# Quantification of Muscle-Derived Signal Interference During Monopolar Needle Electromyography of a Peripheral Nerve Interface in the Rat Hind Limb

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*Abstract*— **High-fidelity signal acquisition is critical for the fundamental control of a neuroprosthesis. Our group has developed a bio-artificial interface consisting of a muscle graft neurotized by a severed nerve in a rat hind limb model. This regenerative peripheral nerve interface (RPNI) permits nerve signal transmission, amplification, and detection via *in situ* electromyography (EMG). Our study examined the magnitude of signal interference from simultaneously contracting muscles adjacent to our muscle of interest.**

**In eighteen F344 rats, the extensor digitorum longus (EDL) muscle was used to fabricate simulated RPNI constructs of various sizes in which the neurovascular pedicle was preserved, obviating the need for reinnervation or revascularization. After 3 weeks of recovery, *in situ* EMG testing was performed using electrical stimulation of the common peroneal nerve. A recording needle was placed in the EDL muscle with a reference/ground electrode in the contralateral toe webspace, comprising a monopolar recording configuration. The superficial peroneal nerve was transected to further isolate stimulation of the anterior compartment. Recordings from the EDL were performed before and after excision of the tibialis anterior (TA) and extensor hallucis longus (EHL) muscles.**

**After TA/EHL excision, EDL compound muscle action potential (CMAP) peak-to-peak amplitudes were significantly lower by an average of 7.4±5.6(SD) mV, or 32±18%, (paired $t(17)=-5.7$, $p<0.0001$). A significant positive linear correlation was seen between CMAP amplitude and EDL mass both before TA/EHL excision ($r=0.68$, $n=18$, $p<0.01$) and after TA/EHL excision ($r=0.79$, $n=18$, $p<0.0001$). EDL mass did not correlate with differences in CMAP amplitude or area caused by TA/EHL excision.**

**Monopolar needle EMG recordings from the EDL muscle are significantly, but predictively, contaminated by concomitant muscular contractions in the anterior compartment of the rat hind limb. Further investigation of strategies to reduce this signal interference, including electrode choice or configuration, use of bioelectrical insulators, and filtering methods, is warranted to promote high-fidelity signal acquisition for prosthetic control.**

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## I. Introduction

Advanced prosthetic control strategies rely on the successful detection of independent peripheral nerve signals from an amputee's residual limb. Although highly sophisticated prosthetic hands capable of multiple degrees of freedom do exist, their use has been limited as the search continues for an optimal interface between human and machine [1]. Targeted muscle reinnervation (TMR), which employs nerve transfers to reinnervate specific muscle sites, is the most immediately applicable interfacing strategy that has been demonstrated in humans and can increase the number of neural input signals available for prosthetic control [2]. Other strategies include direct brain interfaces, which have also been successfully tested in humans but are generally considered too invasive and high-risk for a population of patients with limb loss [3]. Direct peripheral nerve interfaces involving epineural and intraneural electrodes have been studied as well [4], but these nerve recordings tend to be small and are further degraded by scar formation over time [5].

As a solution, our laboratory is developing a regenerative peripheral nerve interface (RPNI), which bridges the gap between a severed nerve and the electronics of a prosthetic device with a muscle graft [6]. Specifically, an RPNI device consists of a nonvascularized skeletal muscle graft that is implanted with and subsequently neurotized by a transected peripheral nerve. Through the muscle graft, nerve signals can be transmitted, amplified, and further detected by either epimysial or intramuscular electrodes [7]. Unlike TMR, the RPNI strategy is not restricted to the utilization of vascularized muscle within the residual limb or the nearby chest wall, thereby permitting physiologically relevant connections to individually functioning fascicles within the peripheral nerve. Furthermore, by connecting the severed nerve to a muscle graft, the RPNI device also prevents neuroma formation.

