others were silent. However, this activity changed into bursting firing of action potentials upon initiation of walking. The bursting activity was observed to be synchronous with rhythmic stepping during bipedal locomotion, and was consistent until 8 weeks post implantation (Fig 2B). This activity mostly occurred during the swing phase between toe off and heel strike (Fig 2C). Each burst typically consisted of 4.49 $\pm$ 0.87, and 4.35 $\pm$ 0.75 spikes (Mean $\pm$ SD); at 45 and 57 days post implantation, respectively.

C. Evoked afferent activity from sural nerve

The initial implants of REMI electrodes in the sural nerve of 4 animals failed to provide signals despite successful nerve regeneration. Upon examination, we confirmed that the regenerated sural nerve was too small (200-250 µm) to grow accurately through the active sites of a regular size REMI. To address this limitation, we interfaced the sural nerve with HD-FMAs in two animals, wherein the epineurium was incised longitudinally and the nerve manually inserted on the electrode array through a window made in the top half of the REMI conduit (Fig. 3B). Using this strategy we were able to record sensory afferent signals starting at 13 days and consistently up to 61 days post implantation from one animal. Figure 3C shows the raster

plot of spike sorted SU waveforms obtained in response to gentle brushing of the sural nerve receptive field at 31 and 54 days. The same electrode did not show any neural activity in response to noxious thermal stimuli, pinching of skin and application of force using calibrated Von Frey filaments; hence is presumably a Low Threshold Mechanoceptive (LTM) unit. Because the spike discharges were sustained throughout the duration of brushing, it can inferred to be Slowly Adapting (SA)[12].

IV. Discussion And Conclusion

This study demonstrates stable REMI interfacing of the sciatic nerve for 120 days. The wound healing and inflammatory response associated with complete nerve transection is severe [7]. However, we observed the largest number of spontaneous SUs with SNR averaging 4.88 ± 1.91 during the first three weeks after implantation which suggests that the tissue response, even when exacerbated by wound healing, does not prevent signal recording. From 28 to 120 days, a modest reduction in the number of spontaneous SUs was observed. This change is consistent with the fact that skin sensory afferents are not spontaneously active and only depolarize in response to specific stimuli. In contrast to the relative stable quality of recorded signals observed, most sciatic REMIs suffered from mechanical failures within the first 60 days of implantation. This notion was supported by the ability to achieve relatively long-term recordings at 2 and 4 months in animals where recordings were delayed, indicating that the main cause of failure in the REMI was abiotic, primarily due to pedestal detachment and wire breakage. This limitation can be obviated in the future by the use of fully implantable wireless systems.

Redundant muscle and skin nerve fascicles are expected to be available in distal amputations, which can offer more selective targets for peripheral neural interfacing. Here we confirmed the ability of the REMI to interface with muscle fascicles and demonstrated that movement related activity can be recorded during locomotion. In some electrodes we observed two distinct firing pattern of spikes (tonic and bursting) similar to those reported in motor units of fast and slow twitch muscle fibers[13]. While we could not differentiate between motor or proprioceptive axons, in amputee preparations only motor signals would be expected. In contrast to the tibial nerve, REMI implantation in the sural failed due to the small size of this fascicle. This result underlies an important limitation in interfacing of small fascicles. However, fascicular sizes in human amputees are expected to be significantly larger, mitigating some of the concerns associated with this result. Importantly, using a modified insertion approach, we were able to record sensory afferent activity that could be evoked by mechanical stimuli.

In summary, sensory-motor neural activity can be recorded chronically by REMI electrodes with high SNR which serves as a tool for evaluating firing patterns of specific axon types during voluntary movement or sensory stimulation. In turn, this interface can be used to improve motor control and sensory feedback in closed loop systems for robotic prosthesis.

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