We perform monopolar needle electromyography (EMG) to test the viability of the muscle graft used for RPNI construction and its state of reinnervation. Given the importance of differentiating our signal of interest from other signals derived from the adjacent musculature, we set out to quantify the magnitude of such interference within one of our rat hind limb models. This model is located in the hind limb's anterior compartment, which contains the tibialis anterior (TA), extensor hallucis longus (EHL), and extensor digitorum longus (EDL) muscles. Innervated by branches of

the deep peroneal nerve, these muscles serve to dorsiflex the ankle in unison. In this study, we hypothesized that signal interference from concomitant stimulation of the anterior compartment musculature would significantly contaminate monopolar needle recordings from EDL muscles that are used to construct simulated RPNI devices.

## II. Methods

### A. Animals & Experimental Design

Experiments were performed on F344 adult male rats at 10-11 months of age, weighing 400-500g. All animal care, housing, anesthesia, analgesia, surgical procedures, and terminal assessments were conducted in accordance with the *Guide for the Care of Laboratory Animals* [8]. The animal care committee at the University of Michigan approved all protocols. Eighteen rats were studied to create an array of simulated RPNI devices using neurovascular intact EDL muscles (one device per rat), as described below. After three weeks of animal recovery, all EDL constructs underwent monopolar needle EMG recordings upon stimulation of the common peroneal nerve (CPN).

### B. Construction of Simulated RPNI Devices

Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital and given subcutaneous buprenorphine hydrochloric acid for analgesia. The EDL muscle was exposed within the anterior compartment of the lower hind limb. For each construct, a varied amount of EDL muscle was excised, from 0 to 75 mg, while preserving the remaining EDL muscle and neurovascular pedicle, thereby creating an array of simulated RPNI devices of different sizes. Each muscle was then wrapped in a layer of acellular small intestinal submucosa (Surgisis®, Cook Biotech, West Lafayette, Indiana, USA) for biological insulation. Wounds were closed with suture.

### C. Monopolar Needle Electromyography (EMG)

After a three-week recovery, each rat was anesthetized, and the surgical site was re-opened to expose the surface of the simulated RPNI device in the lower hind limb. The common peroneal nerve was isolated in the thigh, and the superficial peroneal nerve was segmentally resected in order to denervate the lateral compartment musculature, minimizing cross-compartment signal interference. The common peroneal nerve also gives rise to the deep peronal nerve which remains intact, innervating each of the three muscles of the anterior compartment (TA, EHL, and EDL). As such, stimulation of the common peroneal nerve results in the simultaneous activation of the TA, EHL, and EDL. A 0.32-mm diameter stainless steel needle electrode (112-812-48TP, Grass Technologies, Warwick, Rhode Island, USA) was placed in the RPNI device at the center of the EDL muscle. The recording reference was shorted to the ground electrode placed in the contralateral first toe web space, thereby creating a monopolar recording configuration. A stimulating bipolar stainless steel hook electrode (501650, Harvard Apparatus, Holliston, Massachusetts, USA) was placed on the proximal CPN. Compound muscle action potential (CMAP) signals were acquired using a multichannel acquisition system (RZ2, Tucker-Davis Technologies (TDT), Alachua, Florida, USA). The signals were fed through an anti-aliasing filter (from 2-Hz to 7.5k-Hz) and a preamplifer before being digitized at 50-kHz for offline analysis in MATLAB (Mathworks, Natick, Massachusetts, USA). Biphasic pulses were generated using the TDT equipment, fed through a current amplifier-stimulus isolator, varied from 5 to 505-μA over 100 pulses, at a repetition rate of 1.0-Hz and phase duration of 0.1-ms. After obtaining the initial recordings, the recording sequence was repeated after surgical excision of the TA and EHL (Fig. 1).

### D. Statistical Analyses

SPSS software (IBM SPSS Statistics 21, Chicago, Illinois, USA) was used for all statistical analyses. Data were analyzed using the paired-samples T-test and Pearson correlation. Statistical significance was set at $p \leq 0.05$.

## III. Results

Monopolar needle EMG was used to record CMAPs from the eighteen simulated RPNI devices that were constructed with the EDL muscle in the anterior compartment of the rat hind limb. Successful CMAP recordings were obtained from all RPNI devices, both before and after surgical excision of the TA and EHL muscles.

Prior to TA/EHL excision, mean CMAP peak-to-peak amplitude was 21.6±9.7(SD) mV, ranging from 9.7 to 46 mV. Mean rectified peak CMAP area (i.e. absolute area under the peak CMAP curve) was 23.4±11.7 mV•ms, ranging from 11.3 to 57.9 mV•ms. Average threshold

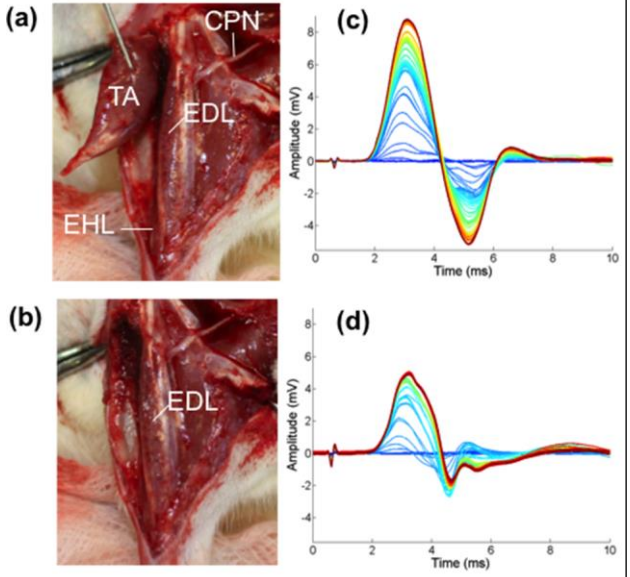


Figure 1. (a) *In situ* appearance of anterior compartment musculature with TA (*retracted*) and EHL (*hidden deep to EDL*) before excision and (b) after excision of TA and EHL. (c) Example of a monopolar needle CMAP recording from an EDL construct (simulated RPNI) upon electrical stimulation of the common peroneal nerve (CPN) prior to and (d) after excision of TA and EHL.

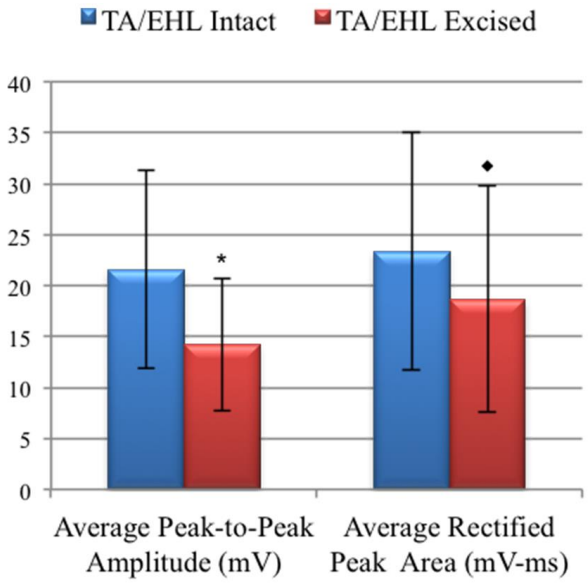


Figure 2. CMAP peak-to-peak amplitudes and rectified peak areas were recorded via monopolar needle EMG of simulated RPNI devices (n=18), before and after excision of the TA and EHL muscles. Both CMAP parameters were significantly lower after TA and EHL excision: paired $t(17)=5.7$, *$p<0.0001$ for amplitude; paired $t(17)=2.8$, •$p=0.012$ for area. Error bars represent standard deviations (SD).

stimulation current was 143±70 μA. Average latency was 3.21±0.53 msec.

After TA/EHL excision, mean CMAP amplitude fell to 14.1±6.5 mV, ranging from 6.8 to 28.1 mV. In addition, mean rectified peak CMAP area fell to 18.7±11.1 mV•ms, ranging from 8.5 to 44.4 mV•ms. Average threshold stimulation current was 183±102 μA. Average latency was 3.56±0.53 msec. Overall, amplitudes were significantly lower after TA/EHL excision by an average of 7.4±5.6 mV (32±18%): paired $t(17)=5.7$, $p<0.0001$ (Fig. 2); while rectified peak areas were significantly lower by an average of 4.8±7.2 mV•ms (19±31%).

Notably, both CMAP amplitude and rectified area significantly correlated with the mass of the simulated RPNI in a linear fashion. After TA/EHL excision, these significant relationships were not only maintained, but also strengthened. The correlation between amplitude and mass is illustrated in Fig. 3: $r=0.68$, $n=18$, $p<0.01$ with TA/EHL intact; $r=0.79$, $n=18$, $p<0.0001$ with TA/EHL excised. The correlation between rectified area and mass is illustrated in Fig. 4: $r=0.70$, $n=18$, $p=0.001$ with TA/EHL intact; $r=0.89$, $n=18$, $p<0.0001$ with TA/EHL excised. RPNI mass did not correlate with the absolute differences in amplitude or area caused by TA/EHL excision.

## IV. Discussion

The above findings support our hypothesis that signal interference from concomitant stimulation of adjacent musculature significantly contaminates monopolar needle EMG recordings from RPNI devices that are located in the anterior compartment of the rat hind limb. Indeed, up to 60% of RPNI maximal amplitudes in this model can be attributed to the simultaneous activation of the tibialis anterior (TA) and extensor hallucis longus (EHL) muscles. In this study, we have successfully quantified the magnitude of EMG signal interference from adjacent, innervated rat hind limb musculature through surgical excision of the interfering muscles. This study underscores an important obstacle that must be addressed in the quest for high-fidelity signal acquisition required for voluntary prosthetic control in humans, where the continuous presence of simultaneously contracting muscles is a reality, and surgical excision of these muscles is not an option.

From an experimental methodology standpoint, our findings prompt the testing and use of other electrode types and/or configurations that can focus the detection field on the signal-of-interest and filter out extraneous bio-signals [9]. We employed the method of monopolar needle EMG in our experiment, as it is a common strategy used for the electrophysiological assessment of muscle and nerve disorders in a variety of animal and human studies [10]. The monopolar needle typically consists of stainless steel or platinum, with an insulating material covering the whole needle except the conductive conical tip. An advantage of this type of needle is that electrical activity can be recorded from all directions, resulting in a relatively large detection field [11]. The caveat is that its field may be too large in certain cases. Concentric needles, which have smaller conductive areas facing just one direction, may be utilized more effectively for these applications by reducing the amount of extraneous signal detected [12].

RPNI signal detection may also be improved by switching to a bipolar recording strategy, which may increase signal selectivity [13]. The application of a biocompatible

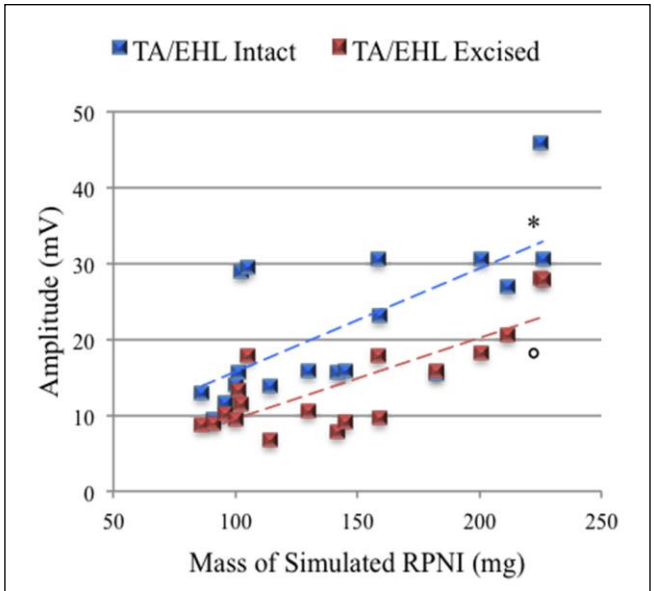


Figure 3. A significant positive correlation between CMAP peak-to-peak amplitudes and simulated RPNI mass was present before and after TA and EHL excision. Pearson correlation coefficients: $r=0.68$, $n=18$, *$p<0.01$ with TA/EHL intact; $r=0.79$, $n=18$, °$p<0.0001$ with TA/EHL excised.

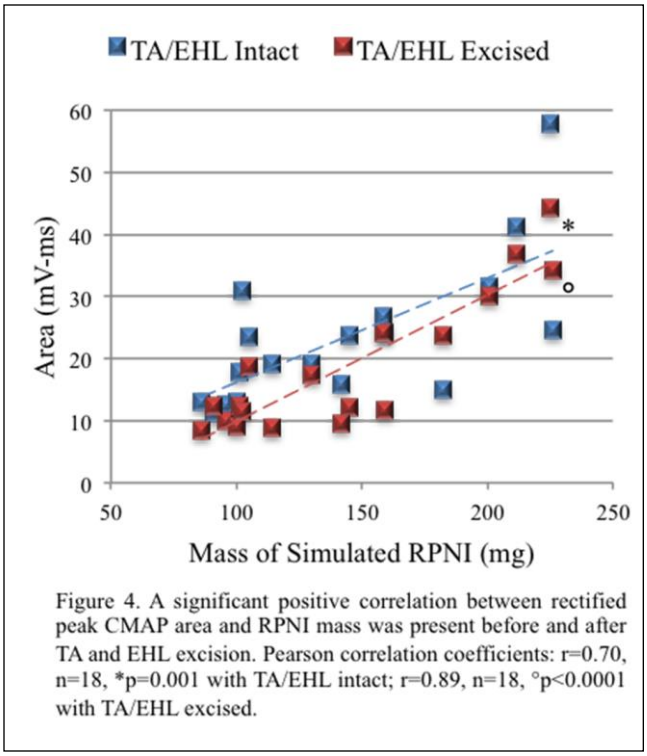


Figure 4. A significant positive correlation between rectified peak CMAP area and RPNI mass was present before and after TA and EHL excision. Pearson correlation coefficients: r=0.70, n=18, *p=0.001 with TA/EHL intact; r=0.89, n=18, °p<0.0001 with TA/EHL excised.

insulating material around the device may also improve signal isolation [14]. Furthermore, post-acquisition digital filtering methods may also be employed during signal processing. By refining signal detection, the relationship between RPNI signal strength and existing independent variables may be further delineated. In this study, for example, not only was RPNI mass a significant predictor of CMAP peak-to-peak amplitude, but the correlation was further strengthened by removing the interfering muscles. Unmasking the exact impact of these variables on RPNI signal properties is crucial for RPNI optimization, guiding its translation into humans.

Study limitations include the focus on only the anterior compartment muscles of the rat hind limb. Our hope is that this report can be expanded to other animal models by raising awareness that significant signal interference can arise from adjacent muscles during monopolar needle EMG. Another limitation is that the recorded potentials were evoked by direct electrical nerve stimulation in the anesthetized rat. Perhaps voluntary muscle contractions in the alert rat during normal low-level activity may not result in such a magnitude of signal interference. Finally, we observed a high variability in absolute differences in RPNI signal amplitude and area after excision of TA/EHL, with no detectable relationship with RPNI mass. It would seem that excising the same two muscles from rats of similar weights would result in similar decreases in recorded amplitudes and areas. One explanation is that needle placement after TA/EHL excision may have been in a slightly different position compared to the initial recordings, picking up different motor units. Another explanation is that a small amount of nerve damage during muscle excision could have led to slight but variable deficits in signal transmission, despite our meticulous surgical technique.

## V. Conclusion

Monopolar needle EMG, though widely used in research, may not be the ideal recording strategy for our RPNI model in the rat hind limb, as signals are significantly contaminated by activation of adjacent muscles. The RPNI is meant to serve as a bio-artificial interface connecting severed nerves to an advanced prosthesis, permitting voluntary motor control for humans who have sustained limb loss. As such, the presence of surrounding muscles is an inevitable reality and will need to be addressed in order to translate our technology into humans.

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